We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS

BOOK
CITATION
INDEX

INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Cerebral Ischemia Induces Neuronal Vulnerability and Astrocytic Dysfunction in Stroke-Prone Spontaneously Hypertensive Rats

Kazuo Yamagata

Additional information is available at the end of the chapter

Abstract

Stroke-prone spontaneously hypertensive (SHRSP) rats develop severe hypertension, and more than 95% of them die of cerebral stroke. Cerebral ischemia or hypoxia and/or subsequent oxygen reperfusion strongly induces neuronal damage in SHRSP rats. The biochemical features of brain cells such as neuronal cells and astrocytes of SHRSP rats might contribute to the strong tendency of SHRSP rats to suffer strokes. In SHRSP rats, the production of hydroxyl radicals was strongly elevated after reperfusion. Neuronal expression of thioredoxin (*Txn1*) and *Bcl2* genes was significantly reduced in SHRSP rats compared with Wistar Kyoto (WKY) rats. In SHRSP rats, the susceptibility of neuronal cells to death is partly due to an insufficiency of mitochondrial redox regulation and a deficiency of the apoptosis-inhibitory protein Bcl-2. Antioxidant vitamin E may regulate the expression of redox and apoptosis-related proteins in neuronal damage. In astrocytes isolated from SHRSP rats, the cells' proliferative ability and expression of vascular cell adhesion molecule-1 (VCAM-1) and high-mobility group box 1 (HMGB1) are strongly increased compared with those in the WKY rat strain. Astrocytic lactate production, an energy source for neuronal cells, was reduced in SHRSP rats in comparison with the WKY rat strain. SHRSP astrocytes reduced their production of glial cell line–derived neurotrophic factor (GDNF) and L-serine compared to WKY astrocytes during hypoxia and reoxygenation (H/R). Furthermore, sphingosine-1-phosphate (S1P) reduced the expression of GDNF in primary SHRSP rat astrocytes. On the other hand, production of L-serine and the expression of alanine/serine/cysteine/threonine transporter (ASCT1) were lower in SHRSP than in WKY rat astrocytes after exposure to arginine vasopressin (AVP). In this chapter, we describe the neuronal vulnerabilities and astrocytic dysfunctions of SHRSP rats induced by cerebral ischemia.

Keywords: astrocyte, apoptosis, GDNF, neuronal cell death, SHRSP

1. Introduction

Stroke involves cerebral infarction and hemorrhaging and is associated with very high mortality. Stroke causes a loss of brain function due to an insufficient blood supply to the brain. The stroke-prone spontaneously hypertensive (SHRSP) rat is an experimental model of human malignant hypertension (>200 mm Hg), and this rat strain has a high incidence of cerebrovascular disease [1, 2]. Namely, SHRSP rats develop severe hypertension of more than 200 mm Hg, and more than 95% die of stroke [1, 2]. In SHRSP rats, the increase of sodium intake accelerates the rise of blood pressure (BP), and cerebral ischemia induces the appearance of cerebral vasogenic edema [3]. Therefore, SHRSP rats are widely used as a model of human stroke [2].

Studies of SHRSP rats may provide considerable useful information regarding human strokes and should indicate genetic susceptibility of particular types of cerebrovascular diseases [4]. Indeed, this strain shares features with human lacunar stroke [5]. Twenty minutes of cerebral ischemia in SHRSP rats induced a large efflux of glutamate, causing strong delayed neuronal death in region CA1 of the hippocampus, whereas the parental strain of SHRSP rats, Wistar Kyoto (WKY) rats, lacked these characteristics under the same conditions [6]. The hippocampal neurons of SHRSP rats were innately vulnerable to ischemic stimulation, and the Ca^{2+} channel blockers prevented neuronal cell death in SHRSP rats [7]. The production of hydroxyl radicals by neurons was strongly elevated after reperfusion of SHRSP rats. In neuronal cells, expression of the thioredoxin gene (*Txn1*) and the *Bcl2* gene was significantly reduced in SHRSP rats compared with WKY rats [8]. We showed that SHRSP rat neurons were more vulnerable than WKY rat neurons during cerebral ischemia-hypoxia [9, 10]. In these findings, we noticed that unknown factors other than a hereditary weakness in the neurons themselves played additional roles in accelerating cell death in SHRSP rats during cerebral ischemia [9, 10]. On the other hand, lactate production from astrocytes (an energy source for neuronal cells) was reduced in SHRSP rat cells in comparison with the WKY rat strain [11]. Moreover, astrocytes from SHRSP rats reduced lactate production, glial cell line–derived neurotrophic factor (GDNF), and ʟ-serine in comparison with WKY rat astrocytes during hypoxia and reoxygenation (H/R) [12]. In addition, the release of ʟ-serine and the expression of lactate transporter were lower in SHRSP rats than in WKY rat astrocytes after exposure to arginine vasopressin (AVP) [13].

Cerebral ischemia promotes blood-brain barrier (BBB) destruction, increases edema, and increases nervous system cell death. In particular, the reperfusion after cerebral ischemia rapidly generates a large quantity of reactive oxygen species (ROS), but the pathogenic mechanism of SHRSP rats in stroke is not well understood. In SHRSP rats, endothelial injury is induced at multiple sites following BBB leakage. Ultimately, this results in vessel rupture [14, 15]. Thus, we asked whether there were significant differences between the functions of WKY and SHRSP rat neurons and astrocytes during cerebral ischemia. Here, we present an overview of the cellular characteristic of SHRSP rat and WKY rat neurons and astrocytes during cerebral ischemia.

