

Review Article

Glycogen as a Putative Target for Diagnosis and Therapy in Brain Pathologies

Jean-François Cloix and Tobias Hévor

Neurobiology Laboratory, University of Orléans, Chartres Street, 45067 Orléans Cedex 2, France

Correspondence should be addressed to Jean-François Cloix, jean-francois.cloix@univ-orleans.fr

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Brain glycogen, a glucose polymer, is now considered as a functional energy store to the brain. Indeed, when neurons outpace their own possibilities to provide themselves with energy, astrocytic metabolism is in charge of feeding neurons, since brain glycogen synthesis is mainly due to astrocyte. Therefore, malfunctions or perturbations of astrocytic glycogen content, synthesis, or mobilization may be involved in processes of brain pathologies. This is the case, for example, in epilepsies and gliomas, two different situations in which, brain needs high level of energy during acute or chronic conditions. The purpose of the present paper is to demonstrate how brain glycogen might be relevant in these two pathologies and to pinpoint the possibilities of considering glycogen as a tool for diagnostic and therapeutic approaches in brain pathologies.

1. Introduction

According to the present understanding of blood-brain barrier (BBB) and the contribution of astrocyte end-feet to the BBB, the nutrients coming from blood stream to brain have to cross various barriers before reaching neurons. The first of them is the BBB itself, that is to say, the endothelial cells of the capillaries, whose cell contacts are constituted by tight junctions, which limited the mass of compounds that can cross over the BBB to about 1 kDa; otherwise, compounds can pass BBB through cell capillaries themselves. After nutrients have crossed BBB, the second barrier they have to pass is extensions of astrocytes, named end-feet, which contribute to BBB, whilst no tight junction exists between end-feet of astrocytes themselves and with other cells. However, considering the possibilities of the presence of nutrients in the extracellular spaces, such nutrients may directly enter target cells through specific transporters. Nevertheless, we consider that most of these nutrients have to enter astrocyte end-feet before reaching cells that need them.

Among nutrients needed by the brain for its functions, glucose is one of the most important, as it constitutes the basis of various metabolisms and energy production that

brain, and particularly neurons, need. Moreover, the brain is largely dependent upon glucose. Indeed when glucose supply to human brain is depressed, the brain cannot survive more than 3–6 minutes. The brain side of capillaries is covered by end-feet of astrocytes. Therefore, any nutrients that passed the BBB thereafter might enter astrocytes end-feet first. However, glucose can be detected in extracellular spaces [1–6] indicating that not all glucose enters astrocyte end-feet. Nevertheless, in the present approach, only glucose entering astrocyte end-feet will be considered. Glucose transporters, GLUT proteins, deliver glucose from the circulation to the brain. The microvascular endothelial cells of the BBB and astrocytes contain GLUT1, while neurons contain both GLUT1 and GLUT3. GLUT1 exists as two different molecular weight forms (45 and 55 kDa). The difference in their relative molecular weight is accounted for by a differing extent of glycosylation [7]. The 55 kDa GLUT1 is exclusively found in endothelial cells of BBB [8–11]. The 45 kDa form of GLUT1 is found in all glial cells [8, 12, 13], as well as the basolateral and apical membranes of the choroid plexus, and ependyma. Under normal conditions there is very limited *in vivo* expression of 45 kDa GLUT1 in neurons [14–17]. GLUT3 has been localized in the brain almost exclusively to neurons

[13, 18–20]. Nevertheless, the still open question whether glucose enters into neurons directly or whether astrocytes metabolize glucose to feed neurons is not solved yet. Even if small amount of glucose enters neurons as glucose could be indeed detected in extracellular spaces of brain [1–6] and even if neurons contain GLUT1 and GLUT3, it seems quite probable that astrocytes metabolism largely contributes to feeding neurons. To summarize, glucose from blood stream passes the BBB essentially using glucose transporters of capillary cells, that is to say, through cells not through tight junctions that exist between capillary cells [21–24]. Astrocytes get glucose from blood stream through capillary cells and metabolize it down to lactate, which is provided to neurons in which it is used to produce ATP by mitochondria [25–29].

2. Glucose Metabolism

As soon as glucose enters brain cells it is transformed to glucose-6-phosphate (G-6-P) by hexokinase. Thereafter, G-6-P cannot return to extracellular spaces due to its negative charge. G-6-P is then used for cellular metabolism such as glycolysis for energy production. The excess of G-6-P produced by hexokinase or coming from gluconeogenesis might be polymerized into glycogen (Figure 1), which is the glucose store of mammalian cells [30–32]. The G-6-P is first transformed to glucose-1-phosphate (G-1-P), which is further processed to UDP-glucose that is incorporated into preexisting glycogen, the latter step being catalyzed by glycogen synthase (GS). When cell needs energy, it can use the glucose store by depolymerizing glycogen using glycogen phosphorylase (GP), an enzyme that produces G-1-P from glycogen (Figure 1). Glycogen synthesis is a very complex process involving various steps, enzymes and regulations. The step one consists of polymerization of the first 8–10 glucosyl units, step performed by glycogenin [33–36]. Thereafter, GS is in charge of continuing the polymerization process in association with branching enzyme [32, 37]. These steps result in a very large molecule of glycogen corresponding to glucose units associated with both α -1,4 and α -1,6 bounds. Depolymerization of glycogen is performed essentially by GP and debranching enzyme, very efficient enzymes responsible for hydrolyzing of the α -1,4 and α -1,6 bounds. A regulatory protein of an enzymatic complex called protein targeting to glycogen (PTG) has been described [38–40]. It is a huge complex constituted of the PTG itself in association with GS, GP, and phosphoprotein phosphatase 1a (PP1a). Therefore, GS and GP activities are modulated by PTG, which is a regulative subunit of PP1a phosphatase that controlled GS and GP activities by their dephosphorylation, activating GS and inhibiting GP [32].

3. Astrocytes as Energy Providers to Neurons

As already mentioned, BBB constitutes the first barrier that blood glucose and other brain nutrients have to cross before entering brain. Therefore, glucose must cross the endothelial cell of capillaries and the astrocyte end-feet before reaching

neurons. The question of glucose entering neurons is still pending [22, 24, 27, 29, 42–44] as neurons contain GLUT3 [9, 12, 13, 18–20], while they have not direct contacts with capillaries. However, glucose is observable in extracellular space inside the brain [1–6] suggesting a putative direct entry of glucose in neurons through GLUT3. However, it seems likely that glucose enters astrocytes in which it is metabolized to lactate through glycolysis pathway, and astrocytes provide neurons with lactate as fuel [27, 29, 42–45]. Lactate is then transformed to pyruvate, which is used by mitochondria to produce ATP (Figure 1), leading to a substantial energy budget economy for neurons.

