Hypothesis and Proposal

Astrogliopathy as a loss of astroglial protective function against glycoxidative stress under hyperglycemia

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Abstract: Reactive oxygen species (ROS) derived from mitochondria play an essential role in stroke as well as in neurodegenerative disorders. Although hyperglycemia associated with diabetes mellitus is well known to enhance ROS production in vascular endothelial cells, the effects of either acute or chronic high glucose environments on neurons and glial cells remain unclear. Astroglia play a pivotal role in glucose metabolism. Thus, the astroglial metabolic response to high glucose environments is an interesting subject. In particular, the glutathione/pentose phosphate pathway (PPP) system, which is a major defense mechanism against ROS in the brain, contributes to glucose metabolism and is more active in astroglia. We propose that high glucose environments activate PPP through an increased flux to the hexosamine biosynthetic pathway (HBP). HBP is known to induce endoplasmic reticulum (ER) stress under hyperglycemia, resulting in the nuclear translocation of nuclear factor-erythroid-2-related factor 2 (Nrf2), a master regulator of phase 2 detoxifying enzymes including glucose-6-phosphate dehydrogenase that regulates PPP activity, as Nrf2 is reported to be a direct substrate of protein kinase RNA (PKR)-like ER kinase (PERK), a transducer of ER stress. Therefore, the phosphorylation of Nrf2 by hyperglycemia-induced ER stress facilitates Nrf2 translocation through PERK, thus activating the PPP. If acute or chronic hyperglycemia induces PPP activation in astroglia to reduce ROS, reducing the glucose concentration may be accompanied by a risk, which may explain the lack of evidence that strict glycemic control during the acute phase of stroke conveys no beneficial effect.

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Key words：astrocyte, diabetes mellitus, endoplasmic reticulum stress, Keap1/Nrf2, pentose phosphate pathway

Introduction

Brain function is exclusively dependent on the high rates of oxidative metabolism of D-glucose[1][2]. Although an adult brain represents approximately 2% (1,400 g) of the total body weight (70 kg), the cerebral metabolic rate of oxygen (CMR_{oxy}) accounts for 20% of the total oxygen consumption and the cerebral metabolic rate of glucose (CMR_{glc}) accounts for 25% of the total glucose consumption of the body[1][2]. As neuronal energy production depends on mitochondrial oxidative phosphorylation, reactive oxygen species (ROS) derived from mitochondria in neural cells may play a harmful role in the aging process of the brain[3] in the acute phase of stroke[4], and probably in neurodegenerative disorders[5]. Hyperglycemia is well known to enhance ROS production in vascular endothelial cells, resulting in stroke and Binswanger's disease—a vascular cognitive disorder—as manifestations of macroangiopathy and microangiopathy in the brain, respectively[6]～[9]. Moreover, epidemiologic observations suggest that diabetes mellitus is closely associated with an increasing risk of Alzheimer disease (AD)[9][10] and Parkinson disease (PD)[11][12], although the exact mechanism of disease pathogenesis remains to be elucidated. Despite the fact that diabetes mellitus is a risk factor of neurodegenerative disorders, whether a hyperglycemic state *per se* causes direct neuronal cell damage remains controversial[13]～[18]. Even if a hyperglycemic state is harmful to parenchymal cells (i.e., neurons and glial cells), the deteriorating effects to neurons and glial cells do not seem to be as devastating as they are to vascular endothelial cells.

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Oxygen stress in the brain as an indicator of the oxidative metabolism of glucose