2. Cerebral ischemic stress induces neuronal vulnerability in SHRSP rats

2.1. Cell death of neuronal cells isolated from SHRSP rats during ischemia

In SHRSP rats, cerebral ischemia of 20 min duration enhances the production of large amounts of glutamate, causing delayed neuronal cell death in the CA1 region of the hippocampus [6]. Briefly, the neurons of SHRSP rats are more susceptible to H/R states than those of WKY rats [7, 9, 10]. We examined cultured neuronal cells isolated from the brains of SHRSP rats and WKY rats. The cells were cultured for 6–24 h under hypoxic conditions (1% O_2) and subsequently for 1.5–5 h in a reoxygenated state to assess cell viability [9]. None of the neuronal cells were stainable by trypan blue, indicating the absence of cell death in both strains. The majority (65–85%) of neuronal cells survived even after 36 h of hypoxic culture. Following hypoxia, after 1.5 h of reoxygenation, only 10–30% of neurons survived. The percentages of neuronal cell deaths in WKY rats and SHRSP rats were 41% (necrosis, 12%; apoptosis, 29%) and 78% (necrosis, 15%; apoptosis, 63%), respectively. Following hypoxia, 3 h of reoxygenation led to 68% cell death in WKY rats, whereas 99% of the neuronal cells from SHRSP rats were dead. Using the TdT-mediated dUTP nick end labeling (TUNEL) method, we found little or no DNA fragmentation in SHRSP rat neuronal cells after culture in 20% oxygen. In contrast, following 36 h of hypoxia and 3 h of reoxygenation, we noted a markedly increased fragmentation of DNA that was generally localized to areas containing many lipid droplets [9]. We classified the levels of apoptosis in H/R status by a morphological analysis of neuronal cell death [9, 10]. Briefly, we showed the characteristics of neuronal apoptosis in SHRSP rats in **Figure 1**. In the initial stage of apoptosis, neuronal cell axons and dendrites were lost, and many lipid droplets appeared in the neuronal cell body (A). In the next stage of apoptosis, cell shrinkage was
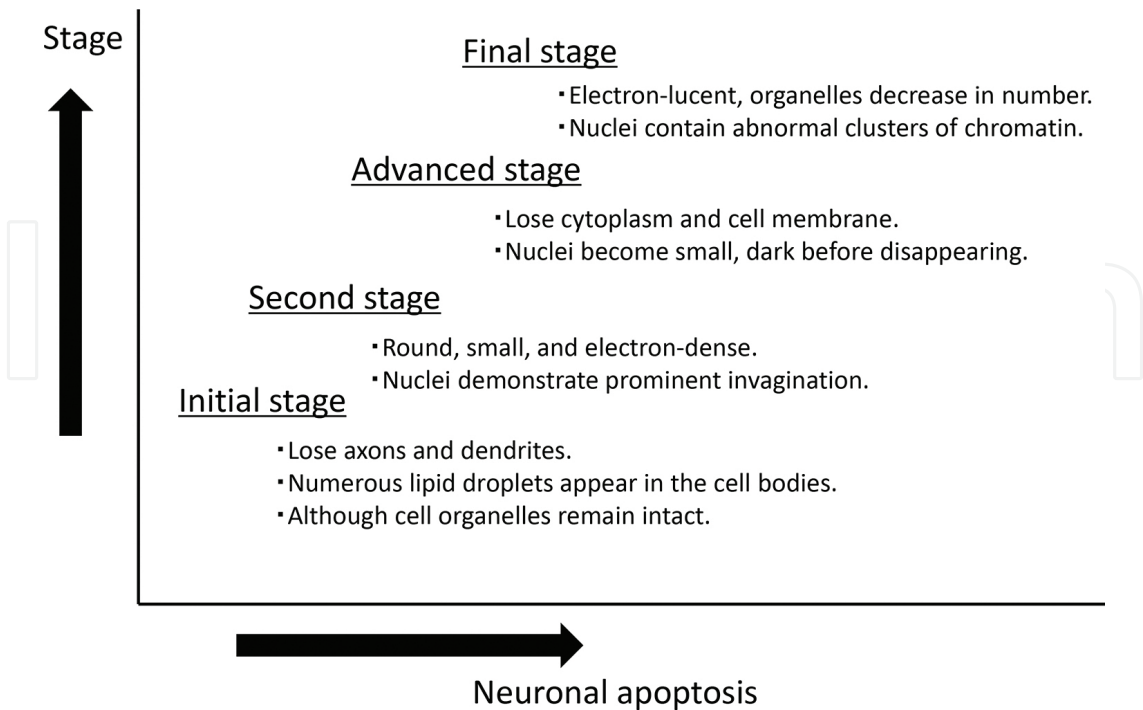

Figure 1. Characteristics of apoptosis in neuronal cells during H/R in SHRSP rats.

observed (B) and, next, continued development of apoptotic morphology (C). Finally, the neuronal cell membrane was lost and the nucleus disappeared (D). These processes eventually led to neuronal cell death. In SHRSP rats, a report demonstrated that the angiotensin II type 1 receptor-activated caspase-3 in the rostral ventrolateral medulla and was involved in sympathoexcitation [16]. These features may be associated with stroke pathogenic mechanisms of SHRSP rats.

2.2. Oxidative stress-induced neuronal cell death and redox changes in SHRSP rats

In the brain, ischemia leads to the rapid generation of a large amount of ROS and induces neuronal cell injury through self-perpetuating reactions. Cerebral ischemia increases the intracellular level of calcium ions and activates calcium-dependent proteases. These reactions activate xanthine dehydrogenase (XDH) and generate xanthine oxidase (XOD). The superoxide anion radicals generated via this pathway cause neuronal cell death in the brain [17]. Free radicals are generated by reoxygenation after cerebral ischemia, and they enhance the injury of brain neuronal cells [18]. These findings suggest that reducing the ROS (such as hydroxyl radicals produced during H/R) would be beneficial for preventing neuronal injury [19]. This might be achieved by increasing the level of antioxidant substances such as vitamin E.

Ischemic stimulation is considered to be the process that most strongly enhances cell death in cerebral ischemia in the SHRSP rat stroke model. In SHRSP rats, ischemic stimulation, i.e., the reoxygenation that occurs after hypoxia, generates a large amount of ROS that lead directly to neuronal death [20]. The expression of the thioredoxin gene (*Txn1*) was significantly reduced in neurons isolated from SHRSP rats compared with WKY rats [8]. Txn protein acts against ROS via its SH group. Furthermore, Txn proteins have many functions that are involved in intracellular signal transduction. For that reason, reduced expression of *Txn1* is associated with an attenuation of the defense system during oxidative stress in SHRSP rats. This in turn causes H/R-induced neuronal cell death. These findings indicated that redox regulatory functions in SHRSP rat neurons were markedly reduced by oxygen stimulation after hypoxia, and such changes may be involved in neuronal vulnerability. From these results, we suggested that the susceptibility of neurons to apoptosis in SHRSP rats is partly due to an insufficiency of mitochondrial thioredoxin and apoptosis-inhibitory proteins.