Two main possibilities exist in relation to physiological conditions to feed neuron with energy. Either glucose could enter neurons or astrocytes metabolism of G-1-P produces pyruvate and lactate being given to neuron. This latter is known as the astrocyte-neuron lactate shuttle hypothesis (ANLSH) of neurons feeding by astrocyte-produced lactate [27, 46–49]. Whilst this ANLSH is still challenged, it seems clear that during pathological conditions such ANLSH is relevant and occurs [25, 27, 29, 43, 44, 50–53]. Normal feeding through direct neuron glucose entry occurs in normal conditions. However, these two mechanisms of neuron feeding are not exclusive to each other and may coexist giving neuron several ways to provide itself with energy.

Considering that ANLSH might occur essentially in pathological conditions, the glycogen astrocyte content and metabolism are relevant processes to provide neurons with energy. Indeed, astrocyte-accumulated glycogen is transformed to G-1-P, that is, processed down to lactate. This latter compound is transferred to neuron using MCTs. Lactate is used by neuron to produce ATP with a fast and economic way compared to normal glycolysis (Figure 1). Therefore, astrocyte glycogen might be regarded as an important molecule to provide energy to neuron, with relevant processing in pathological conditions and may be in normal ones.

We here review previous works performed in our group; the only approach which has not been published yet concerns the one realized on human CCF-STTG-1 cell line. The purpose of the present paper is to demonstrate the relevance of brain glycogen content as a putative target and tool for therapeutic and diagnostic approaches.

4. Involvement of Glycogen in Brain Cancer

Abnormally high glycogen content has been reported in different cancer types, such as gastric tumors [54], thyroid cancer [55], and various brain tumors [56–59]. It has also been reported in tumors induced by the implantation of a rat glioma-derived C6-cell line [56], in spontaneous cat glioma [59], and in cultured human tumor cell lines from tissues of differing origins [58]. In humans, gliomas form the vast majority of malignant brain tumors and are among the most lethal [60]. *Ex vivo* studies of human glioma tumors reveal high GS activity associated with high glycogen content [57, 61]. The putative significance of GS activity on cell proliferation is deduced from the positive correlation of

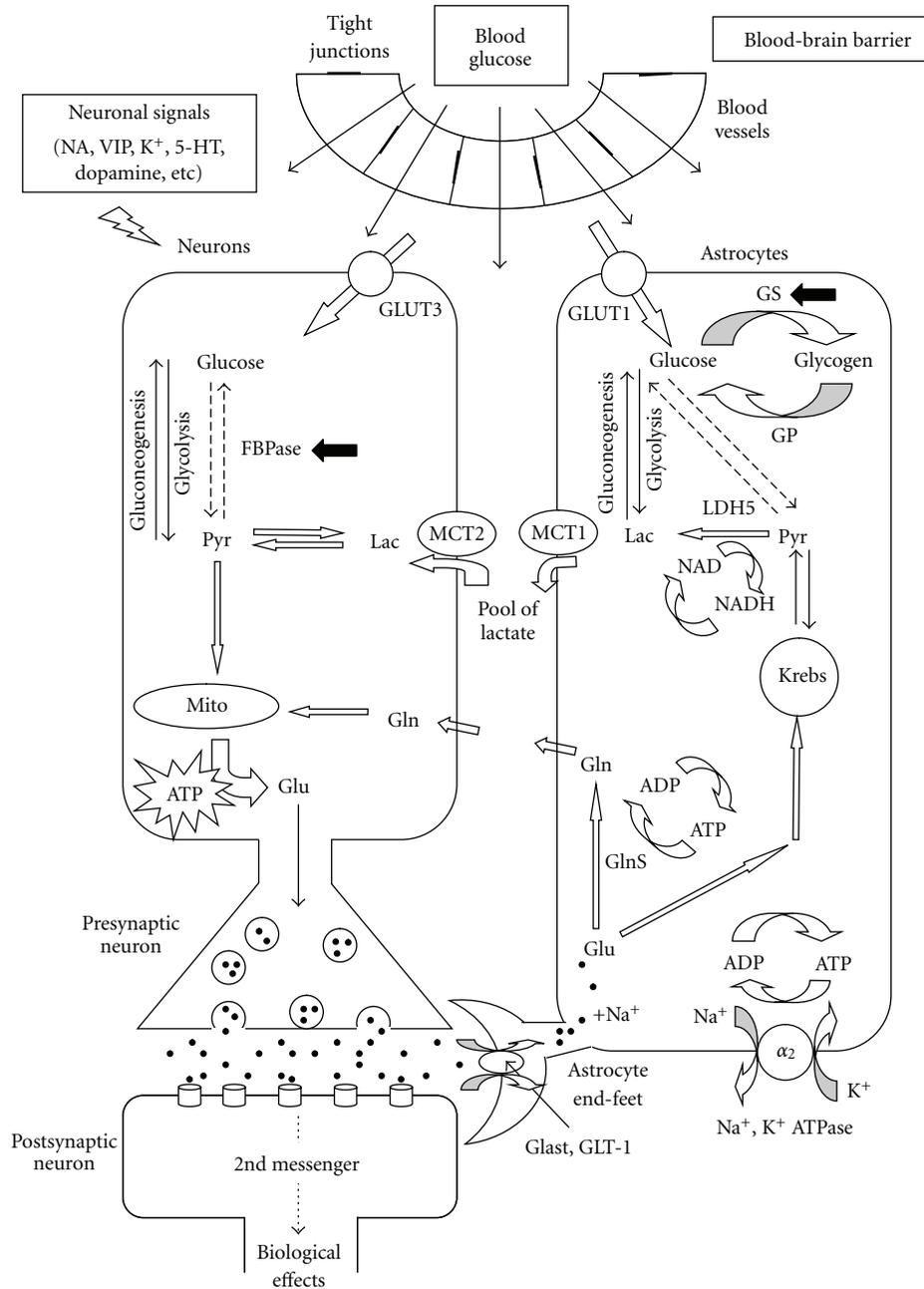


FIGURE 1: Scheme of some glucose pathways, such as glycolysis, gluconeogenesis, glycogenesis, and glycogenolysis in parallel to ANLSH. The two enzymes that will be targeted by antisense strategy are indicated by full horizontal arrow. Schematic representation of glucose-lactate shuttle between neurons and astrocytes (adapted from [28, 41]). FBPase, fructose-1,6-bisphosphatase; GLAST, GLT-1: Na⁺-glutamate co-transporter; Gln: glutamine; GlnS: glutamine synthetase; Glu: glutamate; GLUT, glucose transporter; GP, glycogen phosphorylase; GS, glycogen synthase; 5-HT, serotonin; Krebs: Krebs cycle; Lac: lactate; LDH5: Lactate dehydrogenase 5; MCT: monocarbohydrate transporter; Mito, mitochondria; NA: noradrenaline; Pyr: pyruvate; VIP: vasointestinal peptide.

glycogen content and proliferation in cloned rat astrocytes [62]. In fact, some current front-line strategies that detect, monitor, and treat cancer capitalize on the paradigm that there is a particular “energy budget” that typifies cancer cells [63]. It was suggested that biochemical hallmark of tumor cells was a shift in glucose metabolism from oxidative phosphorylation to aerobic glycolysis [64]. Recently, Michelakis

and coworkers [65] tested whether the small molecule and orphan drug dichloroacetate (DCA), a molecule that increases pyruvate content, can reverse the cancer-specific metabolic and mitochondrial remodeling in glioblastoma. In five patients who had glioblastoma, they prospectively secured baseline and serial tumor tissue, developed patient-specific cell lines of glioblastoma and putative glioblastoma

stem cells, and treated each patient with oral DCA for up to 15 months. They presented indications of clinical efficacy at a dose that did not cause peripheral neuropathy.

