

*Critical Review***Ischemic Stroke and Glucose Intolerance: a Review of the Evidence and Exploration of Novel Therapeutic Targets**Shinichi Harada¹, Wakako Fujita-Hamabe¹, and Shogo Tokuyama^{1,*}¹*Department of Clinical Pharmacy, School of Pharmaceutical Sciences, Kobe Gakuin University,
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Abstract. Stroke is one of the leading causes of death and disability worldwide. It is well known that hyperglycemia and/or diabetes potentially exacerbate the neuronal damage observed following ischemic stroke. Recent reports have shown that hyperglycemia/glucose intolerance may be induced by cerebral ischemic stress, and that normalization of blood glucose levels during the first 48 h of hospitalization appears to confer greater survival outcomes in stroke patients. However, the mechanisms underlying post-ischemic glucose intolerance remain unclear. Here, we review research to date on the mechanisms through which ischemic neuronal damage develops and on the role of post-ischemic glucose intolerance focusing on insulin and adiponectin signaling and communication between the brain and peripheral tissues. The relationship between ischemic neuronal damage and post-ischemic glucose intolerance is also discussed. With respect to therapeutic options, in addition to traditional post-stroke therapies, we also discuss the effect of anti-diabetic drugs and glucose-sensing neuropeptides on the development of the post-ischemic glucose intolerance and neuronal damage. In conclusion, we support the idea for focusing research on the development of post-ischemic glucose intolerance as a new therapeutic target for the stroke patients.

Keywords: cerebral ischemic stress, glucose intolerance, insulin, metformin, orexin-A

1. Introduction

Focal cerebral ischemia (stroke) is one of the leading causes of death and disability worldwide (1). Neuronal damage following ischemic stroke (such as cerebral ischemia) develops as a result of a series of complex pathophysiological events. For instance, glutamate mediated excitotoxicity and inflammation may lead to calcium overload, peri-infarct depolarization, oxidative stress, stress signaling, neurovascular pathophysiology, and neuronal cell death (apoptosis/necrosis) (Fig. 1) (1). In addition, there are two major types of stroke: cerebral ischemia caused by the obstruction of blood vessels and hemorrhagic stroke (2). Many studies have demonstrated the protective effects of synthetic compounds in patients following ischemic stroke (3, 4). In recent decades, al-

though significant breakthroughs have occurred, particularly with respect to the development of advanced therapeutic drugs such as tissue plasminogen activator (t-PA) or edaravone (a free radical scavenger) (5, 6), the limited time window during which these compounds have clinical utility and their adverse side effects restrict their application in practice. Moreover, even with the application of these compounds, it is still difficult to achieve complete return to normal health status without leading to a poor prognosis like long-term physical disability and memory disturbances (7). Therefore, it is still important to explore new therapeutic targets and post-stroke strategies. Recently, we have focused on pathophysiological parameters, specifically pre- or post-ischemic hyperglycemia, as one of the factors influencing prognosis following ischemic stroke.

This review provides an overview of possible mechanisms through which post-ischemic glucose intolerance may develop. Furthermore, we review the evidence for efficacy of post-ischemic glucose intolerance suppression as a potential new therapeutic strategy for ischemic stroke.

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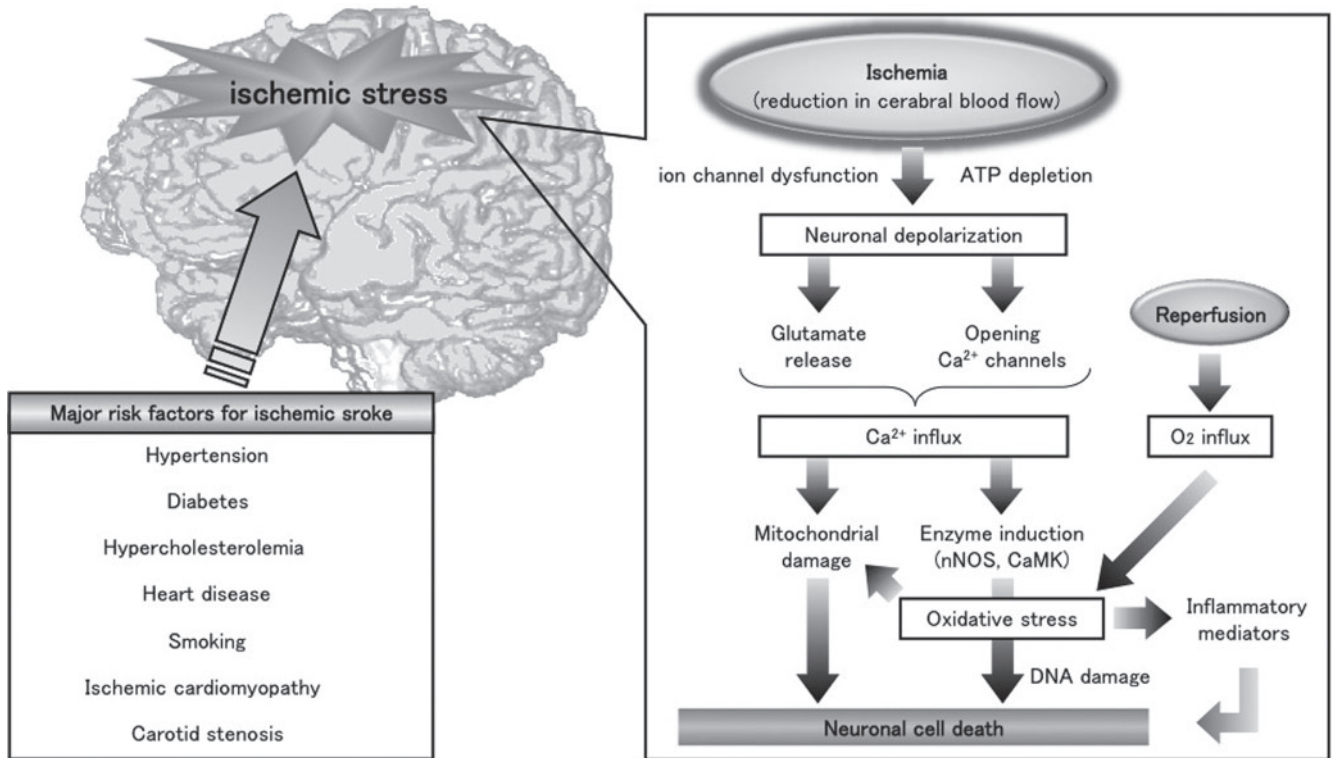


Fig. 1. Major risk factors and the known mechanisms of the development of ischemic stroke.

2. Diabetes mellitus and hyperglycemia as a risk factor of ischemic stroke

2.1. Risk factor of ischemic stroke: diabetes and hyperglycemia

Many pathophysiological determinants such as hypertension, hyperlipidemia, and hyperglycemia are known to be associated with the development of cerebral ischemia and ischemic neuronal damage (Fig. 1) (8). These factors exacerbate the onset of neuronal damage or infarction. Some risk factors, like hypertension and diabetes, impair protective vascular mechanisms that keep cerebral blood flow stable during reductions in blood pressure (cerebrovascular autoregulation), facilitating the occurrence of ischemia (8). In addition to their vascular effects, some risk factors, like aging and diabetes, amplify the intrinsic susceptibility of brain cells to injury and the tissue damage produced by ischemia (1, 8). However, the biological basis of these effects is not well understood. Hyperglycemia and/or diabetes is known to exacerbate ischemic neuronal damage by stimulating vascular inflammation, increasing blood–brain barrier (BBB) permeability, impairing cellular metabolism, and promoting tissue acidosis (9–11). The results of a clinical study found that hyperglycemia was associated with large infarct size, poor clinical outcomes, and a higher

risk of mortality after ischemic stroke. Moreover, this study found that hyperglycemia is an independent predictor of a poor prognosis on the basis of age, diabetic status, and ischemic stroke severity.

2.2. Changes in glucose metabolism after ischemic stroke in clinical study

Whether the patient has a history of diabetes or not, hyperglycemia (that is, glucose intolerance and/or insulin resistance) is reported to be induced after ischemic stroke (12). In addition, 27%–37% of patients admitted to hospital with stroke were shown to have glucose intolerance 3 months after the initial stroke, and approximately one-third of these cases had developed diabetes by this time point (13–15). Furthermore, another systematic review found that even though the elevation of blood glucose levels is moderate in stroke patients, hyperglycemia is associated with mortality and poor functional recovery compared with lower glucose levels (16). Interestingly, nondiabetic ischemic stroke patients have an increased risk of mortality if their blood glucose levels are > 6.1 mM at early time points following the acute phase of ischemic stroke onset (17). In addition, the prevalence of abnormal glucose metabolism after the acute phase of ischemic stroke in 427 Japanese patients without a history of diabetes was 62.8% (18). Of them, a

quarter of these patients had newly diagnosed diabetes (24.8%) and the rest of the patients had either impaired glucose tolerance or impaired fasting blood glucose (FBG) (18). Furthermore, in a recent study, Rosso et al. reported that infarct growth was greater in patients with high blood glucose levels than in patients with low blood glucose levels after ischemic stroke (19). This finding is supported by work showing that patients with ischemic stroke who received glycemic control treatment (normalization of blood glucose to < 7.2 mM) had a 4.6-fold decrease in mortality risk compared with patients with persistent hyperglycemia (20). Thus, even in nondiabetic patients, hyperglycemia after ischemic stroke seems to be associated with a high mortality risk, and a substantial proportion of these patients present with hyperglycemia during the acute phase following ischemic stroke.

