Pharmacological Manipulation of Brain Glycogenolysis as a Therapeutic Approach to Cerebral Ischemia

L. Xu and H. Sun*

Center for Drug Discovery, College of Pharmacy, China Pharmaceutical University, 24 Tongjia Xiang, Nanjing 210009, China

Abstract: Brain ischemia resulting from multiple disease states including cardiac arrest, stroke and traumatic brain injury, is a leading cause of death and disability. Despite significant resources dedicated to developing pharmacological interventions, few effective therapeutic options are currently available. The basic consequence of cerebral ischemia, characterized by energy failure and subsequent brain metabolic abnormalities, enables the protective effects by pharmacological manipulation of brain metabolism. We present here the important roles of brain glycogen metabolism and propose inhibition of glycogenolysis as a therapeutic approach to cerebral ischemia.

Keywords: Brain glycogen, cerebral ischemia, glycogenolysis, inhibitors.

1. INTRODUCTION

Brain is one of the most metabolically active organs in the body, regulating all body functions and activities. The adult human brain accounts for about 20% of the body's oxygen consumption and 15% of the cardiac output in resting state [1]. Energy consumption of the brain is largely devoted to axonal and dendritic transport, intracellular signaling systems and gated natrium influx through plasma membranes [2]. Normally, glucose derived from the blood stream is the principle fuel for the brain [3]. Other substrates such as fatty acid, ketone bodies, lactate and glycogen also make contribution to brain energy metabolism [4-6]. Under conditions where supply transiently cannot meet demand, brain glycogen is rapidly depleted [7], acting as a cellular storage depot for glucose [8], either for the cells own use or for export to other cells [9].

In the adult brain, glycogen is primarily localized in astrocytes, the most abundant glial cells distributed throughout the brain [10]. Neurons have the ability to synthesize glycogen [11], but the synthesis is normally suppressed by the accumulation of the inactive muscle glycogen synthase [12]. Embryonic neural and glia tissue express glycogen but the level of neural expression drops with maturity in younger animals [13]. Astrocytes are divided into two subtypes called fibrous and protoplasmic, which are found in white and grey matter respectively [14]. Most studies find that brain glycogen levels in grey matter are about twice as much as in white matter [15]. An electron microscopic study of barbiturate-induced glycogen accumulation in brain reveals that the greatest accumulation of astrocytic glycogen occurred in areas of high synaptic density and near neuronal perikarya, suggesting a role in the energy dependent process of synaptic transmission [16].

*Address correspondence to this author at the Center for Drug Discovery, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China; Tel: +86-25-83271198; Fax: +86-25-83271198;
E-mail: hbsun2000@yahoo.com

Brain glycogen is an active pool undergoing continuous utilization and resynthesis [17]. The metabolically active glycogen content of human brain is 3–4 μmol/g [18], comparing with the muscle of ~80 μmol/g [19] and in the liver of 200–400 μmol/g [20]. Brain glycogen content is modulated by a number of factors including neurotransmitters, glucose and hormones [21-24]. The key enzymes for the synthesis and degradation of glycogen are glycogen synthase (GS) and glycogen phosphorylase (GP). GS is expressed in astrocytes and neurons with unknown role in neurons [11]. On the contrary, GP is localized predominantly in astrocytes [25, 26], consistent with the distribution of glycogen. GP is a homodimer that exists in three isoforms: the liver (LL), muscle (MM) and brain (BB) isozymes. Both of the muscle isoform and brain isoform are expressed throughout the astrocytes [26]. A relatively inactive form (GPb) and a more catalytically active form (GPa) represent the two states of the enzyme. Control of the activity of GP, and thereby of glycogenolysis, is executed rapidly by phosphorylation and allosteric ligands. Conversion from GPb to GPa is catalyzed by phosphorylase kinase, which is activated by hormonal messengers that raise cAMP or cell calcium. In addition to phosphorylation, GP activity is also regulated by glucose, ATP, glucose-6-phosphate (inhibitors) and AMP (activator) [27]. The muscle isozyme is potently activated by either phosphorylation or AMP while the brain isozyme is predominantly regulated by allosteric control *via* AMP [28].

Brain glycogen storage have a prominent effect during both physiological and pathological circumstances, by converting to lactate and passing it to adjacent neurons or axons where it is used as a fuel for brain cells [29, 30]. Although the precise functions of cerebral glycogen have not yet been firmly identified, it seems to play an important role in events like wakefulness [19, 31], hypoglycemia [32], cerebral ischemia [33, 34], defense against reactive oxygen species [35] and learning [36].

1389-5575/10 $55.00+.00

2. GLYCOGEN METABOLISM IN CEREBRAL ISCHEMIA

A continuous supply of glucose and oxygen from the blood is essential for normal brain function. An interruption of blood flow to the brain for more than ten seconds results in the loss of consciousness and leads to irreversible brain damage [37]. The primary insult that ischemia brings to the cells is glucose and oxygen deprivation, which slows or stops the synthesis of ATP through glycolysis and oxidative phosphorylation [34].