2.3. Protective effects of antioxidant vitamin E in neuronal cell death of SHRSP rats

Vitamin E, which is present in biological membranes, contains a hydroxyl group that reacts with unpaired electrons and can be reduced to form peroxyl radicals. The main antioxidant effect of vitamin E is to rapidly add alkoxy radicals (RO·) and hydrogen to peroxy radicals (ROO·). This is the mechanism of a chain-breaking antioxidant that blocks reactive oxygen metabolic cascades [21, 22].

We demonstrated the preventive effects of vitamin E against neuronal cell death associated with cerebral ischemia-reperfusion, particularly apoptosis, in WKY and SHRSP rat strains [9, 19]. Hypoxic stimuli followed by oxygen reperfusion induced strong neuronal damage in both WKY and SHRSP rats [9, 10]. The rate of neuronal cell death (mainly apoptosis) occurring

during H/R was markedly higher in the neurons of SHRSP rats than in those of WKY rats. Vitamin E is mostly enriched in microsomal cell membranes of mitochondria of the liver and heart. Transport of vitamin E to mitochondria or the microsomes is achieved by vitamin E binding to proteins [23]. Reports demonstrated that the α-tocopherol-binding protein, afamin, transported α-tocopherol across an in vitro model of the BBB. The model consisted of porcine brain capillary endothelial cells and cultured astrocytoma cells [24]. Thus, afamin might function to maintain α-tocopherol homeostasis at the BBB in vivo. Furthermore, scavenger receptor class B type I (SR-BI) facilitates selective uptake of HDL-related α-tocopherol at the BBB [25]. Damage related to oxidative stress alters traffic to neuronal cells and changes the levels of vitamin E in mitochondria. The addition of vitamin E almost completely inhibited neuronal death in SHRSP and WKY rat lines. Vitamin E decreased neuronal cell death in a dose-dependent manner over the range of 10–50 µg/mL. With vitamin E at 50–300 µg/mL, neuronal death was almost completely prevented [9]. In SHRSP rat neurons, we used HPLC to investigate the vitamin E levels in neuronal mitochondria after vitamin E supplementation (20–300 µg/mL) [9]. The accumulation of vitamin E in mitochondria of neuronal cells after 36 h of hypoxia was confirmed. Vitamin E accumulated most effectively into the mitochondria of neuronal cells at 50 µg/mL. When neuronal cell death was induced by reoxygenation for 3 h after 36 h of hypoxia, vitamin E prevented neuronal cell death at 50 µg/mL. Vitamin E prevented stroke and loss of both memory and cognition functions [26]. Vitamin E might have a marked inhibitory effect against neuronal damage after being incorporated into biological membranes, particularly mitochondrial membranes, and capturing the reactive oxygen and free radicals formed.

2.4. Regulation of the Bcl-2 family proteins in neuronal cell death

We pointed out that apoptosis is the likely mechanism responsible for ischemic neuronal death in SHRSP rats [9, 27]. The expression of apoptosis-inducing molecules such as Bax was
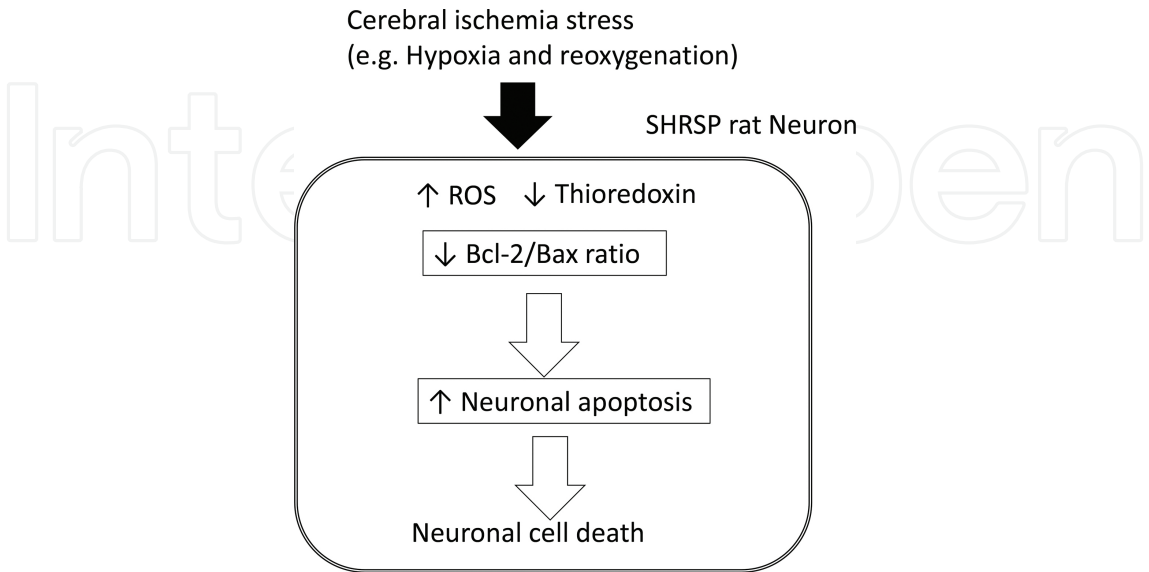

Figure 2. Apoptosis of neuron in SHRSP rats by cerebral ischemia stimulation.

enhanced in injured neuronal cells [8]. On the other hand, in surviving neurons, expression of apoptosis-inhibiting molecules such as Bcl-2 and Bcl-XL was elevated in rat brains following global ischemia [28]. The expression of *Bcl2* mRNA after H/R was investigated by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using cultured neuronal cells isolated from SHRSP and WKY rats [8]. An analysis of *Bcl2* mRNA expression in SHRSP and WKY rats showed that the most remarkable difference occurred after 30 min of reoxygenation. The expression of *Bcl2* mRNA was significantly decreased in SHRSP compared to WKY rats. The vulnerability of neuronal cells to ischemic stress promotes neuronal cell death during stroke in SHRSP rats (**Figure 2**) [8].