With respect to medical treatment following ischemic stroke, t-PA has become standard practice in the acute phase of ischemic stroke care (21); however, hyperglycemia has been shown to decrease the therapeutic efficacy of t-PA in animal models, indicating that using t-PA in patients with hyperglycemia after ischemic stroke may be difficult (21).

2.3. Known mechanisms of the development of post-ischemic glucose intolerance

Several mechanisms underlying hyperglycemia after ischemic stroke have been proposed (22). Specifically, ischemic stroke–related cytokines have been shown to activate the hypothalamus–pituitary–adrenal axis, leading to increased serum glucocorticoid levels and the activation of the sympathetic autonomic nervous system leading to catecholamine release, resulting in excessive glucose production and insulin resistance (23, 24). However, in clinical practice, not all patients with hyperglycemia presenting during the acute phase of ischemic stroke show a significantly elevated catecholamine (25), suggesting that the hyperglycemia observed during the acute phase of ischemic stroke cannot be explained by increased catecholamines alone and that further research on the topic is necessary.

2.4. Interaction of ischemic stroke–induced BBB disruption and glucose metabolism

BBB is one of the most important targets for therapy of cerebral ischemia (26, 27). BBB acts to protect the brain from circulating neurotoxic agents and inflammatory molecules. Furthermore, tight junctions of BBB, as one mode of cell–cell adhesion in epithelial and endothelial cellular sheets, maintain and restrict nutrients and ions in the brain at levels suitable for brain function (28). Tight junction–associated proteins are well known as the occludin family, claudin family, and zonula occludens

family (26, 28). In particular, claudin-5 is a major cell adhesion molecule of tight junctions in brain endothelial cells (29). The destruction of BBB has been considered to be a key step in the disease process of a number of neurological disorders including cerebral ischemia (30). Ischemic stress is reported to alter the expression of the tight junction–associated proteins described above (26). In addition, these changes are due to the ischemic stress–induced up-regulation of matrix metalloproteinase (MMP), a microvascular gelatinase, in particular MMP-2 and MMP-9, and inflammatory molecules such as interleukin-6 and tumor necrosis factor- α (TNF- α), or decrease of caveolin-1, another regulating factor of BBB permeability (30–32).

Furthermore, recent evidences show that diabetes and/or hyperglycemia will provoke molecular changes including imbalance in the MMPs/tissue inhibitors of metalloproteinases cascade that alter the function and structure of blood vessels, resulting in a compromised BBB (8, 30, 33). As we have previously demonstrated, since ischemic stress will activate MMP-2 and MMP-9, and will cause edema, it is possible that post-ischemic glucose intolerance might be involved in disruption of BBB via activation of MMPs and exacerbation of the development of neuronal damage (34).

3. Evidence for the development of post-ischemic glucose intolerance from basic research studies

3.1. Relationship between ischemic neuronal damage and post-ischemic glucose intolerance

Evidence from in vitro research has revealed that glucose itself promotes the development of caspase-dependent apoptosis during re-oxygenation following oxygen and glucose deprivation, and further that it promotes the production of reactive oxygen species, such as radicals, hydrogen peroxide, and peroxynitrite (35). Furthermore, hyperglycemia is known to modify many proteins important for cell survival by advanced glycation, inducing some death-related proteins like high mobility group box 1, which triggers the expression of pro-inflammatory mediators, or downregulation of cytoskeletal proteins that support neuronal cell survival (36, 37). Interestingly, glucose receptors, known as sodium glucose transporter (SGLT) type 3, may act as glucose-sensing receptors, provoking the Na^+ -dependent depolarization of the membrane in response to elevated extracellular glucose (38). In cerebral ischemia, an energy failure triggers the depolarization of the neuronal membrane, and various excitatory neurotransmitters such as glutamate and dopamine are co-released (39). A marked influx of Ca^{2+} into post-synaptic neurons then occurs, which provokes the catastrophic enzymatic process leading to irreversible neu-

ronal injury (40). These findings provide a hypothetical mechanism through which post-ischemic glucose intolerance mediates neuronal damage via the activation of glucose receptors, followed by the modulation, induction, or downregulation of proteins related to cell viability. Interestingly, the inhibition of SGLT by phlorizin, a non-specific SGLT inhibitor, was found to reduce infarct volume in a middle cerebral artery occlusion (MCAO) mouse model (41). These results support our recent findings that ischemic stress-induced glucose intolerance transiently occurred prior to the development of neuronal damage and is possibly involved in the development of neuronal damage.

3.2. Altered blood glucose levels following ischemic stroke in an experimental ischemic stroke model

We have hitherto focused on changes in blood glucose after cerebral ischemic stress using a MCAO mouse

model as a model for ischemic stroke (42). With this ischemic stroke model, infarction began to develop at 6 h, and then peaked on day 3 to day 5 after the MCAO. The observed behavioral abnormalities and memory disturbances were gradually aggravated from day 1 to day 5 following the MCAO. Interestingly, neuronal damage was preceded by elevations in blood glucose levels. That is, FBG levels were significantly elevated at 12 h and day 1 after the MCAO (representing the early phase of the development of ischemic neuronal damage), but then returned to the sham levels on day 3 and decreased to less than the sham levels on day 5 (Fig. 2A). Additionally, the glucose-induced increment of blood glucose levels estimated by the oral glucose tolerance test were significantly enhanced on day 1 compared with the sham group, but returned to sham levels on day 3. However, it was noted that a slight elevation in FBG was observed in every group. This might be due to a physiological re-