The major source of ROS in the brain is thought to be the mitochondrial electron chain in neurons and glial cells$^{19)20)}$, whereas NADPH oxidase seems to be a more important source in vascular endothelial cells$^{20)21)}$. Therefore, ROS production in the brain reflects the high rates of mitochondrial oxidative metabolism of glucose. However, if ROS production under high glucose environments does not increase very much, one possible explanation may be that a high glucose concentration in the extracellular space does not increase the rates of glucose utilization. Obviously, extracellular and intracellular glucose concentrations increase as the blood glucose concentration is elevated because glucose transport to the brain cells from the blood is dependent on facilitated diffusion enabled by glucose transporters$^{1)2)}$. In fact, CMR_{glc}, as measured using the [^{14}C]deoxyglucose method, remains unaltered$^{22)23)}$ in the presence of acute hyperglycemia. Notwithstanding the unaltered total CMR_{glc} in the presence of hyperglycemia, whether the oxidative metabolism of glucose is affected or not remains to be elucidated$^{24)}$, and the effects on each parenchymal cell type have not been examined separately. We reported that chronic (2 weeks) exposure to a high-glucose environment (22 mM) suppresses the oxidative metabolism of glucose as measured using [U-^{14}C]glucose oxidation in astroglia but not in neurons$^{25)}$. As the glycolytic metabolism of glucose predominates in astroglia, while the oxidative phosphorylation of glucose predominates in neurons, the effect of hyperglycemia on glucose metabolism and on glycolysis and oxidative phosphorylation in each cell type is an important issue. In particular, the astroglial glycolytic pathway branches into minor metabolic pathways (Fig. 1) that play various roles in brain pathophysiology: i.e., the polyol pathway, pentose phosphate pathway (PPP) (Fig. 1; A), hexosamine biosynthetic pathway (HBP) (Fig. 1; B), and nonenzymatic pathway, producing methylglyoxal (Fig. 1; C) that in turn generates advanced glycation end products (AGEs).

We hypothesized that brain parenchymal cells possess intrinsic protective mechanisms in the presence of both acute and chronic hyperglycemia. In fact, a high rate of glucose oxidation in neural cells is a potential risk for oxygen stress, even though the total CMR_{glc} remains unaltered$^{22)23)}$ in response to high-glucose environments. Furthermore, if increasing concentrations of glucose enhance these protective mechanisms, reducing the glucose content too rapidly or too drastically may cause the deterioration of such protective mechanisms in the brain. In clinical settings, an elevation in the blood glucose concentration is often observed during the acute phase in stroke patients, regardless of co-existing diabetes mellitus, and is associated with a poorer prognosis$^{26)27)}$. Not including the potential harmful effects of hyperglycemia on brain parenchymal cells observed in animal studies$^{28)}$, the beneficial effects of lowering the blood glucose level during the acute phase of stroke has not been confirmed by clinical trials$^{29)30)}$. Thus, the possible benefit of lowering the blood glucose level during the acute phase of stroke remains controversial. A recent survey of clinical studies suggested that mild hyperglycemia is actually beneficial for lacunar infarction$^{31)}$. These facts may indicate that an appropriate glucose content in the brain is necessary to protect the brain against ischemic cell damage. Furthermore, acute glucose fluctuations in the blood glucose levels, rather than sustained chronic hyperglycemia, are reported to contribute to oxidative stress in type 2 diabetes$^{32)}$, indicating that not increasing but reducing glucose levels may also be associated with an increase in ROS production$^{33)\sim35)}$. Likewise, reducing rather than increasing glucose levels may be more harmful to the brain parenchymal cells.

Role of excessive use of glucose in both resting and activated brain and astroglial glycolysis

Even though the theoretical ratio of CMR_{oxy} to CMR_{glc} for the complete oxidation of glucose is 6, the values measured in human brain during a non-activated steady state are always lower than 6 (i.e., 5.0-5.5$^{1)2)}$), indicating that more glucose is consumed than theoretically expected. This observation has been interpreted as reflecting glucose utilization for the synthesis of neurotransmitters or cellular structural components$^{1)}$. When activated, the local CMR_{glc} increases in distinct regions of the brain, and transient increases in lactate production$^{36)}$ are observed in association with the decrease in $CMR_{oxy}/CMR_{glc}$$^{37)}$, leading to the hypothesis that activated brain tissue utilizes glucose to produce ATP through glycolysis, rather than oxidative phosphorylation$^{38)\sim40)}$. Recent findings have shown that distinct regions of the brain are continuously activated even during the resting state, i.e., a default mode network$^{41)}$, implying that brain function may not be dependent on the complete oxidation of glucose in either the resting or activated state. These facts imply that the glycolytic pathway is necessary not only for ATP production, but also for other important roles.