5. Involvement of Brain Glycogen in Epilepsies

5.1. Generality on Epilepsies. Convulsions, which are the visible signs of epilepsies, display numerous forms such as absences, partial to generalized seizures. World Health Organization (WHO) recognizes at least 40 forms of epilepsies [66–71] that correspond to a sudden and temporary synchronization of neurons' activities whose origins are not well understood [72–77]. Medical imagery of epilepsies is based on various mechanisms; the uptake of glucose analogs represents one of them [78–80]. This allowed demonstrating that epileptic foci are hypermetabolic during ictal period and hypometabolic during interictal period. Such a hypometabolism of epileptic foci might be due to intrinsic increase in brain energy capacities production as a consequence of various cell alterations such as glycolysis, gluconeogenesis, or glycogenesis, the latter resulting in accumulation of glycogen. Such an increase in glycogen content is very difficult to demonstrate in human. However it has been reported that brain biopsies obtained from hippocampus of epileptic patients contained high glycogen content compared to grey and white matter from same patients [81]. During seizures, when neurons need a lot of energy as they are starved by their high activities, this energy might come from different sources: blood supply, neuron glycolysis and astrocyte source. When the first two are outpaced, the astrocyte glycogen might be mobilized, and therefore astrocyte glycolysis of the G-1-P derived from glycogen might generate lactate, which is transferred to neurons. Neurons are therefore provided with a metabolite that can be directly used by mitochondria for ATP production without further processing (Figure 1).

5.2. Model of Epilepsy That Associates Seizures and Elevated Brain Glycogen. Among the various epileptic or seizures-induced models, one of the chemical-induced models associated both crisis and a specific increase in brain glycogen content [82–89]. This model corresponds to seizures induced by a derivative of methionine, methionine sulfoximine (MSO). Animals, essentially rat and mouse, developed seizures after intraperitoneal administration of a single dose of MSO, and such seizures look like the human “grand mal”, which is the most striking and invalidating form of human epilepsy. Animals developed epileptiform seizures 6–8 hours after MSO dosing (preconvulsive period) seizures are recurrent during 24–48 hours (convulsive or ictal period), and animals recover normal behavior (postconvulsive period) [82, 84, 90]. High level of glycogen and the highest augmentation of glycogen are specifically localized in cerebral cortex and cerebellum, are demonstrable as soon as preconvulsive period, and are specifically localized to astrocytes [82, 84, 86, 90, 91]. Moreover, the action of MSO on metabolism alterations is also demonstrable *in vitro* in cultured rat and mouse astrocytes. These data demonstrate that such

metabolic effects are not consequences of seizures [82, 84, 85, 90]. Alteration of glycogen content is also observed in other models of induced seizures, such as, for example, in seizures induced by homocysteic acid [92]. Accumulation of glycogen was essentially due to astrocytes metabolism, nevertheless, in progressive myoclonus epilepsy inclusion bodies, resembling abnormal glycogen, accumulate in this disease [93]. Moreover, it has been reported recently that mouse neurons have enzymatic machinery for synthesizing glycogen [94].

Some actions of MSO on glutamate receptors and inhibition of glutamine synthetase resulting in putative accumulation of glutamate have been reported as responsible for MSO-dependent seizures [95, 96]. Nevertheless, some reports [97–99] describe a decrease in the brain glutamate content after the administration of a convulsive dose of MSO in rats, rather than the expected increase. Moreover, chronic inhibition of brain glutamine synthetase by MSO did not induce seizures in mice [100]. Therefore, the precise mechanism responsible for MSO-dependent seizures is not well defined and needs more investigations.

6. Objectives

The present work was designed to suggest that brain glycogen, that is, astrocyte glycogen, is a relevant source and store of energy in which neurons can find a support to their needs. Moreover, we tried to contribute to demonstrate that brain glycogen has to be considered as a tool for diagnostic and therapeutic approaches. Therefore, we developed tools to target glycogen synthesis and used those to demonstrate that astrocytic glycogen is involved in gliomagenesis. In addition, we selected two lines of mice that differed by their seizures latency to MSO, compound being specifically a brain glycogenic agent, and used this mouse model to suggest that brain glycogen is involved in epileptogeny. We strongly suggest that brain glycogen is largely involved in these two brain pathologies, gliomagenesis, and epilepsy, at least in a mouse model.

7. Tools Designed for Targeting Glycogen in Brain Pathologies

The summary of tools designed in order to target gluconeogenesis and glycogenesis is presented in Figure 2. They were constructed according to previous described methods [101, 102]. Two plasmids were constructed with antisense cDNA in order to decrease both gluconeogenesis and glycogenesis. We developed antisense cDNA of fructose-1,6-bisphosphatase (FBPase) (*pRK5-PBF*) to target gluconeogenesis and antisense cDNA of GS (*pRK5-SG*) to target glycogenesis alone. A third plasmid was also designed in order to increase glycogenesis; it corresponded to the full length encoding GS cDNA (*pRK5-GS*). The empty plasmid was used as a control (*pRK5-Neo*).

7.1. *pRK5-PBF* Plasmid (Figure 2(a)). Total cDNAs were prepared by reversed transcription of total mRNA pools