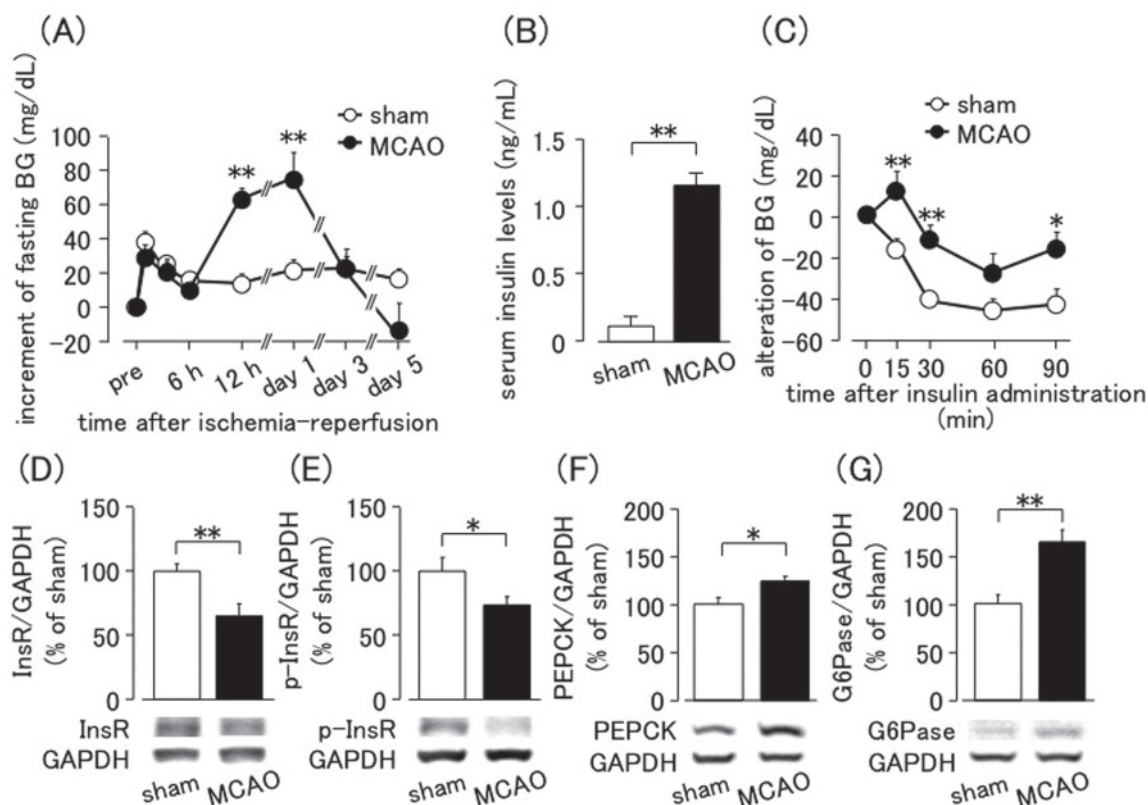


Fig. 2. The development of post-ischemic glucose intolerance and insulin resistance after cerebral ischemic stress. A: The increment of fasting blood glucose levels (FBG) after cerebral ischemic stress. ** $P < 0.01$ vs. sham (12 h and day 1), $n = 8 - 17$. B: Increment of serum insulin levels on day 1 after cerebral ischemic stress. ** $P < 0.01$ vs. sham, unpaired Student's t -test, $n = 7 - 22$. C: Time course of insulin tolerance test on day 1 after cerebral ischemic stress. Mice were administered insulin (0.9 U/kg, i.p.). * $P < 0.05$, ** $P < 0.01$, $n = 7 - 10$. Results of panels A - C were modified from Ref. 42 with permission. D - G: Changes in the expression levels of total and tyrosine-phosphorylated insulin receptor, PEPCK, and G6Pase after cerebral ischemic stress. Representative western blots showing total insulin receptor (InsR) (D), tyrosine-phosphorylated InsR (p-InsR) (E), PEPCK (F), G6Pase (G), and GAPDH levels in the liver on day 1 after MCAO. The relative expression levels were analyzed by determining the ratio of each protein relative to GAPDH. * $P < 0.05$, ** $P < 0.01$, $n = 8 - 16$. All results (A - G) are presented as means \pm S.E.M.

sponse to surgery, the so-called “stress hyperglycemia” mediated by chemical mediators such as catecholamines (epinephrine and norepinephrine), glucagon, glucocorticoids, and cytokines such as TNF- α and IL-1 released from stimulated sympathetic nerve terminals (e.g., the hypothalamic–pituitary–adrenal axis) (16). Furthermore, we have considered that the decrease in FBG and the disappearance of the oral glucose-induced elevation of blood glucose on day 5 may be due to a decrease in physiological function, involving failure of glucose or lipid metabolism, leading to death.

It is worth noting that elevated blood glucose levels during the second phase after cerebral ischemic stress were confirmed only in the MCAO group, but not in the sham group (Fig. 2A). This suggests that the post-ischemic hyperglycemia observed at 12 h and day 1 after MCAO are not merely due to mechanical stress, but rather due to a number of causes including insulin resistance and impaired insulin secretion.

3.3. Alterations in insulin signaling after ischemic stroke

3.3.1. Impaired insulin sensitivity after ischemic stroke

Insulin exerts regulatory effects on glucose metabolism via the downstream signaling of the insulin receptor (InsR), including activation of the InsR substrate (IRS), which activates the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and enhances glucose uptake and glycogenesis (43). Furthermore, it was recently reported that forkhead box O1 and peroxisome proliferator-activated receptor (PPAR)- γ coactivator 1 α are involved in insulin signaling pathways (44, 45). In addition, gluconeogenesis, which is catalyzed by several enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphate (G6Pase), is also highly regulated by insulin (46, 47). We found that the basal serum insulin levels were significantly higher at 12 h and day 1 in the MCAO group compared with the sham group (Fig. 2B), suggesting post-ischemic glucose intolerance might be due to impaired insulin sensitivity. Indeed, in the insulin tolerance test, the insulin-induced (0.9 U/kg, i.p.) decrease in FBG levels was significantly smaller than in the sham group on day 1 after the MCAO (Fig. 2C). Interestingly, following the glucose loading test (2 g/kg, p.o.), serum insulin levels did not change in the MCAO group (42). That is, the glucose-induced stimulation of insulin secretory activity observed in the sham group completely disappeared after ischemic stroke, suggesting the possible involvement of insufficient insulin secretion as a mechanism underscoring the development of post-ischemic glucose intolerance.

3.3.2. Possible mechanisms of impaired insulin sensitivity

In the peripheral tissues, there are many insulin-targeting organs including liver, skeletal muscle and adipose tissues (48). Although some studies have highlighted the importance of insulin signaling as a growth factor to protect against ischemic stress in the central nervous system (CNS) (49), very few studies have investigated post-ischemic changes in InsR expression and intracellular signaling in these peripheral organs. We found that hepatic InsR and p-InsR protein expression levels were significantly lower in animals from the MCAO group than in the sham group on day 1 after MCAO (Fig. 2: D and E) (50). In addition, in skeletal muscle, the InsR and p-InsR levels tended to be lower in the MCAO group than in the sham group (50). This decrease in p-InsR was due to a decrease in total InsR because p-InsR/InsR was not altered between the sham and MCAO group.

Insulin stimulation and activation of its receptor in the liver suppresses the transcription of gluconeogenic enzymes, particularly PEPCK and G6Pase, through activation of PI3K and Akt (51). After ischemic stroke, we have demonstrated that hepatic PEPCK and G6Pase levels are significantly higher in the MCAO group than in the sham group (Fig. 2: F and G) (50). Hence, post-ischemic hyperglycemia or decrease in whole-body insulin sensitivity after ischemic stroke may be due to decreased hepatic InsR expression and to upregulation of gluconeogenesis (42).

In skeletal muscle, insulin signaling is known to activate the translocation of the insulin-dependent glucose transporter 4 (GLUT4) into the plasma membrane (52). After ischemic stroke, glucose utilization in skeletal muscle may be downregulated.

3.3.3. Interaction between impaired insulin sensitivity and ion transporters

It is important to point out that insulin can regulate ion transporters (53 – 55). Alterations in insulin signaling may disrupt the balance of various transporters such as the Na/H exchanger, the Na-K-2Cl co-transporter, and Na/K ATPase activity, leading to dysregulation of ion handling in multiple organ systems (53 – 55). This suggests that these mechanisms may be involved in the development of post-ischemic glucose intolerance. In particular, the loss of Na/K ATPase activity produces membrane depolarization, opening NMDA-receptor channels and favoring the massive Ca²⁺ influx through channels gated by glutamate receptors (55). These findings suggest that impaired ion transport brought about by cerebral ischemic stress-induced insulin resistance may be involved in the development of post-ischemic glucose intolerance and neuronal damage.

3.4. Altered adiponectin signaling after ischemic stroke

3.4.1. Adipokines and glucose metabolism

Adipose tissue is not just an inert organ for energy storage. It also secretes proinflammatory cytokines and synthesizes a wide range of biologically active molecules known as adipokines (56). Adipokines mainly consist of adiponectin, leptin, TNF- α , and resistin (57), and they play an important function in regulating glucose metabolism. For example, TNF- α and resistin activate the c-Jun N-terminal kinase and nuclear factor- κ B pathways and exacerbate insulin resistance, type-2 diabetes, and related complications (58).

Leptin has undoubtedly been one of the most studied adipokine since this protein was first characterized in 1994. Leptin is a well-known regulator of food intake and energy expenditure via hypothalamic-mediated effects. However, it is increasingly appreciated that this adipokine has many additional effects, often as a consequence of direct peripheral actions. These include angiogenesis, hematopoiesis, lipid, and carbohydrate metabolism and effects on the reproductive, cardiovascular, and immune systems. Leptin deficiency induces severe insulin resistance, hyperinsulinemia, hyperglycemia, and enlarged fatty liver (59).

3.4.2. Adiponectin and glucose metabolism

Adiponectin, an insulin-sensitizing adipokine, is released by adipocytes and targets a multitude of different cell types. Prominent target cells are hepatocytes, cardiac myocytes, pancreatic beta cells, and podocytes (60). The genes encoding two related receptors, AdipoR1 and AdipoR2, have been cloned; and these receptors may mediate many of the actions of adiponectin, including its insulin sensitizing, anti-inflammatory, and antiapoptotic functions (61). Decreased adiponectin is implicated in the development of insulin resistance in obesity, which is reversed by the replenishment of adiponectin, whereas overexpression of adiponectin from adipose tissue results in improvements in systemic insulin sensitivity (60, 61). In the case of patients with ischemic cerebrovascular disease, significantly lower levels of plasma adiponectin have been reported (62). In addition, pre-administration with a nonselective β -adrenergic receptor blocker, propranolol, could block the release of adipokines and also suppress increased blood glucose levels after MCAO (63), suggesting there is a potential role of adiponectin in hyperglycemia in the acute phase of ischemic stroke.