Glial cells in the brain have long been thought to outnumber neurons by a factor of 10, although the ratio may be lower$^{42)}$. Among glial cells, astroglia play a pivotal role in glucose metabolism for energy production in the brain$^{43)}$. At least one half or more of the total glucose utilization in the brain has been ascribed to astroglia$^{44)\sim46)}$. In fact, we previ-

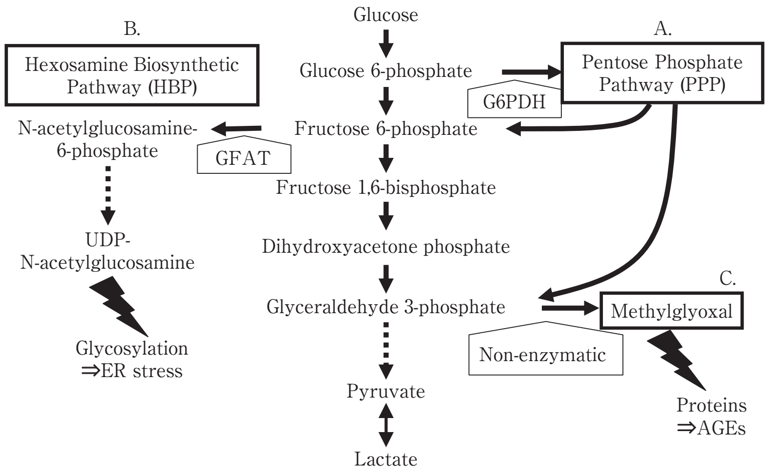


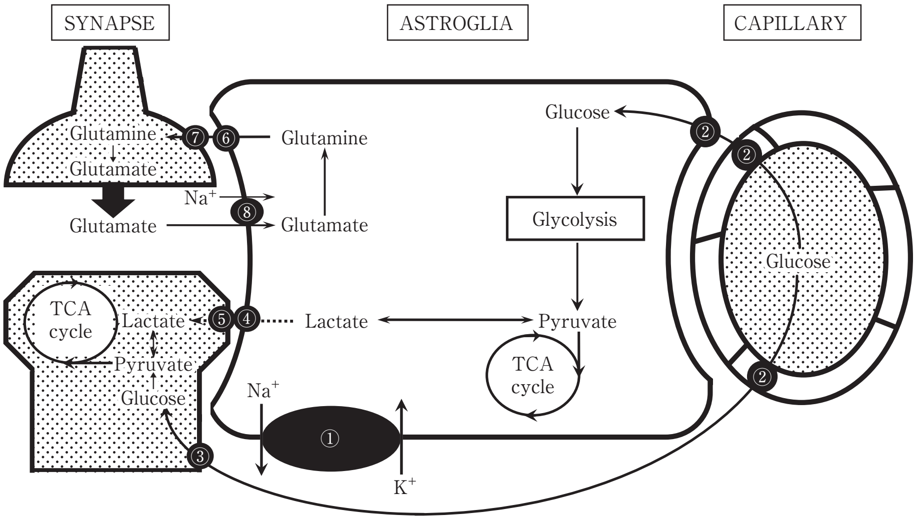
Fig. 1 Three pathways branching from glycolysis; pentose phosphate pathway (PPP), hexosamine biosynthetic pathway (HBP), and generation of advanced glycation end products (AGEs). The enhanced production of reactive oxygen species (ROS) from mitochondria in neurons may play a pathogenic role in neurons. Astroglia protect neurons from ROS toxicity via the glutathione/pentose phosphate pathway, which branches at glucose 6-phosphate, the first metabolite of glycolysis (A). The PPP activity is regulated by the rate-limiting enzyme glucose-6-phohate dehydrogenase (G6PDH) through both allosteric and transcriptional mechanisms. As the PPP is a kind of shunt pathway, it returns to the original glycolytic pathway at fructose 6-phosphate or glyceraldehyde 3-phosphate.

Another minor pathway of glucose metabolism, the hexosamine biosynthetic pathway (B), regulates the PPP through endoplasmic reticulum (ER) stress, as N-acetylglucosamine formed by HBP induces ER stress. In the HBP, another minor pathway of glucose metabolism that branches from glycolysis, fructose-6-phosphate is converted to N-acetylglucosamine-6-phosphate (GlcN-6-P) by the rate-limiting enzyme glutamine: fructose-6-phosphate amidotransferase (GFAT). GlcN-6-P is subsequently converted into UDP-N-acetyl glucosamine, which acts as a substrate for N- and O-linked protein glycosylation. As flux through the HBP increases with glucose concentration, an excess influx to the HBP causes the abnormal glycosylation of proteins, resulting in hyperglycemia-induced ER stress.