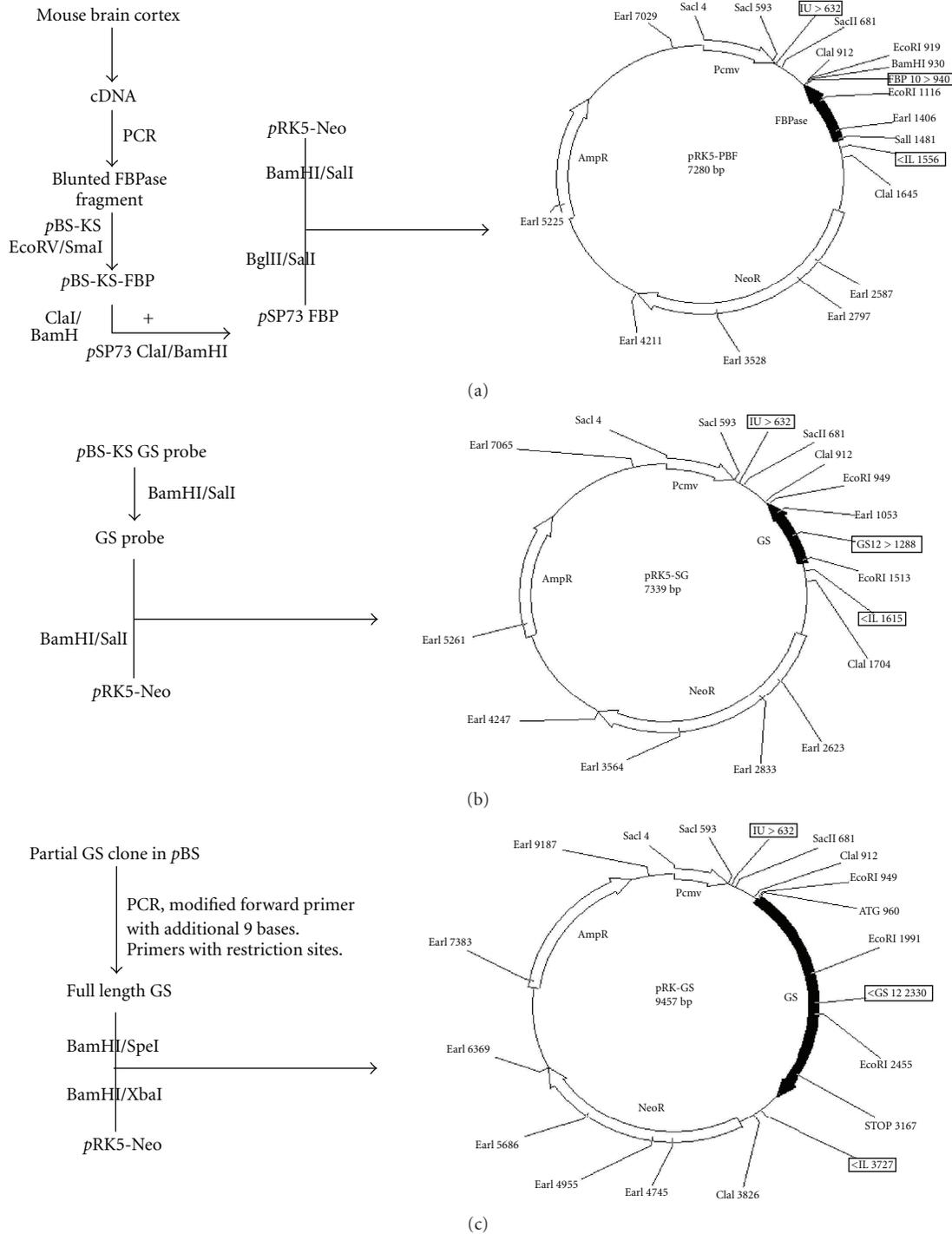


FIGURE 2: Schematic representation of design for building of plasmids. Positions of primers used for verification of both insertion and orientation of insert are indicated in boxes. The sequences of primers used are as follows. IU: 5'-atccacgtcttttgacctc-3', IL: 5'-atgcattgctttatttga-3', FBP10: 5'-tgcacatcagccaccatgga-3', and GS12: 5'-aattcaactggaaaacct-3'.

extracted from C57BL/6J mouse cerebral cortex. The partial FBPFase cDNA was prepared using specific primers as previously described [101]: upper: 5'-caactcctggtcatcaaatgc-3'; lower: 5'-tgcacatcagccaccatgga-3'. The blunt-ended FBPFase amplicon was inserted in the pBS-KS(+) digested by EcoRV and SmaI; antisense orientation was verified by restriction

mapping. Insert was prepared from this plasmid by digestion with ClaI and BamHI and subcloned in the pSP73 digested by ClaI and BamHI. The resulting plasmid was digested using BglII and SalI, and the insert was cloned in the expression plasmid digested with BamHI and SalI. Restriction mapping and PCR using primers specific to the expression plasmid

and to FBPase verified the obtained plasmid, named *pRK5-PBF* containing 528 bp of FBPase in antisense orientation [101, 102].

7.2. *pRK5-SG Plasmid (Figure 2(b)).* An EcoRI probe specific to mouse astrocyte GS was obtained from Pellegri et al. [103]. The probe was removed from the *pBS-KS(+)* by digestion with BamHI and Sall and cloned in antisense orientation in the expression plasmid digested using BamHI and Sall. Restriction mapping and PCR using primers specific to the expression plasmid and to GS verified the obtained plasmid, named *pRK5-SG* containing 564 bp of GS in antisense orientation [102, 103].

7.3. *pRK5-GS Plasmid (Figure 2(c)).* Harmonize by using Pellegri et al. [103] have cloned a partial GS cDNA sequence. In order to obtain full-length coding GS sequence, using PCR we added the nine missing bases. The specific primers we used for such a purpose were as follows, upper primer: 5'-ccagatccATGCCGCGCAGCCGAGTCTCTCTGTG-TC-3' (1/29) (underlined bases correspond to the missing bases starting from ATG codon), lower primer: 5'-tgaactagccaattgGGAAGCCACACAGA-3' (2707/2693). For both primers, small letters corresponded to additional bases for restriction sites used for cloning. The amplicon was digested using BamHI and SpeI and inserted in the expression plasmid digested with BamHI and XbaI. Restriction mapping and PCR using primers specific to the expression plasmid and to GS verified the obtained plasmid, named *pRK5-GS* containing 2655 bp of GS in sense orientation. The sequence of inserts was verified by sequencing *pRK5-GS* various clones. Clones with no sequence errors were only considered and used for transfection of mammalian cells [102, 103].

7.4. Validation of Functionality of the Tools. The validity of the functions of the above-described plasmids was performed by transfection of C57BL/6J astrocytes in cell culture (Figure 3) [102]. Enzyme activities and glycogen content were determined in stably transfected mouse astrocytes. The various plasmids were capable of modifying the corresponding enzyme activities after their permanent transfection in mouse astrocytes (Figure 3). The antisense FBPase decreased significantly the astrocyte glycogen content and FBPase activity, while it did not affect GS activity. This data suggests that FBPase, the last key-enzyme (before G-6-P) in gluconeogenesis, is also implicated in the glycogen synthesis; it also supports the existence of gluconeogenesis in the brain. The antisense GS decreased significantly the glycogen content, FBPase, and GS activities. Conversely, the sense GS increased significantly the glycogen content and GS activity while decreasing FBPase activity. The effect observed on glycogen content with antisense FBPase was not significantly different to that with antisense GS, both decreasing by 60% astrocyte glycogen content [101–103].