3.4.3. Interaction between adiponectin and post-ischemic glucose intolerance

Our latest study showed that the serum adiponectin was significantly decreased in the early phase of cerebral

ischemic stress. These findings suggest that the signaling cascade underlying the adiponectin receptor could be altered during ischemic stress and this may be associated with impaired insulin sensitivity and post-ischemic glucose intolerance (42). As one of the signaling molecules acting on the adiponectin receptor, 5'-adenosine monophosphate-activated protein kinase (AMPK), a serine/threonine kinase, is activated by phosphorylation (pAMPK) and is known as a sensor of energy metabolism mainly expressed in the liver, skeletal muscle, and brain (64, 65). In peripheral tissues, AMPK is known to regulate glucose metabolism by stimulating the translocation of GLUT4 in skeletal muscles and suppressing gluconeogenesis in the liver (66), resulting in a decrease of blood glucose levels. Although it is hypothesized that the peripheral AMPK activation cascade should be suppressed by cerebral ischemic stress, based on our findings, in liver and skeletal muscle, AMPK activation was not affected by cerebral ischemic stress (67). These results suggest that some mechanisms such as downregulation of the PPAR- α signaling pathway, (another downstream pathway of adiponectin receptor), rather than downregulation of hepatic AMPK, may be involved in the development of post-ischemic glucose intolerance (45, 60). Since PPAR- α has a major role in the control of hepatic gluconeogenesis via the regulation of expression and release of the fibroblast growth factor 21, this may be one of the candidate molecules involved in the development of glucose intolerance (45).

3.5. Possible alterations in communication pathways between the brain and peripheral tissues after ischemic stroke

3.5.1. Communication between brain and peripheral tissues

It was recently reported that the autonomic nervous system plays an important role in conveying metabolic information between the CNS and peripheral organs (68). In particular, the hypothalamus is the primary site of convergence and integration for redundant energy status signaling, which includes central and peripheral neural inputs, as well as hormonal and nutritional factors (Fig. 3) (68, 69).

3.5.2. Role of orexins in communication between the brain and peripheral tissues

The orexin family (orexin-A and orexin-B) are a newly identified group of neuropeptides that are mainly expressed in the hypothalamus (70). Collectively, they play roles in many physiological functions, including arousal, energy homeostasis, glucose metabolism, feeding behavior, sleep, and wakefulness (70). Orexins are derived from a single precursor prepro-orexin and act via two

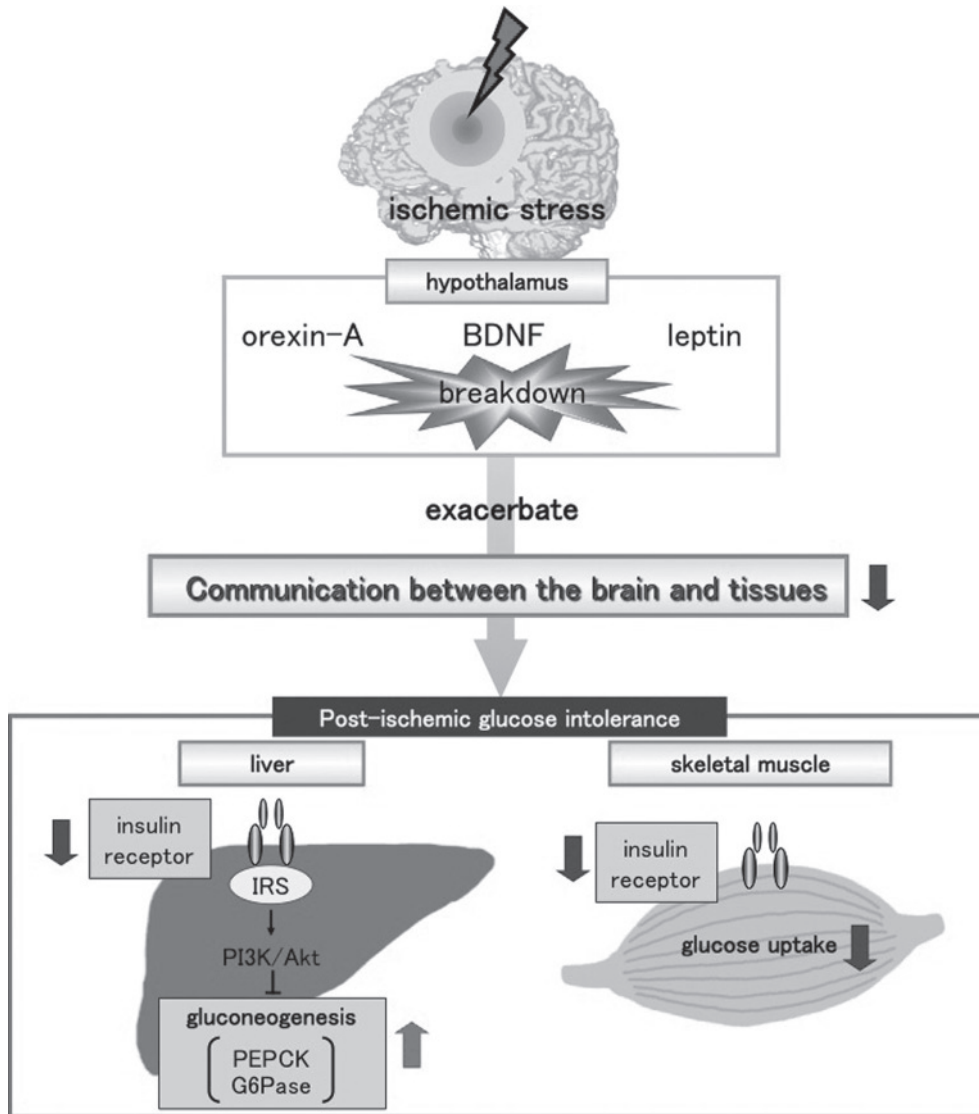


Fig. 3. The possible alteration of communication between brain and tissues after ischemic stroke.

types of G-protein-coupled receptors, the orexin-1 (OX1R) and orexin-2 receptors, which have a seven-transmembrane topology (70). It was recently reported that the autonomic nervous system plays an important role in conveying metabolic information between the hypothalamus and peripheral organs (68, 69). Injection of orexin-A into the ventromedial hypothalamus of mice or rats enhanced insulin-stimulated glucose uptake and glycogenesis in skeletal muscle by activating the sympathetic nervous system (69).

3.5.3. Other neuronal peptides

It has also been reported that microinjection of leptin, a physiologically and clinically important glucose metabolism regulatory hormone (71), into the ventromedial hypothalamic nucleus (VMH) and nearby medial hypo-

thalamic area preferentially increased glucose uptake in skeletal muscle, heart, and brown adipose tissue (72, 73). In addition, activation of the leptin receptor specifically in the VMH resulted in a marked increase in glucose uptake in those peripheral tissues, similar to the effects of intracerebroventricular (i.c.v.) or peripheral administration of leptin (74, 75). On the other hand, Nonomura et al. reported that i.c.v. injection of brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, increases hepatic insulin sensitivity and pancreatic insulin content (76). In addition, the breakdown of this “inter-tissue communication” may be involved in the development of post-ischemic glucose intolerance (Fig. 3). Recently, we have found that the protein expression levels of BDNF and its receptor TrkB in the hypothalamus significantly decreased (77), suggesting the involve-

ment of downregulation of BDNF signaling as one of the mechanisms for the development of post-ischemic glucose intolerance. Involvement of BDNF and post-ischemic glucose intolerance as a possibility should be investigated further.

4. The effectiveness of suppression of post-ischemic glucose intolerance as a new therapeutic strategy against ischemic stroke

4.1. Well-established medical interventions for ischemic stroke

In recent years, efficient treatment strategies during the acute phase of ischemic stroke have been emerged, including thrombolysis with t-PA and removal of free radical with edaravone, a free radical scavenger (5, 78, 79). However, t-PA and edaravone are less effective for patients in the chronic stage because of their narrow therapeutic time window (5, 78, 79). That is, for optimal outcomes, the drug should be administered within 3 h (t-PA) or 24 h (edaravone) after stroke onset. Furthermore, the presence of unintended risks for hemorrhagic transformation also limits the use of t-PA (79, 80). The new oral direct thrombin inhibitor, dabigatran, will be the one that is expected to be safe and have a longer therapeutic time window, while it remains to be determined clinically (81–83).

Furthermore, although many neuroprotective drugs are shown to be effective in animal models, most were found to be ineffective for the treatment of human ischemic stroke. In addition, clinical trials of these compounds ended prematurely because of disruption of normal brain function, suggesting that the development of a therapeutic drug for use in ischemic stroke still requires much work.