A non-enzymatic reaction that generates methylglyoxal (C) produces advanced glycation end products (AGEs), an important source of ROS, resulting in the dysfunction of astroglia (astrogliopathy). AGEs are well-known sources of ROS under a chronic hyperglycemic state. Among the many reactive carbonyl compounds and AGE precursors, methylglyoxal is most likely to contribute to intracellular AGE formation, since it is extremely reactive and constantly produced by the degradation of triose phosphates such as glyceraldehyde-3-phosphate, an intermediate metabolite of glycolysis.

ously reported that the rates of $[^{14}C]$deoxyglucose phosphorylation (an indicator of glucose utilization) in cultured astroglia were twice as high as in cultured neurons[47]. Moreover, a long-lasting debate regarding the astrocyte-neuron lactate shuttle hypothesis (ANLSH)[48] (Fig. 2) suggests that the sites of glucose consumption and lactate production in the activated brain are mainly located in astrocytes[48]～[52], irrespective of the controversy regarding the site of lactate production and neuronal utilization[53]～[60]. If lactate produced by astroglia is transferred to neurons and is consumed completely by the neurons, the ratio of CMR_{oxy}/CMR_{glc} would not decrease when neurons are activated. Recent findings, however, indicate that the astrocytic syncytium conveys lactate from the region where it is produced to the distant region in the brain[61][62], possibly explaining why glucose consumption exceeds oxygen consumption under both resting and activated conditions in the brain. Importantly, however, the benefit of such compartmentation of glucose metabolism in neurons and astroglia in addition to efficient ATP production in neurons remains to be answered.

These facts and controversy may imply that the excess utilization of glucose, compared with oxygen, in the brain as a whole may be of clinical relevance to disease-oriented points of view and that if the glycolytic metabolism of glucose predominates in astrocytes, these cells may play important roles through their glycolytic metabolism in the patho-



①Na$^+$, K$^+$-ATPase ②Glucose transporter 1 (Glut 1) ③Glucose transporter 3 (Glut 3) ④Monocarboxylic acid transporter (MCT) 1&4 (astrocytic form) ⑤Monocarboxylic acid transporter (MCT) 2 (neuronal form) ⑥System N transporter (astrocytic form) ⑦System A transporter (neuronal form) ⑧Na$^+$-dependent glutamate transporter (GLT1, GLAST)

Fig. 2 Flux and compartmentation of glucose metabolism between astroglia and neurons. Glucose supplied by capillaries diffuses into the brain through facilitated diffusion via glucose transporter 1 (Glut 1; ②), which is expressed in the endothelium. Then, glucose is transported into both the astroglia and neurons by glucose transporter 1 and 3 (Glut 3; ③), respectively. Of note, astroglia are anatomically interposed between neurons and capillaries. The astroglial end-feet that envelope the synapse monitor neuronal activities and translate them into metabolic regulation, transmitting signals to the capillaries. Glucose and oxygen are consumed to produce ATP to drive Na$^+$, K$^+$-ATPase (①) and maintain the proper ionic gradients of Na$^+$ and K$^+$ across the cell membrane. Glutamate, an excitatory neurotransmitter released from pre-synaptic neurons, is taken up mainly by astroglia with Na$^+$-dependent glutamate transporters expressed in astroglia (GLT1 or GLAST; ⑧). Increased concentrations of intracellular Na$^+$ in astroglia activate Na$^+$, K$^+$-ATPase, and the enhanced demand of ATP drives both glycolysis and the oxidative metabolism of glucose. The former is more active in astroglia, while the latter is more active in neurons. Therefore, enhanced glycolysis in astroglia results in the increased production of lactate, which is released from astroglia to the extracellular space by monocarboxylic acid transporter (MCT) 1&4 (astrocytic form; ④). Then, lactate is taken up by neurons by MCT 2 (neuronal form; ⑤) to serve as a substrate for the tricarboxylic acid (TCA) cycle; this process is known as the astrocyte-neuron lactate shuttle hypothesis (ANLSH). Glutamate taken up by astroglia can also be a TCA cycle intermediate after conversion to α-ketoglutarate (not shown in this figure) or conversion to glutamine and is recycled back to neurons through the system N transporter (astrocytic form; ⑥) and the system A transporter (neuronal form; ⑦) (glutamate-glutamine cycle).

physiology of brain metabolic diseases such as diabetic encephalopathy[18]. To our regret, however, these important issues with regard to ANLSH have not been addressed from clinical aspects.