8. Effect on Gliomagenesis

The effect of the antisense cDNA of both FBPase and GS was analyzed to determine any potential antiproliferative action

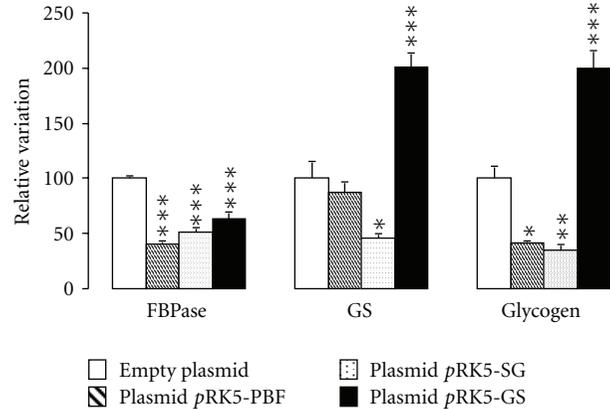


FIGURE 3: Relative variation of FBPase ($n = 8$) and GS ($n = 7$) activities and glycogen content ($n = 12$) of mouse astrocytes after permanent transfection. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus empty plasmid ($n = 10$). Values are expressed as mean \pm SEM, and statistical analysis was made using Student test. Data adapted from Bernard-Hélary et al. [102].

of the inhibition of glycogenesis on glioma cell lines. To such a purpose, plasmids were permanently expressed in cells, and the variations of glycogen level, proliferation, and invasiveness were studied.

8.1. In Vitro and In Vivo Antigliomagenesis in Cell Lines Derived from Glioma. The plasmids mentioned above were permanently transfected in glioma-derived cell lines coming from human and rat gliomas. Fugen-6 transfected cells were selected using 2000 $\mu\text{g}/\text{mL}$ of geneticin. When selection was complete, the geneticin concentration was decreased to 400 $\mu\text{g}/\text{mL}$. The antibiotic was removed from the culture media one passage before making the analyses.

8.1.1. Glioma-Derived Cell Line from Human

Human Derived Cell Lines. Human glioma cells were obtained from ATCC (LGC Promochem, Molsheim, France) and cultured in DMEM medium, as previously described for rat and mouse astrocytes [62, 85, 102, 105]. The CCF-STTG1 cell line was established from a specimen of Grade IV astrocytoma, or glioblastoma, from a 68-year-old Caucasian female.

In Vitro Effects. Transfected cells were cultured and some of their cellular properties were determined. Their capability to induce foci and their doubling time were analyzed and presented in Figure 4. Both antisenses were capable of either significantly decreased number of foci formation (Figure 4(a)) or significantly increased the doubling time (Figure 4(b)). We also determined the activities of FBPase and GS. These activities were significantly lower (data not shown) in transfected CCF-STTG1 cells with either antisense FBPase or GS cDNA when compared to the transfected cells with empty plasmid (*pRK-Neo*). These data demonstrated that the expression of both antisenses in CCF-STTG1 was

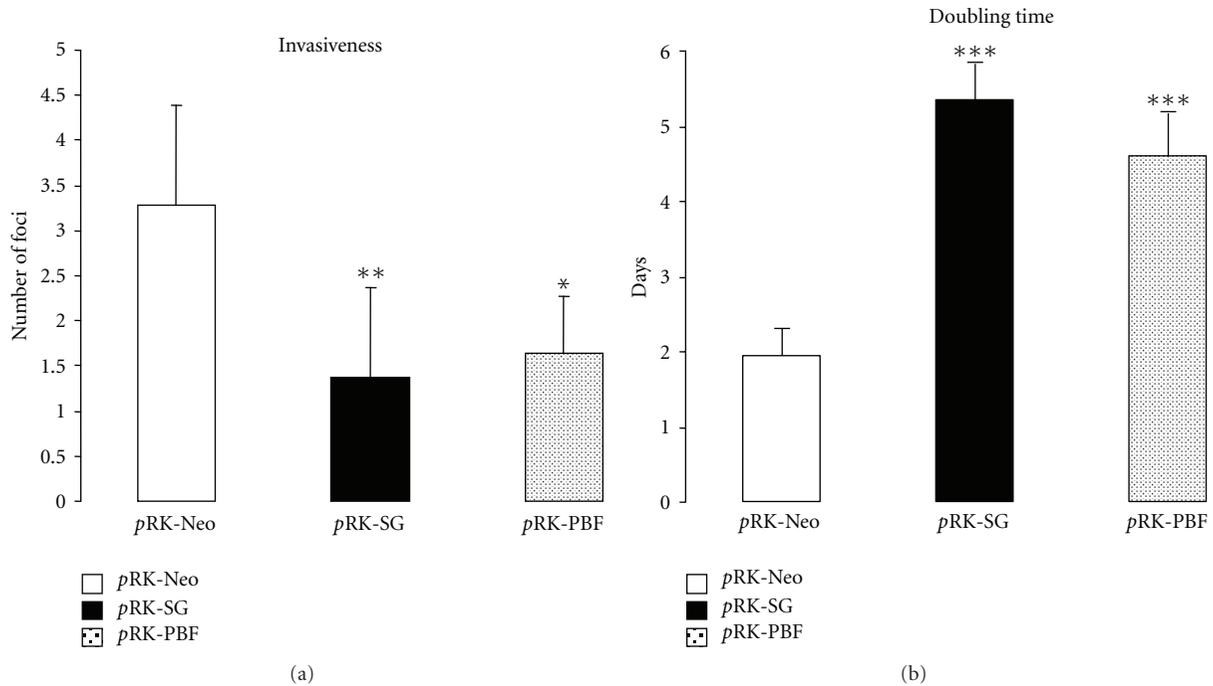


FIGURE 4: *In vitro* effect of FBPase and GS antisense cDNAs expressed in human glioma-derived cell line. Invasiveness (a) was determined in semisolid medium by measuring the number of foci that each cell can induce. Doubling time (b) of various cells is determined using counting of viable cells. Value represents the mean of 5 experiments (corresponding to 3 sets of transfection) \pm SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus empty plasmid, and statistical analysis was made using Student test. Data adapted from Blin et al. [100].

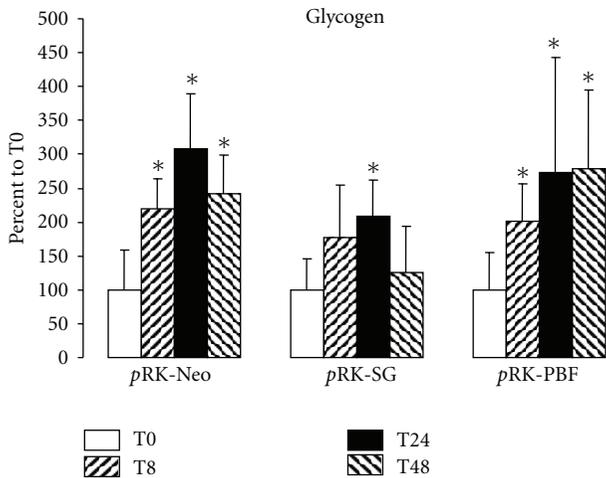


FIGURE 5: *In vitro* effect of FBPase and GS antisense cDNAs expression on glycogen content of human glioma-derived cell line. Cells were harvested at various times after medium was renewed. Value represents the mean of 5 experiments (corresponding to 3 sets of transfection) \pm SEM. * $P < 0.05$, versus T0, and statistical analysis was made using Student test. Data adapted from Blin et al. [100].