Based on the above-mentioned problems, in the recent years, many studies have been performed to find methods to extend the therapeutic time window by combination of t-PA and other known drugs. It is reported that reperfusion with t-PA-induced catastrophic hemorrhagic transformation were dramatically decreased by combination therapy with t-PA and edaravone (84). The possible mechanism is that edaravone may inhibit the activation of MMP-9, which can cause hemorrhagic transformation, by scavenging the t-PA-induced or cerebral ischemia-induced reactive oxygen species (84–86). In addition, combination treatment with t-PA and anti-vascular endothelial growth factor (VEGF) neutralizing antibody significantly attenuated MMP-9 activation, degradation of BBB components, and hemorrhagic transformation, since VEGF is associated with the BBB disruption after cerebral ischemia (87). On the contrary, bad results are also reported that combination of t-PA with erythropoi-

etin induces BBB permeability (88). That is, many problems remain to be solved to define the superiority of combination of t-PA and other drugs.

4.2. Suppression of post-ischemic glucose intolerance as a new therapeutic strategy

4.2.1. Use of anti-diabetic drugs — insulin

Clinical research has shown that normalization of blood glucose levels (from 7.5 to 4.5 mM of FBG) during the first 48 h of hospitalization appears to confer better survival outcomes in patients with thromboembolic stroke (20). In addition to hyperglycemic conditions, hypoglycemia after the onset of ischemic stress are also associated with poor prognosis (89). For example, hypoglycemia characterized by less than 3 mM of serum glucose should be avoided during ischemic stress for a good prognosis (89). Consequently, stabilizing blood glucose levels with insulin is required to minimize the size of the infarction in focal cerebral ischemic stress.

Insulin is a typical and traditional therapeutic agent for diabetes mellitus (90). We have shown the efficacy of suppressing post-ischemic hyperglycemia on the development of neuronal damage using insulin. Interestingly, suppression of elevating blood glucose levels during the first 48 h after ischemic stroke through exogenous insulin completely inhibited the development of neuronal damage (42). These results correspond with other findings showing a correlation between blood glucose levels fol-

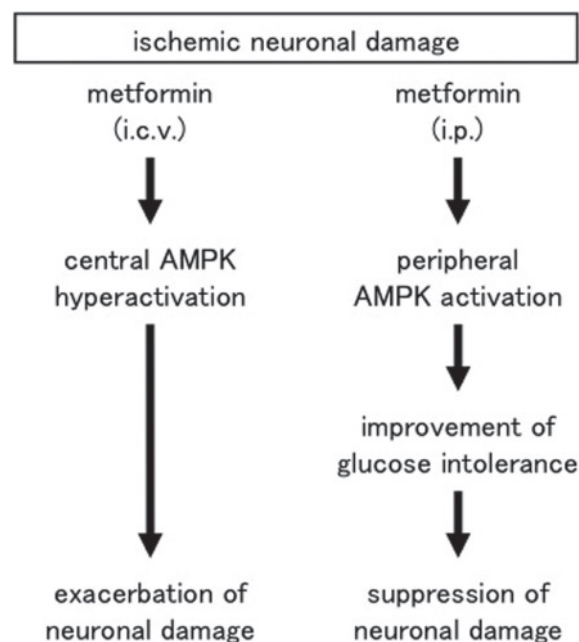


Fig. 4. Schematic illustration of the effect of peripheral or central AMPK activation on the ischemic neuronal damage using metformin. Activation of peripheral AMPK is favorable and activation of central AMPK is harmful on the development of ischemic neuronal damage.

lowing ischemic stress and the size of infarction (89).

4.2.2. Anti-diabetic drugs (metformin)

Metformin, a generic drug, is given as a monotherapy in early stages of type-2 diabetes or in combination with most of the currently available antidiabetic drugs (91). The beneficial effect of metformin on blood glucose levels appears to be a result of complex multifactorial mechanisms: a) through the activation of AMPK followed by decreased hepatic gluconeogenesis leading to reduce glucose output (92); b) increased uptake of glucose by skeletal muscles and white adipocytes (93); and c) through an enhanced metabolic profile with an additional weight reduction capacity (94). As described above, we have already confirmed that the activated state of AMPK in peripheral tissue is normally conserved regardless of whether or not cerebral ischemic stress is present (67). In turn, it is possible that the peripheral activation of AMPK after ischemic stress might suppress ischemic neuronal damage by inhibiting development of post-ischemic glucose intolerance. As expected, it is evi-

denced that administration of intraperitoneal metformin (250 mg/kg) significantly suppressed the development of post-ischemic glucose intolerance and of ischemic neuronal damage. In liver and skeletal muscle, pAMPK was upregulated by systemic metformin administration (Fig. 4); however, there was no alteration of central AMPK activity, which is known to relate to the induction of ischemic neuronal damage (67). When metformin was administrated i.c.v., ischemic neuronal damage was significantly exacerbated (67). These results suggest that the regulation of post-ischemic glucose intolerance by activation of peripheral AMPK (without affecting central AMPK) is of assistance for the suppression of cerebral ischemic neuronal damage (Fig. 4). In addition, metformin as well as insulin might be useful for the medical treatment of ischemic stroke. Importantly, according to the United Kingdom Prospective Diabetes Study 33 and 80, metformin was associated with fewer hypoglycemic attacks than insulin and sulphonylureas, indicating there may be a benefit to using metformin instead of insulin (95).

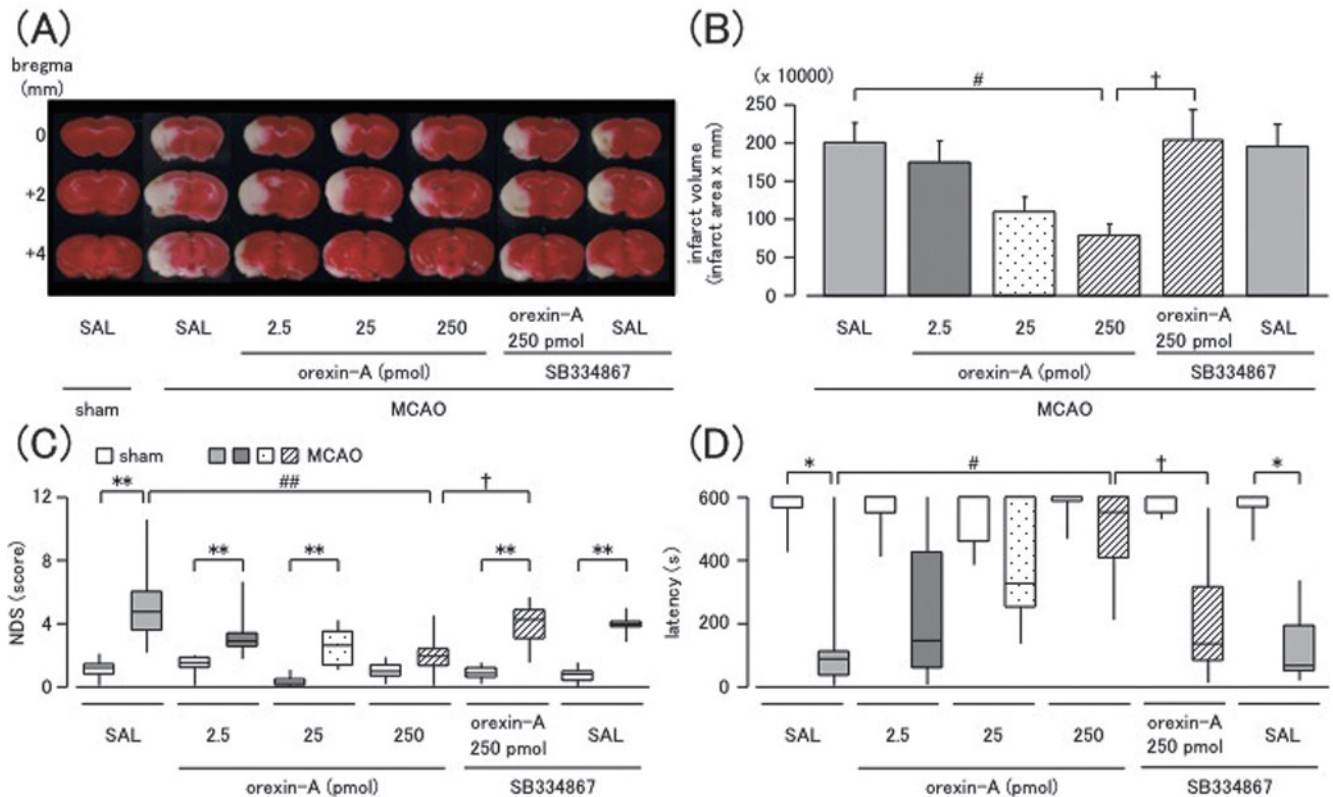


Fig. 5. Effects of orexin-A treatment on ischemic neuronal damage. Mice were treated with orexin-A (2.5, 25, and 250 pmol/mouse, i.c.v.) immediately after MCAO. A: Representative photographs of 2,3,5-triphenyltetrazolium chloride staining on day 3 after MCAO. B: Quantitative analysis of the infarct volume. Results are presented as the mean \pm S.E.M. # $P < 0.05$, † $P < 0.05$, $n = 8 - 17$. C and D: Results of the NDS and the step-through-type passive avoidance learning test on day 3 after MCAO. * $P < 0.05$, ** $P < 0.01$, # $P < 0.05$, ## $P < 0.01$, † $P < 0.05$, $n = 8 - 17$. SAL: saline. NDS: neurological deficit score. Results of panels A – D were modified from Ref. 50 with permission.