When a sudden fall in glucose and oxygen supplement occurs, the brain is protected against cerebral ischemia through a number of autonomic counterregulatory responses. There are potentially alternative substrates to glucose for both glycolysis and oxidation, such as glycogen, lactate and fatty acids [4, 34, 38]. Breakdown of glycogen is especially rapid because the initiation of glycogen catabolism through glycogen phosphorylase to produce glucose-1-phosphate is ATP-independent. Distinct from glucose, oxygen is irreplaceable for aerobic respiration, in which metabolic energy is mainly produced. Consequently, oxygen deprivation immediately induces astrocytes to switch to anaerobic glycolysis to maintain their energy metabolism, which is enhanced by the induction of glycolytic enzymes [39]. Glycolytic rates increased 4- to 7-fold in the model of experimental ischemia mice [40].

Glycogen phosphorylase, the key enzyme controlling glycogen breakdown, is phosphorylation-regulated by phosphorylase kinase. In the initial phases of brain ischemia, the extracellular potassium value rises within a few seconds following induction of ischemia, preceded by rapid Ca^{2+} flux and a fall in ATP content, which triggers the activation of phosphorylase kinase [41-43]. Brain glycogen, therefore, is degraded to meet the energy requirements. During an energy crisis, the rate of glycogenolysis in brain rises 200-fold above that in resting state [44].

Under the anoxic conditions of ischemia, glycogen is metabolized to lactate through anaerobic metabolism; thus, much less ATP is generated per glycose unit. Consequently, brain glycogen is consumed very rapidly and exhausted within 4 min during complete ischemia [40, 45]. Once glycogen content is depleted, brain function fails and may suffer irreversible injury [46].

3. ADVANTAGES OF MANIPULATION OF GLYCOGEN METABOLISM AS A THERAPEUIC APPROACH TO CEREBRAL ISCHEMIA

Knowledge about the pathogenic mechanisms of ischemic brain injury, such as glutamate mediated excitotoxicity, calcium overload, oxidative stress, has led to a host of molecular drug targets. In spite of the availability of different classes of pharmacological agents, their effective uses are limited by weak activities or side effects. The development of successful therapeutic agents against cerebral ischemia still remain challenging [47].

The handicap of energy supply, the beginning factor which leads to the subsequent metabolic disorder, plays a pivotal role in the brain damage caused by cerebral ischemia. Therefore, interference with energy metabolism *via* inhibition of glycogenolysis would be a promising approach to prophylaxis and amelioration. For prophylactic purpose, increasing brain glycogen storage does not have any obvious drawbacks for nervous system function, but could maintain brain function and retard the energy failure in the event of an ischemic episode. Another benefit of inhibiting glycogenolysis comes from the prevention of harms exerted by lactate and other intermediates generated in the over-active glycogenolysis and related glycolysis pathways.

3.1. Improvement of Glycogen Storage

Inhibition of glycogenolysis causes glycogen accumulation under normal conditions [32]. The increasing brain glycogen stores can forestall energy failure and brain injury. During severe brain ischemia, ATP levels in adult rat cerebral fall in parallel with a decrease in glycogen storage [40]. A correlation between glycogen levels and resistance to ischemia has been demonstrated experimentally. Accumulation of glycogen induced by trauma protects the brain during ischemia by serving as an endogenous source of metabolic energy [48]. Prior hypoxic exposure increases brain glycogen and delays energy depletion from a second hypoxia-ischemia in rat [49]. Moreover, the glutamine synthase inhibitor, methinionine sulfoximine (MSO), is reported to increase glycogen storage and reduce cortical infarct size in rats after middle cerebral artery occlusion [50]. Lactate, the primary glycolytic production of glycogen metabolism, can be utilized in the penumbra through aerobic pathway to preserve energy status in either astrocytes or neurons [34]. During reperfusion after transient ischemia, lactate serves as an important neuronal fuel because it is already present in the glucose-depleted brain tissue [51]. It has been suggested that the occurrence of anoxic depolarization, leading to the release of K^+ and glutamate which triggers neuronal death, is primarily dependent on the decrease of ATP from glycolysis [52]. Accordingly, pharmacological means for boosting astrocyte glycogen storage could protect against energy depletion to provide a long-term beneficial effect for brain tissue.

Besides the role as a readily mobilizable storage form of glucose, glycogen may engage in the cellular defense against reactive oxygen species. The disposal of peroxides is dependent on glutathione system. Glutathione disulfide (GSSG) reduction is catalyzed by glutathione reductase, an enzyme which needs reduced nicotinamide adenine dinucleotide phosphate (NADPH) as co-substrate. Therefore, the detoxification of peroxides is linked to the availability and the generation of NADPH [53]. NADPH can be derived from glucose-6-phosphate (G-6-P) by the pentose phosphate pathway. Serving as an endogenous precursor for, glycogen in astroglia-rich cultures exhibits accelerated mobilization after application of peroxides despite of the presence of exogenous glucose [35].

G-6-P can also be produced in an ATP-dependent pathway *via* hexokinase. However, under situations of high demand for G-6-P, supply of ATP and/or the rate of glucose uptake could limit the generation of G-6-P. In contrast to the hexokinase reaction, G-6-P generated by ATP-independent glycogen phosphorolysis might be an effective pathway, coinciding with the report that the glutathione system of per-

oxide detoxification is less efficient in neurons than in astroglial cells [54]. Consequently, increased glycogen storage may benefit the brain through detoxifying reactive oxygen.