3. Characteristics of astrocytes in SHRSP rats during stroke

3.1. Gliosis and the proliferation rate of astrocytes in SHRSP rats

After brain injury, the number of reactive astrocytes increases [29]. These reactive astrocytes have prominent characteristics of growing cells [30]. The growth state of astrocytes in vitro reflects the physiological abnormalities occurring in brain damage. In astrocytes cultured from SHRSP rat brains in 10% fetal bovine serum (FBS), the cell growth rate was faster than astrocytes from WKY rats. For example, the doubling time of cultured astrocytes isolated from SHRSP rats was 21 h, whereas that of WKY rat astrocytes was 30 h [31]. These results indicated the elevated growth capacity of SHRSP rat astrocytes. The greater increase in the numbers of reactive astrocytes in SHRSP rats may have pathological consequences. In addition, reports have indicated enhanced astrocytic reactivity to epidermal growth factor (EGF). SHRSP rat astrocytes responded more strongly to EGF than WKY rat astrocytes, with larger increases in cell number. In the ischemic brain, proliferative astrocytes were found around the infarcted tissue [32]. Likewise, these proliferating astrocytes were immunoreactive for the EGF receptor (EGFR). The increased growth activity of SHRSP rat astrocytes suggests that cerebral vascular lesions in cerebral ischemia may be due to dysfunctional responses to EGF [32].

3.2. Production of neurotrophic factor by SHRSP astrocytes

Astrocytes modulate several functions such as the uptake of glutamate [33] and the induction of the blood-brain barrier (BBB) [34] and induce production of cytokines [35] and neurotrophic factors [36]. In SHRSP rat strain, astrocytic properties relate to the development of brain disorders [37–39]. For example, neuron regeneration is controlled through the production of neurotrophic factors from astrocytes after brain injury [32].

Neuronal vulnerability of SHRSP rats under ischemic conditions was correlated with reduced GDNF production by SHRSP rat astrocytes [13]. The study focused on the production of GDNF under normal conditions and H/R in cultured astrocytes from WKY and SHRSP rat strains. SHRSP rat astrocytes released higher levels of GDNF than did WKY rats under normal oxygen concentrations [13] (**Figure 3**). On the other hand, after hypoxia and 1.5 or 6 h reoxygenation, the expression of GDNF was significantly lower in astrocytes from the SHRSP rat strain than the WKY rat strain [13]. Furthermore, sphingosine-1-phosphate (S1P) [40] and adenosine [41]

reduced the expression of GDNF in primary SHRSP rat astrocytes. S1P is a lysophospholipid released by activated platelets [42, 43], and it enhances the apoptosis of neuronal cells in the central nervous system (CNS) [44]. A report demonstrated that GDNF is a potent factor for the survival of neuronal cells [45]. Thus, in SHRSP rats, upregulation of S1P attenuates GDNF production by astrocytes (**Figure 4**). This reduces neuronal protection mediated by the neurotrophic factor, GDNF. In traumatic injury of the CNS, the attenuated release of GDNF by astrocytes may be involved in neuronal vulnerability in the SHRSP rat strain. Adenosine enhances the levels of several growth factors in ischemic brain tissues, likely as part of a preventive response. In the CNS, adenosine in the extracellular space acts as an intercellular signaling molecule, and at high levels, it induces apoptosis [46]. Briefly, adenosine has both immediate effects such as neurotransmission and neurotrophic effects that enhance changes in cell metabolism and structure and has neuroprotective function [47, 48]. The expression of GDNF was regulated differently in cultured astrocytes from SHRSP compared to WKY rats. The amount of GDNF produced was lower in astrocytes of adenosine-treated SHRSP rats compared with WKY rats. These results indicate that GDNF production is regulated dynamically during reoxygenation and ischemic conditions by S1P and adenosine. Under postischemic reoxygenation conditions, the production of GDNF, neurturin (NTN), and its receptor increases in the brain tissue [49]. The pathogenesis of GDNF released by SHRSP rats is unknown. However, the specific metabolic properties of SHRSP rats may be associated with increased expression of GDNF [13].
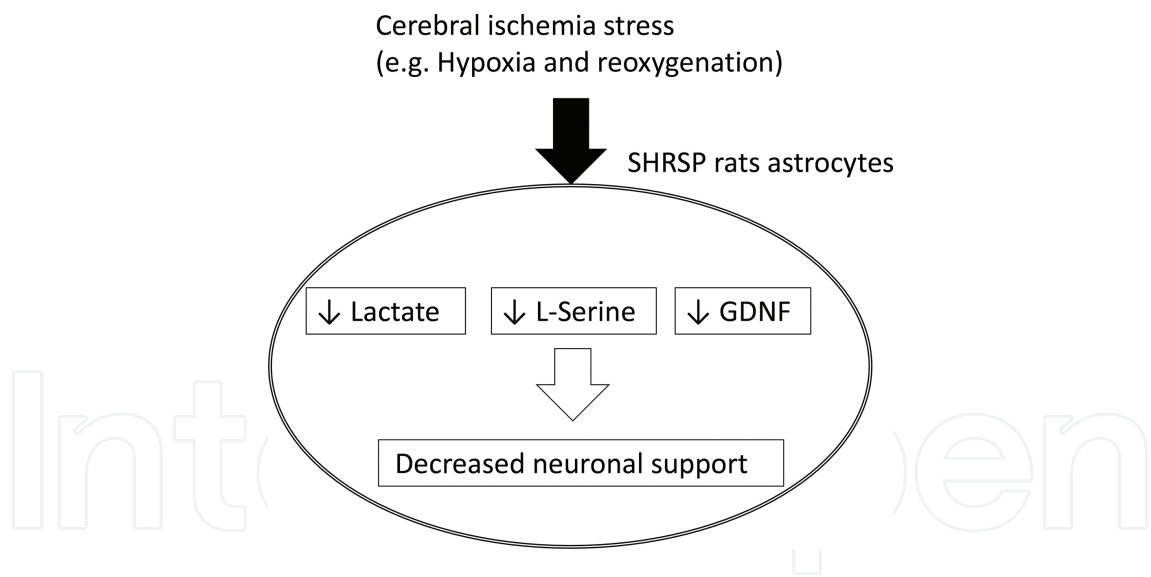

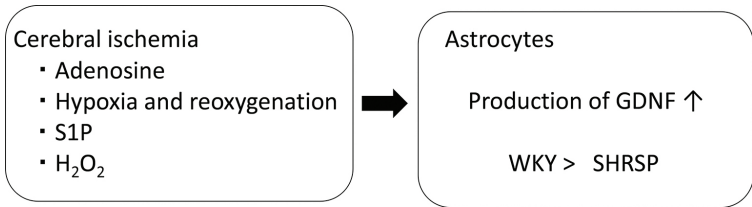**Figure 3.** Alteration of astrocytes in SHRSP rats by cerebral ischemia stimulation.