4.2.3. Endogenous neuropeptides — orexin

Recently, there has been increased demand not only for chemically synthesized compounds, but also for genetically engineered endogenous peptides for the development of therapeutic agents. A neuropeptide with great therapeutic potential for the ischemic stroke patients is orexin-A. It is known that injection of orexin-A into the VMH enhances insulin-stimulated glucose uptake and glycogen synthesis in skeletal muscle by activating the sympathetic nervous system (96). With regard to cerebral ischemia, the gene and protein expression of OX1R was reported to increase in the ischemic hemisphere after focal ischemia in rats (97). In our study, i.c.v. administration of orexin-A (2.5, 25, 250 pmol/mouse) significantly suppressed the development of neuronal damage (infarction, behavioral abnormality, and memory disturbance) on day 3 after ischemic stroke, in a dose-dependent manner (Fig. 5) (50). Furthermore, the development of post-ischemic glucose intolerance on day 1 after the MCAO was significantly suppressed by orexin-A (50). Interestingly, in liver and skeletal muscle, the MCAO-induced decrease in the expression of the InsR and increase of gluconeogenic enzymes (PEPCK and G6Pase) on day 1 after MCAO were returned to control levels by orexin-A. This suggests that orexin-A treatment may lead to the improvement of peripheral insulin sensitivity and to the suppression of post-ischemic glucose intolerance. Furthermore, the effects of orexin-A were reversed by SB334867, a specific OX1R antagonist. In the hypothalamus, orexin receptors are highly expressed in the lateral hypothalamic area (LHA), the periventricular hypothalamic nucleus, VMH, and arcuate nucleus (98, 99). These areas are involved in origins of sympathetic or parasympathetic signals to the liver or skeletal muscle (98, 99). It has been reported that the activation of LHA and VMH neurons could regulate IRS activity and glucose production in the liver and skeletal muscle via parasympathetic and sympathetic neurons, respectively (100). The i.c.v. administration of orexin-A may stimulate these pathways. Furthermore, orexin-A is reported to have neuroprotective effects through improvement of cerebral blood flow after cerebral ischemia (101). These findings provide some insight into the therapeutic effectiveness of this endogenous neuropeptide.

5. Conclusion

Within this review we have clearly demonstrated that post-ischemic glucose intolerance in the early phase of cerebral ischemic stress is one of the triggers for the development of neuronal damage. Importantly, the maintenance of normal blood glucose levels appears necessary for good clinical outcomes, and the use of anti-diabetic

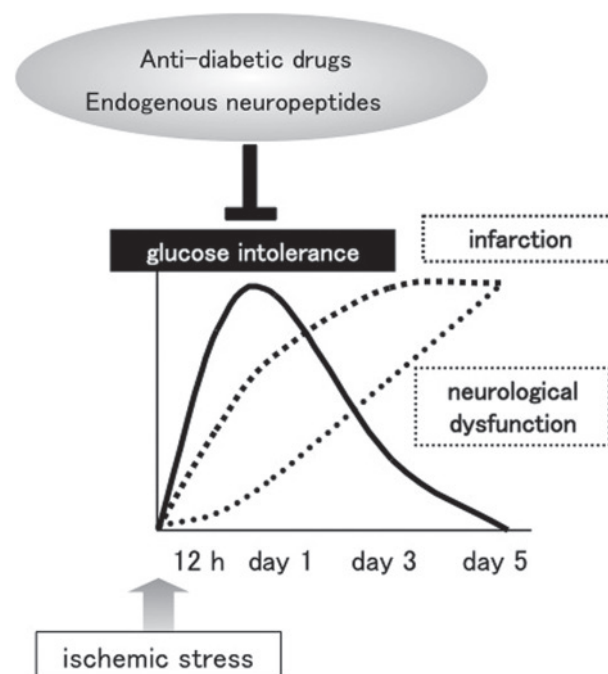


Fig. 6. Graphically-illustrated time course of development of glucose intolerance and neuronal damage after ischemic insult. The post-ischemic glucose intolerance could be one of the exacerbating factors in the development of ischemic neuronal damage. The maintenance of normal blood glucose levels by anti-diabetic drug and neuropeptide is effective for a good prognosis.

drugs and other endogenous neuropeptide may also be effective for the treatment of ischemic stroke (Fig. 6). This review provides valuable insight for the advanced treatment of ischemic stroke and adds to the existing literature about the role of post-ischemic glucose intolerance as a new therapeutic target for use in ischemic stroke patients.

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References

- 1 Moskowitz MA, Lo EH, Iadecola C. The science of stroke: mechanisms in search of treatments. *Neuron*. 2010;67:181–198.
- 2 Tan JR, Koo YX, Kaur P, Liu F, Armugam A, Wong PT, et al. microRNAs in stroke pathogenesis. *Curr Mol Med*. 2011;11: 76–92.
- 3 Davis SM, Lees KR, Albers GW, Diener HC, Markabi S, Karlsson G, et al. Selfotel in acute ischemic stroke: possible neurotoxic effects of an NMDA antagonist. *Stroke*. 2000;31: 347–354.