3.2. Amelioration of Lactic Acidosis

Lactic acidosis is considered to be the main culprit in cerebral ischemia although the mechanisms still remain to be defined. Under ischemic conditions, cerebral oxidative metabolism is suppressed and the generation of ATP is primarily dependent on the glycolytic pathway. However, facing the impaired glucose oxidation, the increased glycolytic rate leads to a buildup of lactate and H^+, which causes pH to fall in the ischemic brain [55]. According to most reports, the neuronal pH of cell cytoplasm of mammals is about 6.98-7.05 [56-58]. During severe ischemia, lactate greatly increases (5–14 times) in core regions [59] and tissue pH could fall to about 6.0 simultaneously [55]. Extreme intracellular lactic acidosis, in which extracellular pH decrease to less than 6.5, may be toxic to cells and induce neuronal death [56]. Patients with larger lactate elevations relatively have larger infarcts and are left with greater neurological deficits [60].

Lactic acidosis induced damage may be mediated primarily by an intracellular Ca^{2+} overload, due to a sequence consisting of activated Na^+-H^+ exchange and resultant reversal of Na^+- Ca^{2+} exchange [61]. It is also suggested that acidosis increases intracellular Ca^{2+} by activating Ca^{2+}-permeable acid sensing ion channels (ASICs) [62, 63]. Ca^{2+} overload induced by acidosis could activate several intricate cell signal cascades that lead to greater damage, either by impairing astrocytic protection of neurons or by killing astrocytes. In focal ischemia, intracerebroventricular injection of ASIC1a blockers or knockout of the ASIC1a gene defends the brain from ischemic injury [62]. Since ASIC-mediated damage is concerned with acid, approaches able to reduce the generation of lactate could be expected to be cerebroprotective. Under ischemic conditions, brain glycogen is an important source of glycolytic substrate. Therefore, inhibition of glycogenolysis would be protective due to limited production of glycolytic substrates and would alleviate lactate overload. Maslinic acid, a glycogen phosphorylase inhibitor, is reported to reduce glycogenolysis and lactate production in cultured cortical astrocytes from cerebral tissue of ischemia rat [64]. However, this can not be the case in the presence of glucose since glycogen shunt is compensated for by an accelerated glycolytic activity when employing glycogen phosphorylase inhibitors. Pellerin *et al*. propose that glycolysis and glycogenolysis could be complementary and are activated as the intensity of activation increases [65]. Thus, lactate production, reflecting glycolytic activity, is enhanced if glycogenolysis is interfered [66].

3.3. Prevention of the Abnormal Oxidation of Catabolite in Mitochondria

The mitochondrial electron transport chain is a principal source of reactive oxygen species within cells [67]. Inhibition of mitochondrial respiration by oxygen deprivation initially interrupts respiratory chain at complex IV [68], where reduction of O_2 to H_2O takes place. It subsequently causes accumulation of reduced intermediates of the respiratory chain and transiently triggers an increase in reactive oxygen species (ROS) due to electron leakage [68]. ROS, such as hydrogen peroxide, superoxide radical, nitric oxide, and peroxynitrite, can provoke damage to lipids, DNA, and proteins, leading to neuronal death [69]. Antioxidants have been mainly used for scavenging free radicals to exert their neuroprotective effects.

When blood flow is lowered, glycolytic catabolites, with much higher levels than normal due to preferential glycolytic activity together with activation of glycogenolysis, would enter in mitochondria and experience abnormal oxidative phosphorylation. Accumulation of catabolites can then affect the efficiency of mitochondria and increase the rate of ROS production and oxygen consumption, further adding to the burden of brain under ischemic conditions. Given the fact that glycolytic catabolites originated from glycogenolysis aggravate ROS generation and oxygen consumption, inhibition of over glycogenolysis could be postulated to attenuate the production and damages of ROS and oxygen deficiency.

4. INHIBITORS OF GLYCOGENOLYSIS

4.1. Maslinic Acid

Maslinic acid (MA) (Fig. **1**) is a natural pentacyclic triterpene present in plants such as Olea europaea and hawthorn. We have first reported that MA represents a new class of GP inhibitors which bind at the allosteric activator site [70]. Enzyme kinetics assay results reveal that MA showes inhibitory activity against rabbit muscle GPa (IC_{50}=28 μM) and rat liver GPa (IC_{50}=99μM) *in vitro* [71]. It also exhibites a dose-dependent inhibitory activity against GP in homogenates of cultured cortical astrocytes with the IC_{50} values of 5.7 μM [64]. It has been previously demonstrated that inactivation of GP could not only reduce glycogenolysis but also stimulate glycogen synthesis [72]. In cultured astrocytes, MA produces a dose-dependent increase in astrocyte glycogen content after 24 hour pre-incubation and blocked glycogen degradation caused by norepinephrine treatment [64]. In a mouse model of ischemia/reperfusion, intragastric administration of MA suppresses the increase of malondialdehyde and lactate concentrations in brain tissue [73]. MA seems to exhibit a protective effect on brain ischemia model, at least in part, through inhibition of glycogen phosphorylase.