Figure 4. Regulation of GDNF in astrocytes of SHRSP rats by cerebral ischemia status.

3.3. Hypoxia and arginine vasopressin (AVP) induce astrocytic dysfunction in SHRSP rats

3.3.1. Altered regulation of L-serine in SHRSP astrocytes

In the CNS, L-serine is generated by astrocytes, and it accelerates neurite outgrowth from ganglion neurons and enhances neuronal survival [50]. L-Serine is synthesized by 3-phospho-glycerate dehydrogenase (3-PGDH) in glial cells but not in neurons [51]. L-Serine released from astrocytes is transported to the extracellular space by neural amino acid transporter alanine/serine/cysteine/threonine transporter (ASCT1) proteins and becomes available to neuronal cells [52]. Induces outgrowth of ganglion neurons and neuronal survival by L-serine from astrocytes. Hence, L-serine synthesis and the expression of ASCT1 protein were essential for neuronal survival and differentiation [53]. On the other hand, the expression of 3-PGDH and ASCT1 proteins is enhanced by excitotoxic damage in the mouse brain hippocampus [54]. In astrocytes isolated from SHRSP rats, glutamate-induced stimulation of L-serine production was reduced [55]. The production of L-serine was regulated by astrocytes in response to molecules such as glutamate, kainic acid (KA), and free radicals and others that induced neurodegenerative disorders [54, 56, 57].

Arginine vasopressin (AVP) induced the effects of inflammatory molecules in traumatic neuronal injury [58]. Furthermore, ischemic conditions such as hypoxia and AVP affect cerebral cell volume [59, 60], ion uptake by cerebral cells via Na/H exchange (NHE) [61], and Na-K-Cl cotransporter (NKCC) activities [62, 63]. AVP and hypoxia contribute to ischemia-induced astrocyte swelling [64]. During cerebral ischemia, astrocyte swelling leads to ischemic neuronal cell death. AVP might contribute to astrocyte swelling induced by hypoxia and reperfusion in SHRSP rats. On the other hand, L-serine generation might be regulated by astrocytes in response to a variety of molecules such as AVP that enhance brain edema in SHRSP rats [12].

3.3.2. Inflammatory regulation and expression of HMGB1 and adhesion molecules in SHRSP astrocytes

High-mobility group box 1 (HMGB1) regulates nucleosomal structure stabilization, modulates inflammation, and is involved in recovery after stroke [65–67]. Hypoxia induces HMGB1 expression in neurons and astrocytes [68]. Furthermore, after hypoxia, HMGB1 enhances breakdown of the blood-brain barrier (BBB) during ischemic injury [69]. These results indicate that HMGB1 is involved in inflammatory responses associated with stroke after ischemia [70]. One report demonstrated that HMGB1 is produced by neuronal cells and glial cells and aggravates ischemic neurodegeneration [68]. In reactive astrocytes, production of HMGB1 accelerates endothelial progenitor cell-mediated neurovascular remodeling during stroke recovery [71]. On the other hand, in oxygen-glucose deprivation or reperfusion, HMGB1 produced from astrocytes induces endogenous neural stem or progenitor cell proliferation [67, 72]. One study examined the expression of AVP-induced HMGB1 in cultured primary astrocytes isolated from WKY, SHR, SHRSP, and SHRpch1_18 rats [12]. AVP induced HMGB1 expression at 50 and 100 nM, and it was significantly higher in SHR, SHRSP, and SHRpch1_18 rat astrocytes than in WKY rat astrocytes. HMGB1 may relate to early stages of the inflammatory response [69]. Reports have indicated that myelin loss is associated with neuroinflamma-

tion [73] and that it induces inflammation after MCA occlusion [74] in the SHRSP rat strain. This characteristic of SHRSP, SHR, and SHRpch1_18 rats is likely an important contributor to enhanced inflammation in astrocytes and could explain how AVP augments the inflammatory reaction and induces neuronal cell death [12].

Following exposure to tumor necrosis factor-alpha (TNF-α), the expression of vascular cell adhesion molecule-1 (VCAM-1) by SHRSP rat astrocytes was increased compared with those from WKY rats. Expression of TNF-α and adhesion molecules are related to the presence of early neurological exacerbation and infarct volume in stroke [75, 76]. TNF-α is generated by microglial cells and infiltrating macrophages following ischemic stroke [77]. In H/R treatment of SHRSP rat astrocytes, the expression of monocyte chemotactic protein-1 (MCP-1) was increased compared with that under normal oxygen. Inhibition or genetic lack of these adhesion molecules decreased infarct volume, edema, and/or mortality in different animal models of ischemic stroke [78]. These enhanced levels of adhesion molecules in H/R and TNF-α treatment may be induced by stroke in SHRSP rats. In SHRSP rats, alteration and attenuation of astrocyte functions promote neuronal cell death during stroke [79].

4. Conclusions

The level of neuronal cell death in SHRSP rats is significantly higher than in the WKY rat strain [9, 10]. In cerebral ischemia, the properties of SHRSP rat neuronal cells, unlike those of WKY rats, might be a factor in the elevated frequency of stroke. Vitamin E reduces neuronal cell damage caused by ROS generated in cerebral ischemia. Thus, antioxidants such as vitamin E could be used as a treatment for oxidative stress-mediated diseases. These antioxidants may regulate redox potential and apoptosis-related proteins. Furthermore, in SHRSP rats, astrocyte properties contribute to the development of brain disorders. In SHRSP rat astrocytes, attenuation and loss of several functions such as GDNF and L-serine were demonstrated under cerebral ischemic stroke conditions (**Figure 4**). In addition, after ischemic reperfusion, generation of MCP-1 is strongly enhanced in SHRSP rat astrocytes [80]. The expression of VCAM-1 and MCP-1 [81] is markedly elevated in SHRSP astrocytes compared with WKY rat astrocytes. Taken together, neuronal vulnerability and altered regulation of the neuronal supportive functions of astrocytes increase the risk of stroke in SHRSP rats.

Supply of oxygen is critical to neuronal cells viability. On the other hand, oxygen reperfusion induces cellular dysfunction, apoptosis, and necrosis. From several animal experiment data, clinical therapy by single-drug treatment may have little effect [82].