The pentose phosphate pathway (PPP), which branches from the glycolytic pathway of glucose (Fig. 1; A), plays neuroprotective roles in concert with glutathione (Fig. 3). The PPP is a minor pathway of glucose metabolism (contributing approximately 2-3%) that generates NADPH, which in turn increases the reduced form of glutathione (GSH) by glutathione reductase to detoxify ROS through the activity of glutathione peroxidase (Fig. 3). The PPP is known to be active in proliferating cells, because PPP yields ribose 5-phosphate for nucleotide biosynthesis leading to DNA and RNA synthesis. In adult brain, resting astroglia have the potential to proliferate under stressed conditions, such as acute stroke and inflammation (i.e., reactive astrocytes express abundant glial fibrillary acidic protein [GFAP] and acquire proliferative activities). Thus, astroglia may possess a higher PPP activity level, compared with neurons. Glucose-6-phosphate dehydrogenase (G6PDH), a rate-limiting enzyme of PPP, is regulated by both allosteric and transcriptional mechanisms[1)2)63)]. Therefore, we hypothesized that both acute and chronic hyperglycemic states activate PPP in astroglia to protect the brain. We focus on endoplasmic reticulum (ER) stress and the Kelchlike ECH-associated protein 1

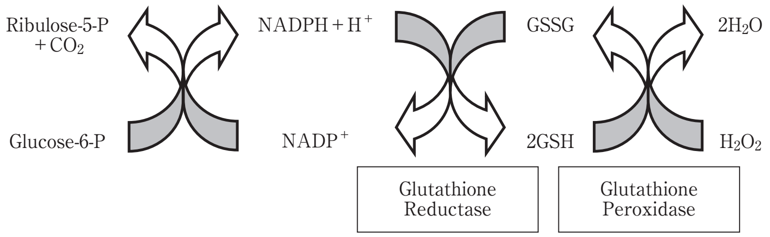


Fig. 3 PPP reduces glutathione to detoxify ROS. In the PPP, glucose-6-phosphate is decarboxylated at the position of carbon 1, generating ribulose-5-phosphate and CO_2. An important role of the PPP is to reduce $NADP^+$ to NADPH; glutathione reductase then converts the oxidized form of glutathione (GSSH) to the reduced form of glutathione (GSH) using NADPH. Glutathione peroxidase detoxifies ROS to form hydrogen peroxide (H_2O_2) using GSH.

(Keap1)/nuclear factor-erythroid-2-related factor 2 (Nrf2) system (Fig. 4), which is a master regulator of phase 2 detoxifying enzymes, including G6PDH[64)～66)].

Regulation of PPP in response to acute and chronic high glucose environments

Acutely increasing concentrations of glucose may increase glycolytic flux, and subsequently the PPP, resulting in the enhancement of the decarboxylation at carbon 1 of D-glucose (Fig. 3), as evidenced by increases in [1-$^{14}CO_2$]glucose-derived $^{14}CO_2$ production. A slight but definite increase in [1-^{14}C] glucose-derived $^{14}CO_2$ production in humans during acute hyperglycemia[24)] indicates PPP activation. Of course, the decarboxylation at carbon 1 may also occur during the tricarboxylic acid (TCA) cycle. The exact measurement of PPP activity requires the measurement of CO_2 production from both [1-^{14}C]glucose-derived $^{14}CO_2$ and [6-^{14}C]glucose-derived $^{14}CO_2$. The difference in these values indicates the PPP activity, because [6-^{14}C]glucose-derived $^{14}CO_2$ strictly indicates TCA cycle activity[67)]. The rates of brain glucose utilization are regulated by an initial step in the phosphorylation of glucose to glucose 6-phosphate by hexokinase. Brain hexokinase is reported to have a low K_m value compared with glucokinase, which has a high K_m[2)68)] and is typically found in the liver. As a result, CMR_{glc} is thought to be constant if the glucose concentration increases above 2 mM or higher[2)]. Irrespective of the fact that CMR_{glc} does not increase in vivo in response to acute hyperglycemia[22)23)], evidence supporting the existence of glucokinase activity in the brain has been reported by several investigators[69)～71)]. Roncerco et al. (2000) reported that approximately 41% of the glucose phosphorylating activity in the cerebral cortex represents glucokinase, while 59% represents hexokinase[70)]. The enhanced production of glucose-6-phosphate by glucokinase upon acutely elevated concentrations of glucose may increase the influx to the PPP, thereby increasing the generation of NADPH and, in turn, facilitating the production of GSH, which may help to eliminate ROS.