4.2. CP-316819

CP-316819 (Fig. **1**), is an indole binding site inhibitor of glycogen phosphorylase developed by Pfizer for type 2 diabetes but terminated for apparent tachyphylaxis [74]. It inhibits human liver GPa and human muscle GPa with IC_{50} values of 47nM and 105 nM respectively [75]. In human liver-derived SK-HEP-1 cells, cell- based IC_{50} value for inhibition of glycogenolysis is 0.56μM [76]. It is reported that rats treated with CP-316819 have an 88 ± 3% increase in brain glycogen content. When subjected to hypoglycemia, these rats maintain brain electrical activity 91 ± 14 min longer than those with normal brain glycogen levels and show markedly reduced neuronal death. As an indole carboximide inhibitor of glycogen phosphorylase, CP-316819 increases brain glycogen storage but does not prevent glycogen utilization during 30 min of complete brain ischemia [32]. CP-316819 administration not only lowers lactate efflux but

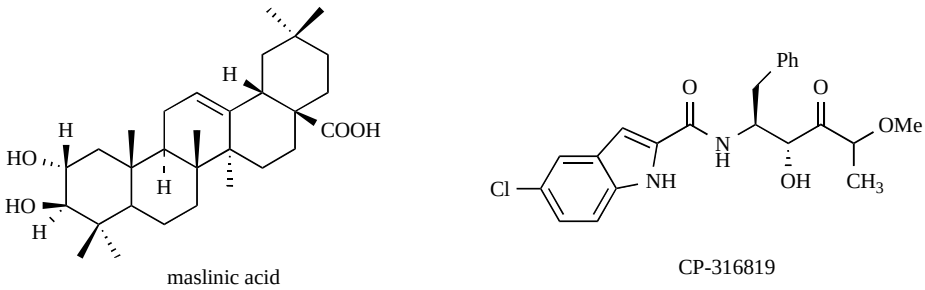

Fig. (1). Structures of maslinic acid and CP-316819.

also unaffectes muscle function during short-term maximal and submaximal intensity contraction, despite the impairment of prolonged low-intensity contraction due to the inhibition of muscle glycogen phosphorylase [77]. These results suggest the potential protective effects of CP-316819 on ischemic brain which has a high energy demand like the conditions just as hypoglycemia and intensity exercise.

4.3. Magnesium Sulfate

Magnesium ion is an important cathodolyte, participating in organic metabolism as prosthetic group or cofactor of many enzymes. Magnesium shows a neuroprotective effect in several models of cerebral ischaemia [78]. Low levels of serum magnesium are found in patients shortly after a stroke has occurred [79]. Although the reason of such deficiency and the benefit for magnesium administration as a therapeutic intervention are still unclear, a number of possible mechanisms have been suggested, including noncompetitive blockade of N-methyl-D-aspartate (NMDA) receptors [80], antagonist of calcium channel [81] and modulation of brain energy metabolism [82].

Uridine diphosphate glucose (UDP-glc), an important substrate in glycogen synthesis, is a product generated from the reaction of glucose-1-phosphate with uridine triphosphate (UTP) catalyzed by glucose-1-phosphate uridyltransferase (UGPase) [83]. X-ray structure of UGPase in the presence of magnesium and UDP-glc showes magnesium to bind at active site, supporting the role of the conserved magnesium ion related to glycogen synthesis [84]. Levin *et al.* [82] report that excess magnesium also inhibits glycogenolysis and lactate efflux. Magnesium sulfate treatment improves the electroencephalogram (EEG) and decreases the levels of malondialdehyde and lactate after cerebral ischemia in rabbits [85]. Gerbils subjected to focal cerebral ischemia with the pretreatment of magnesium sulfate demonstrate significant attenuation in the elevation of lactate (150% of the baseline) and decrease in infarct size compared to the controls [86]. These results suggest that the neuroprotective actions of magnesium sulfate might be related to amelioration of acidosis by inhibiting glycogenolysis.

CONCLUSION

Glycogen is likely a viable and important glucose reservoir, whose metabolism is involved in physiological and pathological conditions. Strategies for direct inhibition of hepatic glycogenolysis have been proposed as a potential approach to diabetic therapy, while the role of brain glycogen has been universally dismissed partially due to its relatively low concentration. Accompanied with the influence of ischemia on brain metabolism extensively being investigated, the substantial involvement of brain glycogen metabolic abnormality in the development of cerebral ischemia has been suggested. Consequently, pharmacological interference with disorders in brain glycogen metabolism sheds some light in cerebral ischemia therapy. However, more *in vitro* and *in vivo* studies are warranted to verify the efficacy and safety of this therapeutic approach based on manipulation of brain glycogen metabolism under cerebral ischemia. Further basic studies on the function and regulation of brain glycogen are urged to provide valuable insight into this new therapeutic approach.

ACKNOWLEDGEMENTS

Some research work was made possible by grants supported by National Natural Science Foundation of China (grants 30672523 and 90713037).

REFERENCES

[1] Buzsáki, G.; Kaila, K.; Raichle, M. Inhibition and brain work. *Neuron*, **2007**, *56*, 771-783.

[2] Ames, A. CNS energy metabolism as related to function. *Brain Res. Rev.*, **2000**, *34*, 42-68.

[3] McCall, A.L. Cerebral glucose metabolism in diabetes mellitus. *Eur. J. Pharmacol.*, **2004**, *490*, 147-158.

[4] Ebert, D.; Haller,R.G.; Walton, M.E. Energy contribution of octanoate to intact rat brain metabolism measured by ^{13}C nuclear magnetic resonance spectroscopy. *J. Neurosci.*, **2003**, *23*, 5928-5935.

[5] Prins, M.L. Cerebral metabolic adaptation and ketone metabolism after brain injury. *J. Cereb. Blood Flow Metab.*, **2008**, *28*, 1-16.