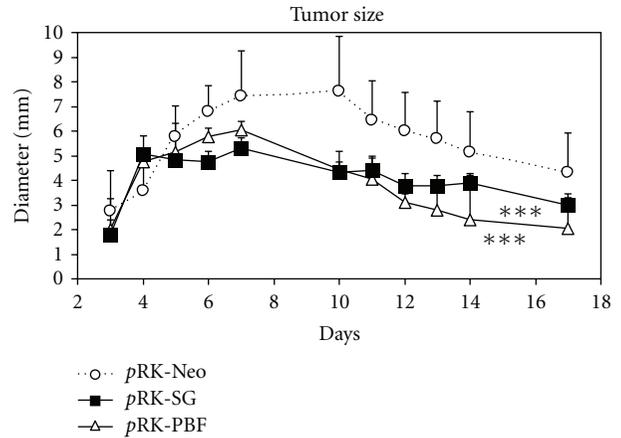


FIGURE 6: *In vivo* effect of FBPase and GS antisense cDNAs expression on tumor implantation of human glioma-derived cell line. Ten NMRI mice were irradiated by 6 Gy 24 hours before subcutaneous injection of 10^7 transfected cells. Tumor size was determined using a caliper square. Value represents the mean \pm SEM, corresponding to 3 sets of transfection. *** $P < 0.001$ versus empty plasmid, and statistical analysis was made using repeated ANOVA test for 10 mice. Data adapted from Blin et al. [100].

effective and capable to induce inhibition of the corresponding en-zyme activities and to decrease the *in vitro* studied cell pro-perties proliferative capacities.

Invasiveness was determined as the capability of transfected cells to induce foci in a semisolid medium (Figure 4(a)). Both antisenses were able to significantly in-

hibit the foci formation, GS antisense being more efficient than that of FBPase. Conversely, the doubling time, determined by counting number of cells after seeding as a function of time, was significantly increased by both antisenses. Therefore, the antiproliferative action seems to be mediated by an action on the capability of cells to divide (Figure 4(b)), in

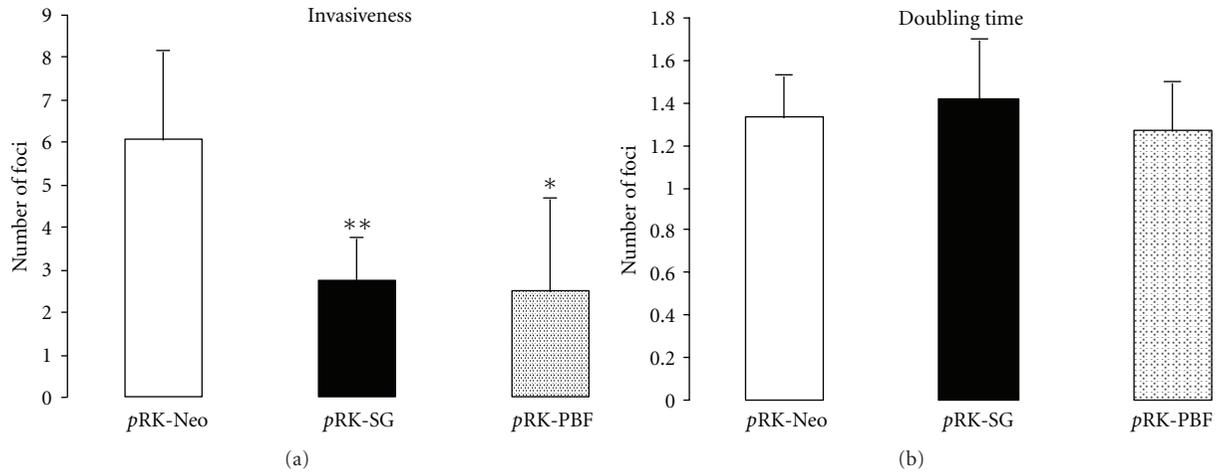


FIGURE 7: *In vitro* effect of FBPase and GS antisense cDNAs expressed in rat glioma-derived cell line. Invasiveness (a) was determined in semisolid medium by determining the number of foci that each cell can induce. Doubling time (b) of the various cells is determined using counting of viable cells. Value represents the mean of 8 experiments (corresponding to 3 sets of transfection) \pm SEM. * $P < 0.05$, ** $P < 0.01$ versus empty plasmid, and statistical analysis was made using Student test. Data adapted from Ardourel et al. [104].

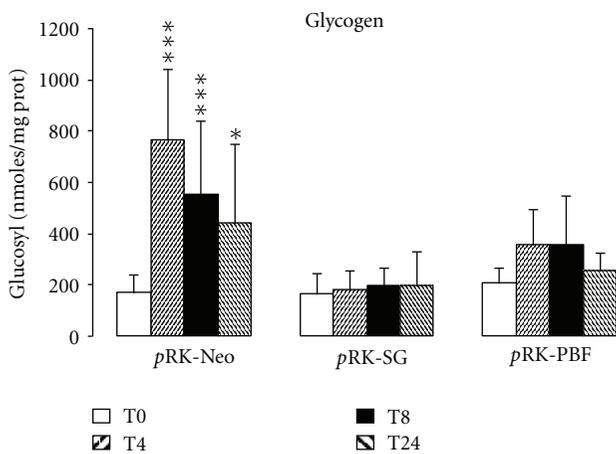


FIGURE 8: *In vitro* effect of FBPase and GS antisense cDNAs expression on glycogen content of rat glioma-derived cell line. Cells were harvested at various times after medium was renewed. Value represents the mean of 7 experiments (corresponding to 3 sets of transfection) \pm SEM. * $P < 0.05$, *** $P < 0.001$ versus empty plasmid, and statistical analysis was made using Student test. Data adapted from Ardourel et al. [104].

relation to the accessibility to glycogen-dependent energy as demonstrated by the effect of GS antisenses on cell glycogen content (Figure 5). Indeed, the capacity of synthesizing glycogen is significantly decreased by GS cDNA antisense expression. Conversely, the data demonstrated an absence of significant effect of FBPase cDNA antisense. Last result may be explained by the quite low effect of anti-FBPase on glycogen content, and therefore, glycogen synthesis is difficult to inhibit in CCF-STTG1 transfected cells, while it is easily inhibited in cultured mouse astrocytes (Figure 3). If this is true, the effects of anti-FBPase may be difficult to