Conflict of interest

None declared.

Acknowledgements

Abbreviations

BBB	blood-brain barrier
CNS	central nervous system
GDNF	glial cell line–derived neurotrophic factor
ROS	reactive oxygen sepsis
HMGB1	high-mobility group box 1
NHE	Na/H exchange
NKCC	Na-K-Cl cotransporter
S1P	sphingosine 1-phosphate
SHRSP	stroke-prone spontaneously hypertensive
VCAM-1	vascular cell adhesion molecule-1
WKY	Wister Kyoto

Author details

Kazuo Yamagata

Address all correspondence to: kyamagat@brs.nihon-u.ac.jp

Department of Food Bioscience and Biotechnology, College of Bioresource Sciences, Nihon University (NUBS), Fujisawa, Kanagawa, Japan

References

[1]   Okamoto K, Yamori Y, Nagaoka K. Establishment of the stroke-prone spontaneously hypertensive rats (SHR). Circ Res 1974: 35 (suppl 1); 1143–53.

[2] Yamori Y, Nagaoka A, Okamoto K. Importance of genetic factors in hypertensive cerebrovascular lesions: an evidence obtained by successive selective breeding of stroke-prone and-resistant SHR. Jpn Circ J 1974: 38; 1095–100.

[3] Nagaoka A, Iwatsuka H, Suzuoki Z, Okamoto K. Genetic predisposition to stroke in spontaneously hypertensive rats. Am J Physiol 1976: 230; 1354–59.

[4] Nabika T, Cui Z, Masuda J. The stroke-prone spontaneously hypertensive rat: how good is it as a model for cerebrovascular diseases? Cell Mol Neurobiol 2004: 24; 639–46.

[5] Bailey EL, Smith C, Sudlow CL, Wardlaw JM. Is the spontaneously hypertensive stroke prone rat a pertinent model of sub cortical ischemic stroke? Int J Stroke 2011: 6; 434–4.

[6] Gemba T, Matsunaga K, Ueda M. Changes in extracellular concentration of amino acids in the hippocampus during cerebral ischemia in stroke-prone SHR, stroke-resistant SHR and normotensive rats. Neurosci Lett 1992: 135; 184–8.

[7] Yasui M, Kawasaki K. Vulnerability of CA1 neurons in SHRSP hippocampal slices to ischemia, and its protection by Ca^{2+} channel blockers. Brain Res 1994: 642; 146–52.

[8] Yamagata K, Tagami M, Ikeda K, et al. Altered gene expressions during hypoxia and reoxygenation in cortical neurons isolated from stroke-prone spontaneously hypertensive rats. Neurosci Lett 2000: 284; 131–4.

[9] Tagami M, Yamagata K, Ikeda K, et al. Vitamin E prevents apoptosis in cortical neurons during hypoxia and oxygen reperfusion. Lab Invest 1998: 78; 1415–29.

[10] Tagami M, Ikeda K, Yamagata K, et al. Vitamin E prevents apoptosis in hippocampal neurons caused by cerebral ischemia and reperfusion in stroke-prone spontaneously hypertensive rats. Lab Invest 1999: 79; 609–15.

[11] Yamagata K, Tagami M, Ikeda K, et al. Reduced production of lactate during hypoxia and reoxygenation in astrocytes isolated from stroke-prone spontaneously hypertensive rats. Neurosci Lett 2000: 296; 113–16.

[12] Yamagata K, Yamamoto M, Kawakami K, et al. Arginine vasopressin regulated ASCT1 expression in astrocytes from stroke-prone spontaneously hypertensive rats and congenic SHRpch1_18 rats. Neuroscience 2014: 267; 277–85.

[13] Yamagata K, Tagami M, Ikeda K, et al. Differential regulation of glial cell line-derived neurotrophic factor (GDNF) mRNA expression during hypoxia and reoxygenation in astrocytes isolated from stroke-prone spontaneously hypertensive rats. Glia 2002: 37; 1–7.

[14] Braun H, Bueche CZ, Garz C, et al. Stases are associated with blood-brain barrier damage and a restricted activation of coagulation in SHRSP. J Neurol Sci 2012; 322: 71–6.

[15] Schreiber S, Bueche CZ, Garz C, Braun H. Blood brain barrier breakdown as the starting point of cerebral small vessel disease?—new insights from a rat model. Exp Transl Stroke Med 2013: 5;4.

[16] Kishi T, Hirooka Y, Konno S, et al. Angiotensin II type 1 receptor-activated caspase-3 through ras/mitogen-activated protein kinase/extracellular signal-regulated kinase in the rostral ventrolateral medulla is involved in sympathoexcitation in stroke-prone spontaneously hypertensive rats. Hypertension 2010: 55; 291–7.

[17] Thompson-Gorman SL, Zweier JL. Evaluation of the role of xanthine oxidase in myocardial reperfusion injury. J Biol Chem 1990: 265; 6656–63.

[18] Bolli R. Oxygen-derived free radicals and myocardial reperfusion injury: an overview. Cardiovasc Drugs Ther 1991: 5 (Suppl 2); 249–68.

[19] Yamagata K, Tagami M, Yamori Y. Neuronal vulnerability of stroke-prone spontaneously hypertensive rats to ischemia and its prevention with antioxidants such as vitamin E. Neuroscience 2010: 170; 1–7.

[20] Negishi H, Ikeda K, Nara Y, Yamori Y. Increased hydroxyl radicals in the hippocampus of stroke-prone spontaneously hypertensive rats during transient ischemia and recirculation. Neurosci Lett 2001: 306; 206–08.

[21] Packer L, Landvik S. Vitamin E in biological systems. Adv Exp Med Biol 1990: 264; 93–103.

[22] Chow CK. Vitamin E and oxidative stress. Free Radic Biol Med 1991: 11; 215.

[23] Manor D, Morley S. The alpha-tocopherol transfer protein. Vitam Horm 2007: 76; 45–65.

[24] Kratzer I, Bernhart E, Wintersperger A, et al. Afamin is synthesized by cerebrovascular endothelial cells and mediates alpha-tocopherol transport across an in vitro model of the blood-brain barrier. J Neurochem 2009: 108; 707–18.