[6] Ransom, B.R.; Fern, R. Does astrocytic glycogen benefit axon function and survival in CNS white matter during glucose deprivation? *Glia*, **1997**, *21*, 134-141.

[7] Rolf, G. Glycogen: The forgotten cerebral energy store. *J. Neurosci. Res.*, **2003**, *74*, 179-183.

[8] Öz, G.; Kumar, A.; Rao, J.P.; Kodl, C.T.; Chow, L.; Eberly, L.E.; Seaquist, E.R. Human Brain Glycogen Metabolism During and After Hypoglycemia. *Diabetes*, **2009**, *58*, 1978-1985.

[9] Claudia, Z.; Dieter, L. Regulation of glial metabolism studied by ^{13}C-NMR. *NMR Biomed.*, **2003**, *16*, 370-399.

[10] Morgenthaler, F.D.; Koski, D.M.; Kraftsik, R.; Henry, P.G.; Gruetter, R. Biochemical quantification of total brain glycogen concentration in rats under different glycemic states. *Neurochem. Int.*, **2006**, *48*, 616-622.

[11] Pellegri, G.; Rossier, C.; Magistretti, P.J.; Martin, J.L. Cloning, localization and induction of mouse brain glycogen synthase. *Mol. Brain Res.*, **1996**, *38*, 191-199.

[12] Vilchez, D.; Ros, S.; Cifuentes, D.; Pujadas, L.; Vallès, J.; García-Fojeda, B.; Criado-García, O.; Fernández-Sánchez, E.; Medrano-Fernández, I.; Domínguez, J.; García-Rocha, M.; Soriano, E.; Rodríguez de Córdoba, S.; Guinovart, J. J. Mechanism suppressing

glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. *Nat. Neurosci.,* **2007**, *10*, 1407-1413.

[13] Magistretti, P.J., Sorg, O., Martin, J.L. Regulation of glycogen metabolism in astrocytes: physiological, pharmacological and pathological aspects. In *Astrocytes: Pharmacology and Function;* Murphy, S., Ed; Academic Press, San Diego, **1993**; pp. 243-265.

[14] García-Marína, V.; García-Lópeza, P.; Freire, M. Cajal's contributions to glia research. *Trends Neurosci.,* **2007**, *30*, 479-487.

[15] Brown, A.M.; Ransom, B.R. Astrocyte glycogen and brain energy metabolism. *Glia*, **2007**, *55*, 1263-1271.

[16] Phelps, C.H. Barbiturate-induced glycogen accumulation in brain. An electron microscopic study. *Brain Res.,* **1972**, *39*, 225-234.

[17] Swanson, R.A. Physiologic coupling of glial glycogen metabolism to neuronal activity in brain. *Can. J. Physiol. Pharmacol.,* **1992**, *70 (Suppl)*, S138-144.

[18] Öz, G.; Seaquist, E.R.; Kumar, A.; Criego, A.B.; Benedict, L.E.; Rao, J.P.; Henry, P.G.; Van De Moortele, P.F.; Gruetter, R. Human brain glycogen content and metabolism: implications on its role in brain energy metabolism. *Am. J. Physiol. Endocrinol. Metab.,* **2007**, *292*, 946-951.

[19] Kong, J.; Shepel, P.N.; Holden, C.P.; Mackiewicz, M.; Pack, A.I.; Geiger, J.D. Brain glycogen decreases with increased periods of wakefulness: Implications for homeostatic drive to sleep. *J. Neurosci.,* **2002**, *22*, 5581-5587.

[20] Roden, M.; Petersen, K.F.; Shulman, G. Nuclear Magnetic Resonance Studies of Hepatic Glucose Metabolism in Humans. *Recent Prog. Horm. Res.,* **2001**, *56*, 219-238.

[21] Mellerup, E.T.; Rafaelsen, O.J. Brain glycogen after intracisternal insulin injection. *J. Neurochem.,* **1969**, *16*, 777-781.

[22] Pellerin, P.; Stolz, M.; Sorg, O.; Martin, J.L.; Deschepper, C.F.; Magistretti, P.J. Regulation of energy metabolism by neurotransmitters in astrocytes in primary culture and in an immortalized cell line. *Glia,* **1997**, *21*, 74-83.

[23] Quach, T.T.; Rose, C.; Schwartz, J.C. [^{3}H]Glycogen hydrolysis in brain slices: responses to neurotransmitters and modulation of noradrenalines receptors. *J. Neurochem.,* **1978**, *30*, 1335-1341.

[24] Cardinaux, J.R.; Magistretti, P.J. Vasoactive intestinal peptide, pituitary adenylate cyclase-activating peptide, and noradrenaline induce the transcription factors CCAAT/enhancer binding protein (C/EBP)-beta and C/EBP delta in mouse cortical astrocytes: involvement in cAMP-regulated glycogen metabolism. *J. Neurosci.,* **1996**, *16*, 919-929.

[25] Ignacio, P.C.; Baldwin, B.A.; Vijayan, V.K.; Tait, R.C.; Gorin, F.A. Brain isozyme of glycogen phosphorylase: immunohistological localization within the central nervous system. *Brain Res.,* **1990**, *529*, 42-49.

[26] Pfeiffer-Guglielmi, B.; Fleckenstein, B.; Jung, G.; Hamprecht, B. Immunocytochemical localization of glycogen phosphorylase isozymes in rat nervous tissues by using isozyme-specific antibodies. *J. Neurochem.,* **2003**, *85*, 73-81.