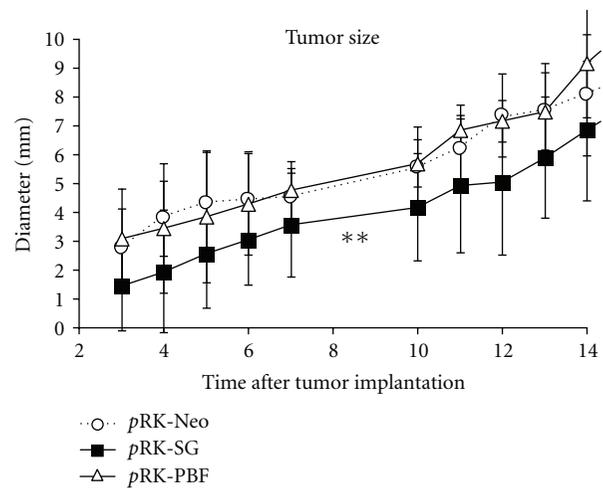


FIGURE 9: *In vivo* effect of FBPase and GS antisense cDNAs expression on tumor implantation of rat glioma-derived cell line. Ten NMRI mice were injected subcutaneously with of 5×10^5 transfected cells. Tumor size was determined using a caliper square. Value represents mean \pm SEM corresponding to 3 sets of transfections. ** $P < 0.01$ versus empty plasmid, and statistical analysis was made using Student test for ten mice. Data adapted from Ardourel et al. [104].

determine, but may be effective. This assumption needs more investigation.

In Vivo Effects. The subcutaneous evolution of implanted human tumors in immunodepressed mice is presented in Figure 6. The tumor size is significantly decreased by the expression of both GS and FBPase cDNA antisenses in human CCF-STTG1 cell line when compared to the empty plasmid. No significant difference could be found between the expression of antisenses of GS and FBPase.

Overall, these data strongly suggest that the inhibition of synthesis of both GS and FBPase in human glioma-derived cell line might induce an inhibition of both *in vivo* and *in vitro* proliferation. However, regarding the putative action of inhibition of GS it seems clear that such an effect could be at least the result of inhibition of glycogen synthesis and, therefore, the energy depending upon glycogen utilization. Nevertheless, this putative explanation might not be valid for inhibition of FBPase, as in this human cell line no effect was demonstrable on glycogen content. It might be possible that both glycogen synthesis and gluconeogenesis would be relevant in the antiproliferative effect on human glioma-derived cell line.

8.1.2. Glioma-Derived Cell Line from Rat

Rat-Derived Cell Lines. Rat C6 glioma cells were obtained from ATCC (LGC Promochem, Molsheim, France) and cultured in DMEM medium, as previously described for rat and mouse astrocytes [62, 85, 102, 105]. Benda et al. cloned the glial cell strain, C6, from a rat glial tumor induced by N-nitrosomethylurea [106].

In Vitro Effects. As for human glioma-derived cell line, the transfection of rat glioma-cell line, C6 cells, induces an inhibition of the capability of these cells to form foci (Figure 7). Indeed, both antisense cDNAs of GS and FBPase induce an inhibition of the corresponding enzyme activity (data not shown) in addition to inhibition of foci formation when compared to empty plasmid (Figure 7(a)). Conversely to human cell, these effects seem not to be mediated by any action of doubling time of these transfected cells. Indeed, the antisense cDNAs of GS and FBPase do not induce a significant change in the capacity of cell to divide (Figure 7(b)). Moreover, glycogen synthesis (Figure 8) could be involved in the action of inhibition of proliferation of rat glioma-derived cell by various antisenses. Indeed, both antisense cDNAs of either GS or FBPase induce a significant decrease in glycogen synthesis of the transfected rat glioma-derived cell line.

In Vivo Effects. The subcutaneous evolution of implanted rat tumors in immunodepressed mice is presented in Figure 9. The tumor size is significantly decreased by the expression of GS cDNA antisense, but not by FBPase cDNA antisense, in rat C6 cell line when compared to the empty plasmid. Indeed, the size of the subcutaneous tumor obtained with transfected cells by antisense GS cDNA is always significantly lower than that obtained with empty plasmid or plasmid containing cDNA antisense of FBPase.

In the case of rat glioma-derived cell line, we can probably conclude that glycogenesis is involved in the control of both *in vivo* and *in vitro* proliferation of rat glioma-derived cell line.

8.2. Putative Conclusion. In conclusion, we can assume that targeting either glycogen synthesis or gluconeogenesis can affect the proliferation of glioma-derived cell lines, at least in

the cell lines we used. Therefore, if transposition of obtained *in vitro* and in living mice to brain tumors of human beings is valid, brain glycogen might constitute a therapeutic target in order to decrease the development of brain tumor. Moreover, when it will be possible to follow the brain glycogen variation using medical imagery [41, 107–110], such a hypothesis may find an experimental confirmation.

9. Selection of Lines of Mouse Based upon their Latency to Seizures Induced by Methionine Sulfoximine

9.1. Selection of MSO-Fast and MSO-Slow Mouse Lines. After MSO administration to mice or rats, glycogen accumulates in large amount in brain cortices and cerebellum during the preconvulsive period [88, 89, 91]. The glycogen content remains high during the convulsive period and up to the postconvulsive period. Moreover, MSO induces accumulation of glycogen in mouse and rat astrocytes both *in vivo* [82, 84, 86, 90, 91] and *in vitro* [82, 85, 111]. This *in vitro* data, in addition to the *in vivo* effect before onset of seizures, clearly suggested that the brain variation of glycogen is not a consequence of epilepsy. Causal links between glycogen accumulation and seizure remain an open and pending question. We have demonstrated that both seizures latency and glycogen accumulation are depending upon genetic background in mice, and when inbred mice can accumulate glycogen in brain cortices after MSO dosing and can mobilize accumulated glycogen during seizures (C57BL/6J), the latency of MSO-dependent seizures is high as compared to inbred mice that cannot mobilize glycogen (CBA/J) [82, 90] (Figure 10). We hypothesized, therefore, that the two MSO effects, that is, elevation of glycogen and its mobilization during seizures, might constitute a way to postpone seizures [82, 90] and/or a mean to struggle against the epileptic status and the unwilling consequences of epilepsies. Whether this hypothesis is valid may have therapeutic consequences for patient suffering from epilepsies and needs experimental approaches for being validated.

In order to validate such a hypothesis, we have selected two lines of mice on the basis of their latencies to MSO-dependent seizures [112]. Three intercrosses of eight inbred mouse lines produce the generation 1 (G1) for MSO challenging. The selection of MSO-sensitive and MSO-resistant mouse from G1 was performed by a single injection of 75 mg/kg MSO and observation of the first generalized seizure. Mice with the fastest latency were crossed with care not to cross sister with brother; they were named as MSO-Fast. Mice with the longest latency were crossed with care not to cross sister with brother; they were named as MSO-Slow. Such a process was conducted for 7 generations, and additional 3 generations were performed with increased selection pressure, by decreasing administered MSO dose for MSO-Fast and increasing it for MSO-Slow. After these steps, phenotypes considered as established, inbreeding was performed using 8 sister-brother crosses for each line.