- 4 Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, et al. NXY-059 for the treatment of acute ischemic stroke. *N Engl J Med*. 2007;357:562–571.
- 5 Yamaguchi T, Mori E, Minematsu K, Nakagawara J, Hashi K, Saito I, et al. Alteplase at 0.6 mg/kg for acute ischemic stroke within 3 hours of onset: Japan Alteplase Clinical Trial (J-ACT). *Stroke*. 2006;37:1810–1815.
- 6 Higashi Y. Edaravone for the treatment of acute cerebral infarction: role of endothelium-derived nitric oxide and oxidative stress. *Expert Opin Pharmacother*. 2009;10:323–331.
- 7 Li J, Zeng Z, Viollet B, Ronnett GV, McCullough LD. Neuroprotective effects of adenosine monophosphate-activated protein kinase inhibition and gene deletion in stroke. *Stroke*. 2007;38:2992–2999.
- 8 Goldstein LB, Bushnell CD, Adams RJ, Appel LJ, Braun LT, Chaturvedi S, et al. Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:517–584.
- 9 Dietrich WD, Alonso O, Busto R. Moderate hyperglycemia worsens acute blood-brain barrier injury after forebrain ischemia in rats. *Stroke*. 1993;24:111–116.
- 10 Folbergrova J, Memezawa H, Smith ML, Siesjo BK. Focal and perifocal changes in tissue energy state during middle cerebral artery occlusion in normo- and hyperglycemic rats. *J Cereb Blood Flow Metab*. 1992;12:25–33.
- 11 Widmer H, Abiko H, Faden AI, James TL, Weinstein PR. Effects of hyperglycemia on the time course of changes in energy metabolism and pH during global cerebral ischemia and reperfusion in rats: correlation of ¹H and ³¹P NMR spectroscopy with fatty acid and excitatory amino acid levels. *J Cereb Blood Flow Metab*. 1992;12:456–468.
- 12 Matz K, Keresztes K, Tatschl C, Nowotny M, Dachenhausenm A, Brainin M, et al. Disorders of glucose metabolism in acute stroke patients: an underrecognized problem. *Diabetes Care*. 2006;29:792–797.
- 13 Gray CS, Scott JF, French JM, Alberti KG, O'Connell JE. Prevalence and prediction of unrecognised diabetes mellitus and impaired glucose tolerance following acute stroke. *Age Ageing*. 2004;33:71–77.
- 14 Kernan WN, Viscoli CM, Inzucchi SE, Brass LM, Bravata DM, Shulman GI, et al. Prevalence of abnormal glucose tolerance following a transient ischemic attack or ischemic stroke. *Arch Intern Med*. 2005;165:227–233.
- 15 Vancheri F, Curcio M, Burgio A, Salvaggio S, Gruttadauria G, Lunetta MC, et al. Impaired glucose metabolism in patients with acute stroke and no previous diagnosis of diabetes mellitus. *QJM*. 2005;98:871–878.
- 16 Capes SE, Hunt D, Malmberg K, Pathak P, Gerstein HC. Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview. *Stroke*. 2001;32:2426–2432.
- 17 Vriesendorp TM, Roos YB, Kruij ND, Biessels GJ, Kappelle LJ, Vermeulen M, et al. Efficacy and safety of two 5 day insulin dosing regimens to achieve strict glycaemic control in patients with acute ischaemic stroke. *J Neurol Neurosurg Psychiatry*. 2009;80:1040–1043.
- 18 Urabe T, Watada H, Okuma Y, Tanaka R, Ueno Y, Miyamoto N, et al. Prevalence of abnormal glucose metabolism and insulin resistance among subtypes of ischemic stroke in Japanese patients. *Stroke*. 2009;40:1289–1295.
- 19 Rosso C, Attal Y, Deltour S, Hevia-Montiel N, Lehericy S, Crozier S, et al. Hyperglycemia and the fate of apparent diffusion coefficient-defined ischemic penumbra. *AJNR Am J Neuroradiol*. 2011;32:852–856.
- 20 Gentile NT, Seftchick MW, Huynh T, Kruus LK, Gaughan J. Decreased mortality by normalizing blood glucose after acute ischemic stroke. *Acad Emerg Med*. 2006;13:174–180.
- 21 Pandolfi A, Giaccari A, Cilli C, Alberta MM, Morviducci L, De Filippis EA, et al. Acute hyperglycemia and acute hyperinsulinemia decrease plasma fibrinolytic activity and increase plasminogen activator inhibitor type 1 in the rat. *Acta Diabetol*. 2001;38:71–76.
- 22 Guyomard V, Jamieson EI, Myint PK. Glucose blood levels as a therapeutic target in acute ischaemic stroke setting. *Curr Top Med Chem*. 2009;9:1261–1277.
- 23 Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med*. 1995;332:1351–1362.
- 24 Chan O, Inouye K, Akirav E, Park E, Riddell MC, Vranic M, et al. Insulin alone increases hypothalamo-pituitary-adrenal activity, and diabetes lowers peak stress responses. *Endocrinology*. 2005;146:1382–1390.
- 25 van Kooten F, Hoogerbrugge N, Naarding P, Koudstaal PJ. Hyperglycemia in the acute phase of stroke is not caused by stress. *Stroke*. 1993;24:1129–1132.
- 26 Jiao H, Wang Z, Liu Y, Wang P, Xue Y. Specific role of tight junction proteins claudin-5, occludin, and ZO-1 of the blood-brain barrier in a focal cerebral ischemic insult. *J Mol Neurosci*. 2011;44:130–139.
- 27 Yan J, Zhang Z, Shi H. HIF-1 is involved in high glucose-induced paracellular permeability of brain endothelial cells. *Cell Mol Life Sci*. In press.
- 28 Wolburg H, Lippoldt A. Tight junctions of the blood-brain barrier: development, composition and regulation. *Vascul Pharmacol*. 2002;38:323–337.
- 29 Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, et al. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J Cell Biol*. 2003;161:653–660.
- 30 Kumari R, Willing LB, Patel SD, Baskerville KA, Simpson IA. Increased cerebral matrix metalloproteinase-9 activity is associated with compromised recovery in the diabetic db/db mouse following a stroke. *J Neurochem*. 2011;119:1029–1040.
- 31 Gu Y, Zheng G, Xu M, Li Y, Chen X, Zhu W, et al. Caveolin-1 regulates nitric oxide mediated matrix metalloproteinases activity and blood-brain barrier permeability in focal cerebral ischemia and reperfusion injury. *J Neurochem*. In press.
- 32 Zhang Y, Peng F, Gao B, Ingram AJ, Krepinsky JC. High glucose-induced RhoA activation requires caveolae and PKC β 1-mediated ROS generation. *Am J Physiol Renal Physiol*. In press.
- 33 Takeda S, Sato N, Uchio-Yamada K, Sawada K, Kunieda T, Takeuchi D, et al. Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and A β deposition in an Alzheimer mouse model with diabetes. *Proc Natl Acad Sci U S A*. 2010;107:7036–7041.
- 34 Shichi K, Fujita-Hamabe W, Harada S, Mizoguchi H, Yamada K, Nabeshima T, et al. Involvement of matrix metalloproteinase-mediated proteolysis of neural cell adhesion molecule in the development of cerebral ischemic neuronal damage. *J Pharmacol Exp Ther*. 2011;338:701–710.

- 35 Serra-Perez A, Verdaguer E, Planas AM, Santalucia T. Glucose promotes caspase-dependent delayed cell death after a transient episode of oxygen and glucose deprivation in SH-SY5Y cells. *J Neurochem.* 2008;106:1237–1247.
- 36 Cronberg T, Rytter A, Asztely F, Soder A, Wieloch T. Glucose but not lactate in combination with acidosis aggravates ischemic neuronal death in vitro. *Stroke.* 2004;35:753–757.
- 37 Faraco G, Fossati S, Bianchi ME, Patrone M, Pedrazzi M, Sparatore B, 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.
- 38 Diez-Sampedro A, Hirayama BA, Osswald C, Gorboulev V, Baumgarten K, Volk C, et al. A glucose sensor hiding in a family of transporters. *Proc Natl Acad Sci U S A.* 2003;100:11753–11758.
- 39 Singh V, Carman M, Roeper J, Bonci A. Brief ischemia causes long-term depression in midbrain dopamine neurons. *Eur J Neurosci.* 2007;26:1489–1499.
- 40 Turski L, Huth A, Sheardown M, McDonald F, Neuhaus R, Schneider HH, et al. ZK200775: a phosphonate quinoxalinedione AMPA antagonist for neuroprotection in stroke and trauma. *Proc Natl Acad Sci U S A.* 1998;95:10960–10965.
- 41 Vemula S, Roder KE, Yang T, Bhat GJ, Thekkumkara TJ, Abbruscato TJ. A functional role for sodium-dependent glucose transport across the blood-brain barrier during oxygen glucose deprivation. *J Pharmacol Exp Ther.* 2009;328:487–495.
- 42 Harada S, Fujita WH, Shichi K, Tokuyama S. The development of glucose intolerance after focal cerebral ischemia participates in subsequent neuronal damage. *Brain Res.* 2009;1279:174–181.
- 43 Rask-Madsen C, King GL. Endothelium-dependent delivery of insulin to muscle interstitium. *Cell Metab.* 2011;13:236–238.
- 44 Cheng Z, Guo S, Copps K, Dong X, Kolipara R, Rodgers JT, et al. Foxo1 integrates insulin signaling with mitochondrial function in the liver. *Nat Med.* 2009;15:1307–1311.
- 45 Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, et al. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. *Diabetes.* 2009;58:250–259.
- 46 Hanson RW, Reshef L. Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu Rev Biochem.* 1997;66:581–611.
- 47 Postic C, Dentin R, Girard J. Role of the liver in the control of carbohydrate and lipid homeostasis. *Diabetes Metab.* 2004;30:398–408.
- 48 Lin HV, Accili D. Reconstitution of insulin action in muscle, white adipose tissue, and brain of insulin receptor knock-out mice fails to rescue diabetes. *J Biol Chem.* 2011;286:9797–9804.
- 49 Auer RN. Insulin, blood glucose levels, and ischemic brain damage. *Neurology.* 1998;51:S39–S43.
- 50 Harada S, Fujita-Hamabe W, Tokuyama S. Effect of orexin-A on post-ischemic glucose intolerance and neuronal damage. *J Pharmacol Sci.* 2011;115:155–163.
- 51 De Souza CT, Frederico MJ, da Luz G, Cintra DE, Ropelle ER, Pauli JR, et al. Acute exercise reduces hepatic glucose production through inhibition of the Foxo1/HNF-4alpha pathway in insulin resistant mice. *J Physiol.* 2010;588:2239–2253.
- 52 Huang S, Czech MP. The GLUT4 glucose transporter. *Cell Metab.* 2007;5:237–252.
- 53 Sauvage M, Maziere P, Fathallah H, Giraud F. Insulin stimulates NHE1 activity by sequential activation of phosphatidylinositol 3-kinase and protein kinase C zeta in human erythrocytes. *Eur J Biochem.* 2000;267:955–962.
- 54 Zhao H, Hyde R, Hundal HS. Signalling mechanisms underlying the rapid and additive stimulation of NKCC activity by insulin and hypertonicity in rat L6 skeletal muscle cells. *J Physiol.* 2004;560:123–136.
- 55 Aragno M, Parola S, Brignardello E, Mauro A, Tamagno E, Manti R, et al. Dehydroepiandrosterone prevents oxidative injury induced by transient ischemia/reperfusion in the brain of diabetic rats. *Diabetes.* 2000;49:1924–1931.
- 56 Spiegelman BM, Flier JS. Obesity and the regulation of energy balance. *Cell.* 2001;104:531–543.
- 57 Antuna-Puente B, Fève B, Fellahi S, Bastard JP. Adipokines: the missing link between insulin resistance and obesity. *Diabetes Metab.* 2008;34:2–11.
- 58 Ndisang JF. Role of heme oxygenase in inflammation, insulin-signalling, diabetes and obesity. *Mediators Inflamm.* 2010;2010:359732.
- 59 Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature.* 1999;401:73–76.
- 60 Matsuzawa Y. Adiponectin: a key player in obesity related disorders. *Curr Pharm Des.* 2010;16:1896–1901.
- 61 Yamauchi T, Hara K, Kubota N, Terauchi Y, Tobe K, Froguel P, et al. Dual roles of adiponectin/Acrp30 in vivo as an anti-diabetic and anti-atherogenic adipokine. *Curr Drug Targets Immune Endocr Metabol Disord.* 2003;3:243–254.
- 62 Chen MP, Tsai JC, Chung FM, Yang SS, Hsing LL, Shin SJ, et al. Hypoadiponectinemia is associated with ischemic cerebrovascular disease. *Arterioscler Thromb Vasc Biol.* 2005;25:821–826.
- 63 Wang YY, Lin SY, Chuang YH, Chen CJ, Tung KC, Sheu WH. Adipose proinflammatory cytokine expression through sympathetic system is associated with hyperglycemia and insulin resistance in a rat ischemic stroke model. *Am J Physiol Endocrinol Metab.* 2011;300:E155–E163.
- 64 Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cell Metab.* 2005;1:15–25.
- 65 Ronnett GV, Ramamurthy S, Kleman AM, Landree LE, Aja S. AMPK in the brain: its roles in energy balance and neuroprotection. *J Neurochem.* 2009;109 Suppl 1:17–23.
- 66 Hayashi T, Hirshman MF, Kurth EJ, Winder WW, Goodyear LJ. Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes.* 1998;47:1369–1373.
- 67 Harada S, Fujita-Hamabe W, Tokuyama S. The importance of regulation of blood glucose levels through activation of peripheral 5'-AMP-activated protein kinase on ischemic neuronal damage. *Brain Res.* 2010;1351:254–263.
- 68 Yamada T, Katagiri H. Avenues of communication between the brain and tissues/organs involved in energy homeostasis. *Endocr J.* 2007;54:497–505.
- 69 Imai J, Katagiri H, Yamada T, Ishigaki Y, Suzuki T, Kudo H, et al. Regulation of pancreatic beta cell mass by neuronal signals from the liver. *Science.* 2008;322:1250–1254.
- 70 Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that