[27] Agius, L. New hepatic targets for glycaemic control in diabetes. *Best Pract. Res. Clin. Endocrinol. Metab.,* **2007**, *21*, 587-605.

[28] Crerar, M.M.; Karlsson, O.; Flettrick, R.J.; Hwang, P.K. Chimeric muscle and brain glycogen phosphrylases define protein domain governing isozyme-specific responses to allosteric activation. *J. Biol. Chem.,* **1995**, *270*, 13748-13756.

[29] Shulman, R.G.; Hyder, F.; Rothman, D.L. Cerebral energetics and the glycogen shunt: neurochemical basis of functional imaging. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 6417-6422.

[30] Tekkok, S.B.; Brown, A.M.; Westenbroek, R.; Pellerin, L.; Ransom, B.R. Transfer of glycogen-derived lactate from astrocytes to axons *via* specific monocarboxylate transporters supports mouse optic nerve activity. *J. Neurosci. Res.,* **2005**, *81*, 644-652.

[31] Morgenthaler, F.D.; Lanz, B.R.; Petit, J.M.; Frenkel, H.; Magistretti, P.J.; Gruetter, R. Alteration of brain glycogen turnover in the conscious rat after 5h of prolonged wakefulness. *Neurochem. Int.,* **2009**, *55*, 45-51.

[32] Suh, S.W.; Bergher, J.P.; Anderson, C.M.; Treadway, J.L.; Fosgerau, K.; Swanson, R.A. Astrocyte glycogen sustains neuronal activity during hypoglycemia: studies with the glycogen phosphorylase inhibitor CP-316,819 ([R-R*,S*]-5-chloro-N-[2-hydroxy-3-(methoxymethylamino)-3-oxo-1-(phenylmethyl)propyl]-1H-indole-2-carboxamide). *J. Pharmacol. Exp. Ther.,* **2007**, *321*, 45-50.

[33] Gerald, A.D.; Leif, H. Astrocytic contributions to bioenergetics of cerebral ischemia. *Glia,* **2005**, *50*, 362-388.

[34] Rossi, D.J.; Brady, J.D.; Mohr, C. Astrocyte metabolism and signaling during brain ischemia. *Nat. Neurosci.,* **2007**, *10*, 1377-1386.

[35] Rahman, B.; Kussmaul, L.; Hamprecht, B.; Dringen, R. Glycogen is mobilized during the disposal of peroxides by cultured astroglial cells from rat brain. *Neurosci. Lett.,* **2000**, *290*, 169-172.

[36] Marie, E.G.; Damian, G.A.; Leif, H. Inhibition of glycogenolysis in astrocytes interrupts memory consolidation in young chickens. *Glia*, **2006**, *54*, 214-222.

[37] Raichle, M.E. The pathophysiology of brain ischemia. *Ann. Neurol.,* **1983**, *13*, 2-10.

[38] Hertz, L. Bioenergetics of cerebral ischemia: a cellular perspective. *Neuropharmacology*, **2008**, *55*, 289-309.

[39] Niitsu, Y.; Hori, O.; Yamaguchi, A.; Bando, Y.; Ozawa, K.; Tamatani, M.; Ogawa, S.; Tohyama, M. Exposure of cultured primary rat astrocytes to hypoxia results in intracellular glucose depletion and induction of glycolytic enzymes. *Mol. Brain Res.,* **1999**, *74*, 26-34.

[40] Lowry, O.H.; Passonneau, J.V.; Hasselberger, F.X.; Schulz, D.W. Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J. Biol. Chem.*, **1964**, *239*, 18-30.

[41] Folbergrov, J.; Minamisawa, H.; Ekholm, A.; Siesj, B.K. Phosphorylase a and labile metabolites during anoxia: correlation to membrane fluxes of K^+ and Ca^{2+}. *J. Neurochem.,* **1990**, *55*, 1690-1696.

[42] Subbarao, K.V.; Stolzenburg, J.U.; Hertz, L. Pharmacological characteristics of potassium-induced glycogenolysis in astrocytes. *Neurosci. Lett.,* **1995**, *196*, 45-48.

[43] Robert, A.G.; James, A.F. Mechanisms of cyclic AMP regulation in cerebral anoxia and their relationship to glycogenolysis. *J. Neurochem.*, **1980**, *34*, 1309-1318.

[44] Dienel, G.A.; Cruz, N.F. Astrocyte activation in working brain: energy supplied by minor substrates. *Neurochem. Int.,* **2006**, *48*, 586-595.

[45] Swanson, R.A.; Sagar, S.M.; Sharp, F.R. Regional brain glycogen stores and metabolism during complete global ischaemia. *Neurol. Res.,* **1989**, *11*, 24-28.

[46] Auer, R.N. Progress review: hypoglycemic brain damage. *Stroke*, **1986**, *17*, 699-708.

[47] Zaleska, M.M.; Mercado, M.L.T.; Chavez, J.; Feuerstein, G.Z.; Pangalos, M.N.; Wood, A. The development of stroke therapeutics: Promising mechanisms and translational challenges. *Neuropharmacology*, **2009**, *56*, 329-341.