Glutathione synthesis is also reported to be more active in astroglia than in neurons[71)73)]. As mitochondrial oxidative phosphorylation is an important source of ROS in the brain, astroglial glutathione may play a key role in protecting neurons from oxidative damage. In fact, ROS production is much higher in cultured neurons than in cultured astroglia[74)], and neuronal ROS seems to be derived from the mitochondrial electron chain, rather than NADPH oxidase[74)]. GSH in astroglia is released to the extracellular space to reduce ROS, and released GSH is then hydrolyzed by the γ-glutamyltranspeptidase present on the external surfaces of astrocytes, producing the dipeptide cysteinyl-glycine that is then hydrolyzed by the aminopeptidase N to release cysteine and glycine. These amino acids with glutamine released from astroglia make available all the necessary precursors for neuronal GSH synthesis[66)]. Acute hyperglycemia increases ROS production in the endothelium[75)], and neuronal cultures in our laboratory also exhibited the enhanced production of ROS under acutely increasing glucose concentrations. In contrast, ROS production in astroglia was reduced by increasing glucose concentrations. Interestingly, in mixed cultures of neurons and astroglia, ROS production remained constant as the glucose concentrations were elevated. We speculated that increases in astroglial GSH may reduce neuronal ROS production[33)～35)]. In fact, Asanuma et al. (2010) demonstrated that zonisamide, which has been used clinically as an anti-epileptic drug, augments astroglial GSH synthesis and resultant increases in cysteine transfer to dopaminergic neurons confer a novel protective mechanism against neuronal degeneration through quenching ROS and dopamine quinone in PD model in vivo[76)]. In vivo diabetic model animals, astroglial response also does, indeed, occur by changing GFAP or S100b expression in an early phase of high glucose stress[77)～79)]. Although the interpretation of astroglial GFAP or S100b expression is difficult, these reports emphasizes