- regulate feeding behavior. *Cell*. 1998;92:573–585.
- 71 Toda C, Shiuchi T, Lee S, Yamato-Esaki M, Fujino Y, Suzuki A, et al. Distinct effects of leptin and a melanocortin receptor agonist injected into medial hypothalamic nuclei on glucose uptake in peripheral tissues. *Diabetes*. 2009;58:2757–2765.
 - 72 Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. *Diabetes*. 1999;48:287–291.
 - 73 Shiuchi T, Nakagami H, Iwai M, Takeda Y, Cui T, Chen R, et al. Involvement of bradykinin and nitric oxide in leptin-mediated glucose uptake in skeletal muscle. *Endocrinology*. 2001;142:608–612.
 - 74 Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ. Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature*. 1997;389:374–377.
 - 75 Wang JL, Chinookoswong N, Scully S, Qi M, Shi ZQ. Differential effects of leptin in regulation of tissue glucose utilization in vivo. *Endocrinology*. 1999;140:2117–2124.
 - 76 Nonomura T, Tsuchida A, Ono-Kishino M, Nakagawa T, Taiji M, Noguchi H. Brain-derived neurotrophic factor regulates energy expenditure through the central nervous system in obese diabetic mice. *Int J Exp Diabetes Res*. 2001;2:201–209.
 - 77 Harada S, Fujita-Hamabe W, Tokuyama S. Ameliorating effect of hypothalamic brain-derived neurotrophic factor against impaired glucose metabolism after cerebral ischemic stress in mice. *J Pharmacol Sci*. 2012;118:109–116.
 - 78 Krzyt ND, Biessels GJ, Devries JH, Roos YB. Hyperglycemia in acute ischemic stroke: pathophysiology and clinical management. *Nat Rev Neurol*. 2010;6:145–155.
 - 79 Edaravone Acute Infarction Study Group. Effect of a novel free radical scavenger, edaravone (MCI-186), on acute brain infarction. Randomized, placebo-controlled, double-blind study at multicenters. *Cerebrovasc Dis*. 2003;15:222–229.
 - 80 Montaner J, Molina CA, Monasterio J, Abilleira S, Arenillas JF, Ribo M, et al. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. *Circulation*. 2003;107:598–603.
 - 81 Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, et al. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med*. 2009;361:1139–1151.
 - 82 Diener HC, Connolly SJ, Ezekowitz MD, Wallentin L, Reilly PA, Yang S, et al. Dabigatran compared with warfarin in patients with atrial fibrillation and previous transient ischaemic attack or stroke: a subgroup analysis of the RE-LY trial. *Lancet Neurol*. 2010;9:1157–1163.
 - 83 Hori M, Connolly SJ, Ezekowitz MD, Reilly PA, Yusuf S, Wallentin L. Efficacy and safety of dabigatran vs. warfarin in patients with atrial fibrillation –sub-analysis in Japanese population in RE-LY trial. *Circ J*. 2011;75:800–805.
 - 84 Yamashita T, Kamiya T, Deguchi K, Inaba T, Zhang H, Shang J, et al. Dissociation and protection of the neurovascular unit after thrombolysis and reperfusion in ischemic rat brain. *J Cereb Blood Flow Metab*. 2009;29:715–725.
 - 85 Lukic-Panin V, Deguchi K, Yamashita T, Shang J, Zhang X, Tian F, et al. Free radical scavenger edaravone administration protects against tissue plasminogen activator induced oxidative stress and blood brain barrier damage. *Curr Neurovasc Res*. 2010;7:319–329.
 - 86 Kimura K, Aoki J, Sakamoto Y, Kobayashi K, Sakai K, Inoue T, et al. Administration of edaravone, a free radical scavenger, during t-PA infusion can enhance early recanalization in acute stroke patients - A preliminary study. *J Neurol Sci*. In press.
 - 87 Kanazawa M, Igarashi H, Kawamura K, Takahashi T, Kakita A, Takahashi H, et al. Inhibition of VEGF signaling pathway attenuates hemorrhage after tPA treatment. *J Cereb Blood Flow Metab*. 2011;31:1461–1474.
 - 88 Zechariah A, ElAli A, Hermann DM. Combination of tissue-plasminogen activator with erythropoietin induces blood-brain barrier permeability, extracellular matrix disaggregation, and DNA fragmentation after focal cerebral ischemia in mice. *Stroke*. 2010;41:1008–1012.
 - 89 Zhu CZ, Auer RN. Optimal blood glucose levels while using insulin to minimize the size of infarction in focal cerebral ischemia. *J Neurosurg*. 2004;101:664–668.
 - 90 Ohkubo Y, Kishikawa H, Araki E, Miyata T, Isami S, Motoyoshi S, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract*. 1995;28:103–117.
 - 91 Kahn SE, Lachin JM, Zinman B, Haffner SM, Aftiring RP, Paul G, et al. Effects of rosiglitazone, glyburide, and metformin on beta-cell function and insulin sensitivity in ADOPT. *Diabetes*. 2010;60:1552–1560.
 - 92 He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, et al. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell*. 2009;137:635–646.
 - 93 Bailey CJ, Turner RC. Metformin. *N Engl J Med*. 1996;334:574–579.
 - 94 Rojas J, Arraiz N, Aguirre M, Velasco M, Bermudez V. AMPK as target for intervention in childhood and adolescent obesity. *J Obes*. 2011;2011:252817.
 - 95 Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*. 2008;359:1577–1589.
 - 96 Shiuchi T, Haque MS, Okamoto S, Inoue T, Kageyama H, Lee S, et al. Hypothalamic orexin stimulates feeding-associated glucose utilization in skeletal muscle via sympathetic nervous system. *Cell Metab*. 2009;10:466–480.
 - 97 Irving EA, Harrison DC, Babbs AJ, Mayes AC, Campbell CA, Hunter AJ, et al. Increased cortical expression of the orexin-1 receptor following permanent middle cerebral artery occlusion in the rat. *Neurosci Lett*. 2002;324:53–56.
 - 98 Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, et al. Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol*. 2001;435:6–25.
 - 99 Trivedi P, Yu H, MacNeil DJ, Van der Ploeg LH, Guan XM. Distribution of orexin receptor mRNA in the rat brain. *FEBS Lett*. 1998;438:71–75.
 - 100 Shimazu T, Ogasawara S. Effects of hypothalamic stimulation on gluconeogenesis and glycolysis in rat liver. *Am J Physiol*. 1975;228:1787–1793.
 - 101 Kitamura E, Hamada J, Kanazawa N, Yonekura J, Masuda R, Sakai F, et al. The effect of orexin-A on the pathological mechanism in the rat focal cerebral ischemia. *Neurosci Res*. 2010;68:154–157.