[48] Otori, T.; Friedland, J.C.; Sinson, G.; McIntosh, T.K.; Raghupathi, R.; Welsh, F.A. Traumatic brain injury elevates glycogen and induces tolerance to ischemia in rat brain. *J. Neurotrauma*, **2004**, *21*, 707-718.

[49] Brucklacher, R.M.; Vannucci, R.C.; Vannucci, S.J. Hypoxic preconditioning increases brain glycogen and delays energy depletion from hypoxia-ischemia in the immature rat. *Dev. Neurosci.,* **2002**, *24*, 411-417.

[50] Swanson, R.A.; Shiraishi, K.; Morton, M.T.; Sharp, F.R. Methionine sulfoximine reduces cortical infarct size in rats after middle cerebral artery occlusion. *Stroke*, **1990**, *21*, 322-327.

[51] Schurr, A.; Payne, R.S.; Miller, J.J.; Tseng, M.T.; Rigor, B.M. Blockade of lactate transport exacerbates delayed neuronal damage in a rat model of cerebral ischemia. *Brain Res.,* **2001**, *895*, 268-272.

[52] Allen, N.J.; Karadottir, R.; Attwell, D. A preferential role for glycolysis in preventing the anoxic depolarization of rat hippocampal area CA1 pyramidal cells. *J. Neurosci.,* **2005**, *25*, 848-859.

[53] Ralf, D.; Petra, G.P.; Johannes, H. Peroxide detoxification by brain cells. *J. Neurosci. Res.,* **2005**, *79*, 157-165.

[54] Dringen, R.; Kussmaul, L.; Gutterer, J.M.; Hirrlinger, J.; Hamprecht, B. The glutathione system of peroxide detoxification is less efficient in neurons than in astroglial cells. *J. Neurochem.,* **1999**, *72*, 2523-2530.

[55] Rehncrona, S. Brain acidosis. *Ann. Emerg. Med.,* **1985**, *14*, 770-776.

[56] Isaev, N.K.; Stelmashook, E.V.; Plotnikov, E.Y.; Khryapenkova, T.G.; Lozier, E.R.; Doludin, Y.V.; Silachev, D.N.; Zorov, D.B. Role of acidosis, NMDA receptors, and acid-sensitive ion channel 1a (ASIC1a) in neuronal death induced by ischemia. *Biochemistry (Mosc),* **2008**, *73*, 1171-1175.

[57] Nedergaard, M.; Goldman, S.A.; Desai, S.; Pulsinelli, W.A. Acid-induced death in neurons and glia. *J. Neurosci.,* **1991**, *11*, 2489-2497.

[58] Tomlinson, F.H.; Anderson, R.E.; Meyer, F.B. Acidic foci within the ischemic penumbra of the New Zealand white rabbit. *Stroke*, **1993**, *24*, 2030-2039.

[59] Sims, N.R.; Anderson, M.F. Mitochondrial contributions to tissue damage in stroke. *Neurochem. Int.*, **2002**, *40*, 511-526.

[60] Graham, G.D.; Blamire, A.M.; Howseman, A.M.; Rothman, D.L.; Fayad, F.B.; Brass, L.M.; Petroff, O.A.C.; Shulman, R.G.; Prichard, J.W. Proton magnetic resonance spectroscopy of cerebral lactate and other metabolites in stroke patients. *Stroke*, **1992**, *23*, 333-340.

[61] Chesler, M. Failure and function of intracellular pH regulation in acute hypoxic-ischemic injury of astrocytes. *Glia*, **2005**, *50*, 398-406.

[62] Xiong, Z.G.; Zhu, X.M.; Chu, X.P.; Minami, M.; Hey, J.; Wei, W.L.; MacDonald, J.F.; Wemmie, J.A.; Price, M.P.; Welsh, M.J.; Simon, R.P. Neuroprotection in ischemia: blocking calcium-permeable acid-sensing ion channels. *Cell*, **2004**, *118*, 687-698.

[63] Yermolaieva, O.; Leonard, A. S.; Schnizler, M.K.; Abboud, F.M.; Welsh, M.J. Extracellular acidosis increases neuronal cell calcium by activating acid-sensing ion channel 1a. *Proc. Natl. Acad. Sci. U.S.A.*, **2004**, *101*, 6752-6757.

[64] Guan, T.; Li, Y.M.; Sun, H.B.; Tang, X.Z.; Qian, Y.S. Effects of maslinic acid, a natural triterpene, on glycogen metabolism in cultured cortical astrocytes. *Planta Med.*, **2009**, *75*, 1141-1143.

[65] Pellerin, L.; Bouzier-Sore, A.K.; Aubert, A.; Serres, S.; Merle, M.; Costalat, R.; Magistretti, P.J. Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia.*, **2007**, *55*, 1251-1262.

[66] Walls, A.B.; Heimburger, C.M.; Bouman, S.D.; Schousboe, A.; Waagepetersen, H.S. Robust glycogen shunt activity in astrocytes: Effects of glutamatergic and adrenergic agents. *Neuroscience.* **2009**, *158*, 284-292.

[67] Walker, D.W.; Hájek, P.; Muffat, J.; Knoepfle, D.; Cornelison, S.; Attardi, G.; Benzer, S. Hypersensitivity to oxygen and shortened lifespan in a Drosophila mitochondrial complex II mutant. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 16382-16387.

[68] Abramov, A.Y.; Scorziello, A.; Duchen, M.R. Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. *J. Neurosci.*, **2007**, *27*, 1129-1138.