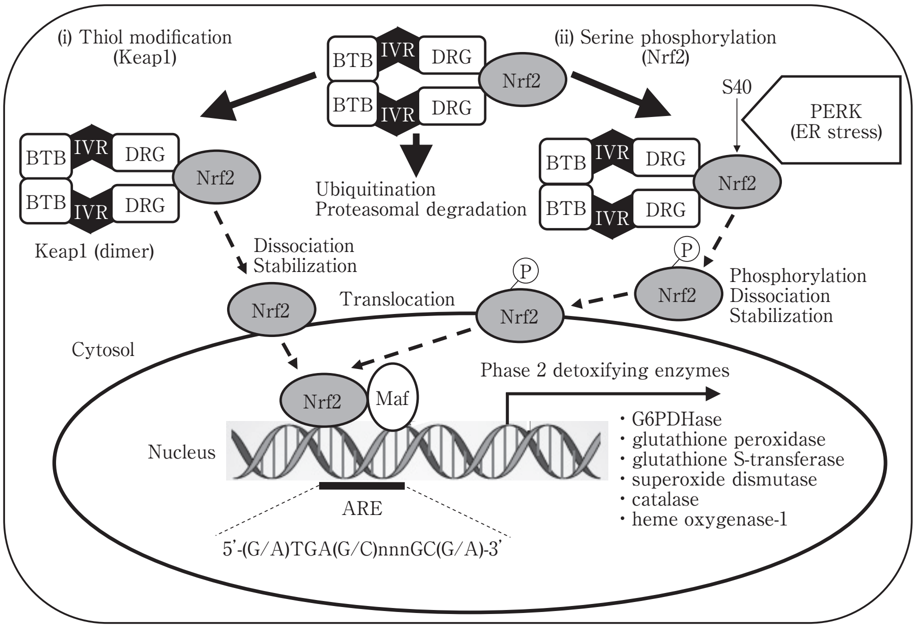


Fig. 4 A model for the Kelchlike ECH-associated protein 1 (Keap1)/nuclear factor-erythroid-2-related factor 2 (Nrf2) interaction and activation (adapted from Vargas and Johnson, 2009[66]). Nrf2 can be activated by at least two mechanisms that include: (i) stabilization of Nrf2 via Keap1 cysteine thiol modification and (ii) phosphorylation of serine residues of Nrf2 by upstream kinases, such as RNA (PKR)-like ER kinase (PERK). Keap1 presents two characteristic domains, the bricabrac, tramtrack and broad complex (BTB) domain and the double glycine repeat (DGR) domain. Keap1 bridges the Cullin-3-based E3 ligase and Nrf2 using its BTB and the central intervening region (IVR) to bind Cullin-3 and its DGR to bind the Nrf2. The BTB domain participates in Keap1 dimerisation. Under basal conditions, Nrf2 is continuously degraded by the ubiquitin/proteasomal system. At least two different mechanisms that facilitate the dissociation of Nrf2 from Keap1, leading to Nrf2 translocation from the cytosol to the nucleus, are known. One involves the modification of thiol residues in the Keap1 protein. Another mechanism for the dissociation of the Keap1/Nrf2 complex is the phosphorylation of serine residues of Nrf2. Nrf2 then translocates to the nucleus and must heterodimerise with members of the Maf proto-oncogene family in order to bind to regulatory elements in the DNA to increases antioxidant response element (ARE)-driven transcription.

adaptive responses of astrocytes under hyperglycemia. Unfortunately, however, the direct evidence that an activation of PPP in astroglia leads to reduced ROS production in the brain in vivo is lacking. Chronic high glucose environments may also increase PPP activities in astroglia through different mechanisms[35]. The G6PDH gene is known to possess antioxidant response elements (AREs), and the Keap1/Nrf2 system (Fig. 4) plays a pivotal role in the transcriptional regulation of G6PDH under stressed conditions[64]～[66]. Nrf2 is a transcriptional factor that is maintained in the cytosol, forming a complex with Keap1 (an anchor protein bound to the cytoskeleton) under unstressed conditions. Keap1/Nrf2 complexes are constantly degraded by a proteasome system; thus, the transcriptional activity of Nrf2 is suppressed under normal physiological environments. At least two different mechanisms that facilitate the dissociation of Nrf2 from Keap 1, leading to Nrf2 translocation from the cytosol to the nucleus, are known. One involves the modification of thiol residues in the Keap1 protein, typically by an increase in ROS production[64][66]. Sulforaphane, a natural isothionate found in broccoli sprouts, is a potent activator of Nrf2 and may also activate Nrf2 via this mechanism[64][80][81]. In fact, sulforaphane reportedly enhances astroglial survival under conditions of oxygen-glucose deprivation[82]. Another mechanism for the dissociation of the Keap1/Nrf2 complex is the phosphorylation of serine residues of Nrf2. Several upstream kinases are reported to phosphorylate Nrf2 at serine 40[64][83]. Cullinan et al[84]. reported that Nrf2 is a direct substrate of PERK, a kinase that acts as a transducer of ER stress. Thus, ER stress could trigger the Keap1/Nrf2 system-dependent transcriptional regulation of various type 2 detoxifying enzymes. Protein kinase C (PKC) is another candidate kinase for Nrf2 phosphorylation. PKC activation induced by the enhanced production of diacylglycerol under high glucose environments is a well known mechanism[6)~8)].

Finally, high glucose environments and ER stress as a regulatory mechanism of the PPP via the Keap1/Nrf2 system should be considered. ER stress exerts a protective mechanism for cell survival by reducing protein synthesis upon the increased production of misfolded proteins in the ER. ER stress plays an important role in the pathogenesis of pancreatic β-cell degeneration in diabetes mellitus[85)], neuronal cell death caused by stroke[86)], as well as neurodegenerative disease[87)]. Although it has been emphasized that ER stress induces cell death, ER stress as a cell-survival signal has not been studied extensively. We hypothesized that HBP, another minor pathway of glucose that also branches from glycolysis (Fig. 1; B), induces ER stress in astroglia as a protective signal in the brain. In the HBP, glucose-6-phosphate is converted to fructose-6-phosphate, which is then converted to N-acetylglucosamine-6-phosphate (GlcN-6-P) by the rate-limiting enzyme glutamine: fructose-6-phosphate amidotransferase (GFAT). GFAT is the rate-limiting enzyme of the HBP responsible for the conversion of L-glutamine and D-fructose 6-phosphate to L-glutamate and D-glucosamine 6-phosphate (GlcN-6-P), and flux through the HBP increases with the glucose concentration. GlcN-6-P is subsequently converted into UDP-N-acetyl glucosamine, which acts as a substrate for N- and O-linked protein glycosylation (Fig. 1; B). Glycosylation is essential to the folding, translocation, function, and stability of many proteins. Recently, a causative role of the excess influx to the HBP in hyperglycemia-induced ER stress has been reported in hepatic cells[88)]. Cellular treatment with glucosamine has been widely used as a tool to investigate the effects of increased HBP flux on a variety of cell signaling pathways. Recent findings using glucosamine suggest that an increased HBP flux in human astroglial cells results in the rapid, short-term phosphorylation of Akt that is likely a result of increased ER stress[89)].