[25] Balazs Z, Panzenboeck U, Hammer A, Sovic A, et al. Uptake and transport of high-density lipoprotein (HDL) and HDL-associated alpha-tocopherol by an in vitro blood-brain barrier model. J Neurochem 2004: 89; 939–50.

[26] Murad LB, Guimaraes MR, Paganelli A, et al. Alpha-tocopherol in the brain tissue preservation of stroke-prone spontaneously hypertensive rats. J Physiol Biochem 2014: 70; 49–60.

[27] Yamagata K, Ichinose S, Tagawa C, et al. Vitamin E regulates SMase activity, GSH levels, and inhibits neuronal death in stroke-prone spontaneously hypertensive rats during hypoxia and reoxygenation. J Exp Stroke Transl Med 2009: 1; 41–8.

[28] Chen J. Apoptosis repressor genes Bcl-2 and Bcl-x-long are expressed in the rat brain following global ischemia. J Cereb Blood Flow Metab 1997: 17; 2–10.

[29] Bramanti V, Tomassoni D, Avitabile M, et al. Biomarkers of glial cell proliferation and differentiation in culture. Front Biosci 2010: 2; 558–70.

[30] Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. Glia 2005: 50; 427–34.

[31] Yamagata K, Nara Y, Tagami M, et al. Demonstration of hereditarily accelerated proliferation in astrocytes derived from spontaneously hypertensive rats. Clin Exp Pharmacol Physiol 1995: 22; 605–9.

[32] Planas AM, Justicia C, Soriano MA, Ferrer I. Epidermal growth factor receptor in proliferating reactive glia following transient focal ischemia in the rat brain. Glia 1998: 23; 120–9.

[33] Gouix E, Leveille F, Nicole O, et al. Reverse glial glutamate uptake triggers neuronal cell death through extrasynaptic NMDA receptor activation. Mol Cell Neurosci 2009: 40; 463–73.

[34] Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 2006: 7; 41–53.

[35] Suzumura A, Takeuchi H, Zhang G, Kuno R, et al. Roles of glia-derived cytokines on neuronal degeneration and regeneration. Ann N Y Acad Sci 2006: 1088; 219–29.

[36] Muller HW, Junghans U, Kappler J. Astroglial neurotrophic and neurite-promoting factors. Pharmacol Ther 1995: 65; 1–18.

[37] Tagami M, Kubota A, Nara Y, et al. Detailed disease processes of cerebral pericytes and astrocytes in stroke-prone SHR. Clin Exp Hypertens A 1991: 13; 1069–75.

[38] Tagami M, Nara Y, Kubota A, et al. Ultrastructural changes in cerebral pericytes and astrocytes of stroke-prone spontaneously hypertensive rats. Stroke 1990: 21; 1064–71.

[39] Tomassoni D, Avola R, Di Tullio MA, Sabbatini M, Vitaioli L, Amenta F. Increased expression of glial fibrillary acidic protein in the brain of spontaneously hypertensive rats. Clin Exp Hypertens 2004: 26(4); 335–50.

[40] Yamagata K, Tagami M, Torii Y, et al. Sphingosine 1-phosphate induces the production of glial cell line-derived neurotrophic factor and cellular proliferation in astrocytes. Glia 2003: 41; 199–206.

[41] Yamagata K, Hakata K, Maeda A, et al. Adenosine induces expression of glial cell line-derived neurotrophic factor (GDNF) in primary rat astrocytes. Neurosci Res 2007: 59; 467–74.

[42] Yatomi Y, Ruan F, Hakomori S, Igarashi Y. Sphingosine-1-phosphate: a platelet-activating sphingolipid released from agonist-stimulated human platelets. Blood 1995: 86; 193–202.

[43] Yatomi Y, Ohmori T, Rile G, et al. Sphingosine 1-phosphate as a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells. Blood 2000: 96; 3431–8.

[44] Moore AN, Kampfl AW, Zhao X, Hayes RL, Dash PK. Sphingosine-1-phosphate induces apoptosis of cultured hippocampal neurons that requires protein phosphatases and activator protein-1 complexes. Neuroscience 1999: 94; 405–15.

[45] Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 1993: 260; 1130–2.

[46] Fields RD, Burnstock G. Purinergic signalling in neuron-glia interactions. Nat Rev Neurosci 2006: 7; 423–36.

[47] Ciccarelli R, Ballerini P, Sabatino G, et al. Involvement of astrocytes in purine-mediated reparative processes in the brain. Int J Dev Neurosci 2001: 19; 395–14.

[48] Rathbone MP, Middlemiss PJ, Gysbers JW, et al. Trophic effects of purines in neurons and glial cells. Prog Neurobiol 1999: 59; 663–90.

[49] Kokaia Z, Airaksinen MS, Nanobashvili A, et al. GDNF family ligands and receptors are differentially regulated after brain insults in the rat. Eur J Neurosci 1999: 11; 1202–16.

[50] Mitoma J, Furuya S, Hirabayashi Y. A novel metabolic communication between neurons and astrocytes: non-essential amino acid l-serine released from astrocytes is essential for developing hippocampal neurons. Neurosci Res 1998: 30; 195–99.

[51] Yamasaki M, Yamada K, Furuya S, et al. 3-Phosphoglycerate dehydrogenase, a key enzyme for l-serine biosynthesis, is preferentially expressed in the radial glia/astrocyte lineage and olfactory ensheathing glia in the mouse brain. J Neurosci 2001: 21; 7691–704.

[52] Zerangue N, Kavanaugh MP. ASCT-1 is a neutral amino acid exchanger with chloride channel activity. J Biol Chem 1996: 271; 27991–4.

[53] Furuya S, Tabata T, Mitoma J, et al. l-Serine and glycine serve as major astroglia-derived trophic factors for cerebellar Purkinje neurons. Proc Natl Acad Sci U S A 2000: 97; 11528–33.

[54] Jeon GS, Choi DH, Lee HN, et al. Expression of l-serine biosynthetic enzyme 3-phosphoglycerate dehydrogenase (Phgdh) and neutral amino acid transporter ASCT1 following an excitotoxic lesion in the mouse hippocampus. Neurochem Res 2009: 34; 827–34.

[55] Yamagata K, Shoji Y, Terashima T, Yokogoshi H. Glutamate reduces secretion of l-serine in astrocytes isolated from stroke-prone spontaneously hypertensive rats. Neuroscience 2006: 143; 729–37.