[69] Moro, M.A.; Almeida, A.; Bolanos, J.P.; Lizasoain, I. Mitochondrial respiratory chain and free radical generation in stroke. *Free Radic. Biol. Med.*, **2005**, *39*, 1291-1304.

[70] Wen, X.A.; Sun, H.B.; Liu, J.; Zhang, P.; Zhang, L.Y.; Hao, J.; Zhang, L.Y.; Ni, P.Z.; Zographos, S.E.; Leonidas, D.D.; Alexacou, KM.; Gimisis, T.; Hayes, J.M.; Oikonomakos, N.G. Naturally Occurring Pentacyclic Triterpenes as Inhibitors of Glycogen Phosphorylase: Synthesis, Structure-Activity Relationships, and X-ray Crystallographic Studies. *J. Med. Chem.*, **2008**, *51*, 3540–3554.

[71] Wen, X.A.; Zhang, P.; Liu, J.; Zhang, L.Y.; Wu, X.M.; Ni, P.Z.; Sun, H.B. Pentacyclic triterpenes. Part 2: Synthesis and biological evaluation of maslinic acid derivatives as glycogen phosphorylase inhibitors. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 722-726.

[72] Aiston, S.; Coghlan, M.P.; Agius, L. Inactivation of phosphorylase is a major component of the mechanism by which insulin stimulates hepatic glycogen synthesis. *Eur. J. Biochem.*, **2003**, *270*, 2773-2781.

[73] Guan, T.; Li, Y.M.; Sun, H.B. Effects of maslinic acid as a novel glycogen phosphorylase inhibitor on cerebral ischemia in mice(in Chinese). *Chin. J. Clin. Pharmacol Ther.*, **2007**, *12*, 381-384.

[74] van Poelje, P.D.; Dang, Q.; Erison, M.D. Fructose-1,6-bisphosphatase as a therapeutic target for type 2 diabetes. *Drug Discov. Today*, **2007**, *4*, 103-109.

[75] Henke, B.R.; Sparks, S.M. Glycogen phosphorylase inhibitors. *Mini-Rev. Med. Chem.*, **2006**, *6*, 845-857.

[76] Ercan-Fang, N.; Treadway, J.L.; Levy, C.B.; Genereux, P.E.; Gibbs, E.M.; Rath, V.L.; Kwon, Y.; Gannon, M.C.; Nuttall, F.Q. Endogenous effectors of human liver glycogen phosphorylase modulate effects of indole-site inhibitors. *Am. J. Physiol. Endocrinol. Metab.*, **2005**, *289*, E366-372.

[77] Baker, D.J.; Greenhaff, P.L.; Maclnnes, A.; Timmons, J.A. The experimental type 2 diabetes therapy glycogen phosphorylase inhibition can impair aerobic muscle function during prolonged contraction. *Diabetes*, **2006**, *55*, 1855-1861.

[78] Vink, R.; Cook, N.L. van den Heuvel, C. Magnesium in acute and chronic brain injury: an update. *Magnes. Res.*, **2009**, *22*, 158S-162S.

[79] Altura, B.T.; Memon, Z.I.; Zhang, A.M.; Cheng, T.P.O.; Silverman, R.; Cracco, R.Q.; Altura, B.M. Low levels of serum ionized magnesium are found in patients early after stroke which result in rapid elevation in cytosolic free calcium and spasm in cerebral vascular muscle cells. *Neurosci. Lett.*, **1997**, *230*, 37-40.

[80] McDonald, J.W.; Silverstein, F.S.; Johnston, M.V. Magnesium reduces N-methyl-d-aspartate (NMDA)-mediated brain injury in perinatal rats. *Neurosci. Lett.*, **1990**, *109*, 234-238.

[81] Iseri, L.T.; French, J.H. Magnesium: Nature's physiologic calcium blocker. *Am. Heart J.*, **1984**, *108*, 188-193.

[82] Levin, R.M.;Haugaard, N.; Hess, M.E. Opposing actions of calcium and magnesium ions on the metabolic effects of epinephrine in rat heart. *Biochem. Pharmacol.*, **1976**, *25*, 1963-1969.

[83] Zea, C.J.; Camci-Unal, G.; Pohl, N.L. Thermodynamics of binding of divalent magnesium and manganese to uridine phosphates: implications for diabetes-related hypomagnesaemia and carbohydrate biocatalysis. *Chem. Cent. J.*, **2008**, *2*, 1-7.

[84] Thoden, J.B.;Holden, H.M. Active site geometry of glucose-1-phosphate uridylyltransferase. *Protein Sci.*, **2007**, *16*, 1379-1388.

[85] Bariskaner, H.; Ustun, M.E.; Ak, A.; Yosunkaya, A.; Ulusoy, H.B.; Gurbilek, M. Effects of magnesium sulfate on tissue lactate and malondialdehyde levels after cerebral ischemia. *Pharmacology*, **2003**, *68*, 162-168.

[86] Lin, J.Y.; Chung, S.Y.; Lin, M.C.; Cheng, F.C. Effects of magnesium sulfate on energy metabolites and glutamate in the cortex during focal cerebral ischemia and reperfusion in the gerbil monitored by a dual-probe microdialysis technique. *Life Sci.*, **2002**, *71*, 803-811.

Received: April 04, 2010 Revised: July 13, 2010 Accepted: July 15, 2010