Recently, we reported that chronic hyperglycemic conditions induce ER stress and nuclear Nrf2 translocation of Nrf 2, resulting in PPP activation in cultured astroglia[35)].

Astrogliopathy as a loss of the protective function of astroglia

Irrespective of these astroglial protective mechanisms, a long-lasting hyperglycemic state may cause the deterioration of astroglial function. A mouse model of type 1 diabetes induced by the administration of streptozotocin showed a marked enhancement of intracellular lipofuscin deposits, characteristic of increased oxidative stress and aging in both the hilus and the subgranular zone and the granular cell layer in the hippocampus[90)]. Recent findings that experimental diabetes increases production of reactive oxygen-nitrogen species and inhibits astrocytic gap junctional communication in tissue culture and brain slices from streptozotocin-diabetic rats suggest that astroglial dysfunction does, indeed, occur after longer period of disease duration irrespective of intrinsic self-defense mechanisms[91)92)]. These observations imply opposite roles of D-glucose: the enhancement of generation and the elimination of ROS under high glucose environments in astroglia. We hypothesized that carbonyl stress and the resultant formation of AGE originated from a non-enzymatic reaction, the third minor pathway of glucose metabolism branching from glycolysis, producing methylglyoxal[93)].

AGEs are a well-known source of ROS under a chronic hyperglycemic state[6)~8)94)] and are found in various intraneuronal protein deposits such as neurofibrillary tangles in AD and Lewy bodies in PD. In AD, AGEs accumulate in an age- and stage-dependent manner in neurons and astroglia and are also increasingly found in neuritic amyloid plaques, indicating an imbalance between the formation and degradation of AGE-modified proteins[95)]. Among the many reactive carbonyl compounds and AGE precursors, methylglyoxal is most likely to contribute to intracellular AGE formation, since it is extremely reactive and constantly produced by the degradation of triose phosphates such as glyceraldehyde-3-phosphate (Fig. 1; C). Furthermore, methylglyoxal levels increase under pathophysiological conditions; for example, when triose phosphate levels are elevated, the expression or activity of glyoxalase I is decreased, as in the case when the concentration of glutathione, the rate-determining co-factor of glyoxalase I, is low. The increased production of methylglyoxal via increased flux to glycolysis in astroglia and the resultant increases in ROS production not derived from the mitochondrial oxidative metabolism of glucose could be a plausible mechanism under high glucose environments. If the adverse effects of high-glucose environments overcome the astroglial intrinsic protective mechanism over the long run, astroglial dysfunction leading to neuronal damage via glycoxidative stress is likely to occur[96)]. The failure of astroglial protective mechanism induced by long-lasting diabetic status seems to be involved in the pathogenesis of central nervous system dysfunction in diabetic patients and some neurodegenerative diseases.

Conclusions

In conclusion, astroglia may exert a neuroprotective role under acute and chronic hyperglycemic conditions associated with diabetes mellitus via different and cooperative regulatory mechanisms. Importantly, both mechanisms depend on appropriate D-glucose contents; thus, maintaining glucose concentrations in a proper range may be relevant for astroglial neuroprotective function. However, long-lasting and/or fluctuating glucose levels may diminish astroglial function via high rates of glycolytic activity in astroglia, resulting in the dysfunction of astroglia ("astrogliopathy"). Maintaining the protective roles of astroglia may be relevant to the development of novel therapeutic strategies against neurological disorders.

Disclosure：The authors do not have any conflicts of interest to disclose.

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