[56] Lehmann A, Hansso E. Kainate-induced stimulation of amino acid release from primary astroglial cultures of the rat hippocampus. Neurochem Int 1988;13(4); 557-61.

[57] Mustafa AK, Kumar M, Selvakumar B, et al. Nitric oxide S-nitrosylates serine racemase, mediating feedback inhibition of d-serine formation. Proc Natl Acad Sci U S A 2007: 104; 2950–5.

[58] Szmydynger-Chodobska J, Fox LM, Lynch KM, Zink BJ, Chodobski A. Vasopressin amplifies the production of proinflammatory mediators in traumatic brain injury. J Neurotrauma 2010: 27; 1449–61.

[59] Brillault J, Lam TI, Rutkowsky JM, Foroutan S, O'Donnell ME. Hypoxia effects on cell volume and ion uptake of cerebral microvascular endothelial cells. Am J Physiol Cell Physiol 2008: 294; C88–96.

[60] O'Donnell ME, Duong V, Suvatne J, Foroutan S, Johnson DM. Arginine vasopressin stimulation of cerebral microvascular endothelial cell Na-K-Cl cotransporter activity is V1 receptor and [Ca] dependent. Am J Physiol Cell Physiol 2005: 289; C283–92.

[61] Lam T, Wise PM, O'Donnell ME. Cerebral microvascular endothelial cell Na/H exchange: evidence for the presence of NHE1 and NHE2 isoforms and regulation by arginine vasopressin. Am J Physiol Cell Physiol 2009: 297; C278–89.

[62] Jayakumar AR, Panickar KS, Curtis KM, Tong XY, Moriyama M, Norenberg MD. Na-K-Cl cotransporter-1 in the mechanism of cell swelling in cultured astrocytes after fluid percussion injury. J Neurochem 2011: 117; 437–48.

[63] Foroutan S, Brillault J, Forbush B, O'Donnell ME. Moderate-to-severe ischemic conditions increase activity and phosphorylation of the cerebral microvascular endothelial cell Na$^+$-K$^+$-Cl$^-$ cotransporter. Am J Physiol Cell Physiol 2005: 289; C1492–501.

[64] Rutkowsky JM, Wallace BK, Wise PM, O'Donnell ME. Effects of estradiol on ischemic factor-induced astrocyte swelling and AQP4 protein abundance. Am J Physiol Cell Physiol 2011: 301; C204–12.

[65] Kim JB, Sig Choi J, Yu YM, et al. HMGB1, a novel cytokine-like mediator linking acute neuronal death and delayed neuroinflammation in the postischemic brain. J Neurosci 2006: 26; 6413–21.

[66] Hayakawa K, Qiu J, Lo EH. Biphasic actions of HMGB1 signaling in inflammation and recovery after stroke. Ann NY Acad Sci 2010: 1207; 50–7.

[67] Lei C, Lin S, Zhang C, et al. High-mobility group box1 protein promotes neuroinflammation after intracerebral hemorrhage in rats. Neuroscience 2013: 228; 190–9.

[68] Faraco G, Fossati S, Bianchi ME, et al. High mobility group box 1 protein is released by neural cells upon different stresses and worsens ischemic neurodegeneration in vitro and in vivo. J Neurochem 2007: 103; 590–603.

[69]   Huang J, Liu B, Yang C, et al. Acute hyperglycemia worsens ischemic stroke-induced brain damage via high mobility group box-1 in rats. Brain Res 2013: 1535; 148–55.

[70]   Schulze J, Zierath D, Tanzi P, et al. Severe stroke induces long-lasting alterations of high-mobility group box 1. Stroke 2013: 44; 246–8.

[71]   Hayakawa K, Pham LD, Katusic ZS, Arai K, Lo EH. Astrocytic high-mobility group box 1 promotes endothelial progenitor cell-mediated neurovascular remodeling during stroke recovery. Proc Natl Acad Sci USA 2012: 109; 7505–10.

[72]   Li M, Sun L, Li Y, Xie C, Wan D, Luo Y. Oxygen glucose deprivation/reperfusion astrocytes promotes primary neural stem/progenitor cell proliferation by releasing high-mobility group box 1. Neurochem Res 2014: 39; 1440–50.

[73]   Jalal FY, Yang Y, Thompson J, Lopez AC, Rosenberg GA. Myelin loss associated with neuroinflammation in hypertensive rats. Stroke 2012: 43; 1115–22.

[74]   Moller K, Boltze J, Posel C, et al. Sterile inflammation after permanent distal MCA occlusion in hypertensive rats. J Cereb Blood Flow Metab 2014: 34; 307–15.

[75]   Rodríguez-Yáñez M, Castillo J. Role of inflammatory markers in brain ischemia. Role of inflammatory markers in brain ischemia. Curr Opin Neurol 2008: 21; 353–7.

[76]   Clausen BH, Lambertsen KL, Babcock AA, et al. Interleukin-1beta and tumor necrosis factor-alpha are expressed by different subsets of microglia and macrophages after ischemic stroke in mice. J Neuroinflammation 2008: 5; 46.

[77]   Lambertsen KL, Clausen BH, Fenger C, et al. Microglia and macrophages express tumor necrosis factor receptor p75 following middle cerebral artery occlusion in mice. Neuroscience 2007: 144; 934–49.

[78]   Yilmaz G, Granger DN. Cell adhesion molecules and ischemic stroke. Neuronal Res 2008: 30; 783–93.

[79]   Yamagata K. Pathological alterations of astrocytes in stroke-prone spontaneously hypertensive rats under ischemic conditions. Neurochem Int 2012: 60; 91–98.

[80]   Sakurai-Yamashita Y, Shigematsu K, Yamashita K, Niwa M. Expression of MCP-1 in the hippocampus of SHRSP with ischemia-related delayed neuronal death. Cell Mol Neurobiol 2006: 26; 823–31.

[81]   Yamagata K, Kitazawa T, Shinoda M, et al. Stroke status evoked adhesion molecule genetic alterations in astrocytes isolated from stroke-prone spontaneously hypertensive rats and the apigenin inhibition of their expression. Stroke Res Treat 2010; 2010. pii: 386389.

[82]   Schaller B, Graf R. Cerebral ischemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. J Cereb Blood Flow Metab 2004: 24(4); 351–371.