Characterization of MSO-Fast and MSO-Slow lines was performed after 6 MSO-challenges, that is, at selection

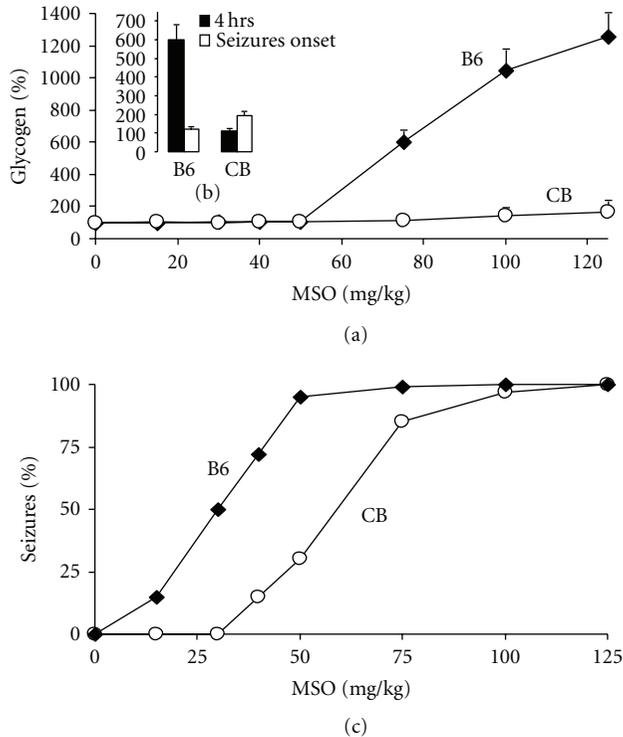


FIGURE 10: MSO effects on C57BL/6J (B6) and CBA/J (CB) strains. (a) and (b) Percent of glycogen variation after various doses of MSO (a) and 75 mg/kg of MSO when mice were sacrificed 4 h after dosing or at the onset of seizures (b). (c) percent of mice that convulses as a function of doses of MSO. Values are expressed as mean \pm SEM of at least 20 mice. Data adapted from Bernard-Hélarly et al. 2002 [82, 90].

generation G6 [112, 113]. After establishment of inbreeding by 10 sister-brother crossings, characterization was again performed, that is, at inbreeding C8 [114]. We presented both data here.

9.2. Epileptic Characteristics of MSO-Fast and MSO-Slow Mouse Lines. The process of selection generated two lines of mice that we call MSO-Fast, for their fast response to MSO in terms of latency, and MSO-Slow, for their very long latency to MSO [112]. We might consider these two lines as MSO sensitive and MSO resistant, respectively. Indeed, EEG of MSO-Fast mice demonstrate an epileptic profile when mice were given a single 75 mg/kg MSO. Conversely, no EEG alteration could be observed in MSO-Slow receiving the same dose of MSO [112]. Data obtained regarding sensitivity toward MSO are summarized in Table 1. All along the selection process, the latency toward MSO-dependent seizures and ED_{50} of MSO significantly varies between generation G1 and G6. Latency decreases and increases for MSO-Fast and MSO-Slow, respectively. The percentage of convulsive mice increases from 80% to 88% for MSO-Fast mice and decreases from 80% to 5% for MSO-Slow ones. The variation of ED_{50} of MSO confirms that the two lines of mice largely differ from each other. The establishment of 8 inbreeding

TABLE 1: Summarized data obtained during selection of MSO-Fast and MSO-Slow lines.

Mouse	Step	n	Latency (min)	Seizures (%)	Death (%)	ED_{50} (mg/kg)
G0	initial	343	420 ± 100	80	37	ND
MSO-Fast	G6	104	320 ± 50	88	12	$36,1 \pm 0,3$
MSO-Slow	G6	80	570 ± 30	5	5	$124,9 \pm 10,3$
MSO-Fast	C8	40	240 ± 20	100	6	41.8 ± 1.6
MSO-Slow	C8	40	600	0	2	>300

Data concerned sensitivity toward MSO (75 mg/kg) of mouse at different levels of generation during the selection process. G0: after mixing of the eight parental strains, G6: after 6 processes of crossing and MSO-challenging, C8: after 8 inbreeding crossings of the selected lines after 10 MSO-challenges. ND: not determined. Data adapted from Cloix et al. [112].

TABLE 2: Summarized latency (min) obtained during characterization of MSO-Fast and MSO-Slow lines at the 6th generation of selection process.

Products	Mouse			
	MSO-Fast	n	MSO-Slow	n
Convulsant				
MSO (75 mg/kg)	$295,6 \pm 102,4^a$	20	ND	
MSO (200 mg/kg)	ND		$347,7 \pm 79,5$	20
KA (25 mg/kg)	$48,8 \pm 23,2^a$	28	$76,8 \pm 46,5$	27
PTZ (75 mg/kg)	$1,24 \pm 0,48^b$	30	$1,18 \pm 0,46$	29
Anticonvulsant				
MK-801 (1 mg/kg)	600 ^c	12	600 ^c	12
VPA (250 mg/kg)	$453,7 \pm 65,1^d$	10	$470,5 \pm 183,6$	10

The anticonvulsants were given to mice 30 minutes before the appropriate dose of MSO. ^a: $P < 0.01$ MSO-Fast versus MSO-Slow. ^b: NS. ^c: The anticonvulsant effect of MK-801 is obvious, but not statistically analyzed as the latency value in its presence has no SEM. Indeed, when the latency is above 10 hours, mice were given a score of 600. ^d: $P < 0.001$ VPA versus its absence in MSO-Fast only. ND: not determined. Data adapted from Cloix et al. [113].

crosses improves such differences between the two lines. The percentage of MSO-induced death of mice in both lines indicates that the selection process in MSO-Fast mice concerns mice that are “less sensitive” toward MSO as the most sensitive ones died during the process of selection. Conversely, MSO-Slow mice are not concerned, as only the resistant mice were crossed.

These two lines of mice differ as well by their sensitivity to other epileptic compounds and to anticonvulsants (Table 2). Indeed, the latency to seizures induced by kainic acid (KA) is significantly lower in MSO-Fast than that in MSO-Slow. In addition, no significant difference between MSO-Fast and MSO-Slow could be observed between the latency to seizures induced by pentylentetrazol (PTZ). Moreover, MK-801 antagonizes MSO-dependent seizures in both MSO-Fast and MSO-Slow lines. Conversely, valproic acid (VPA) partially antagonizes MSO-dependent seizures in MSO-Fast line only. We suggested [112], therefore, that the MSO-dependent seizures might be mediated by an effect of MSO on glutamatergic pathways rather than GABAergic ones.

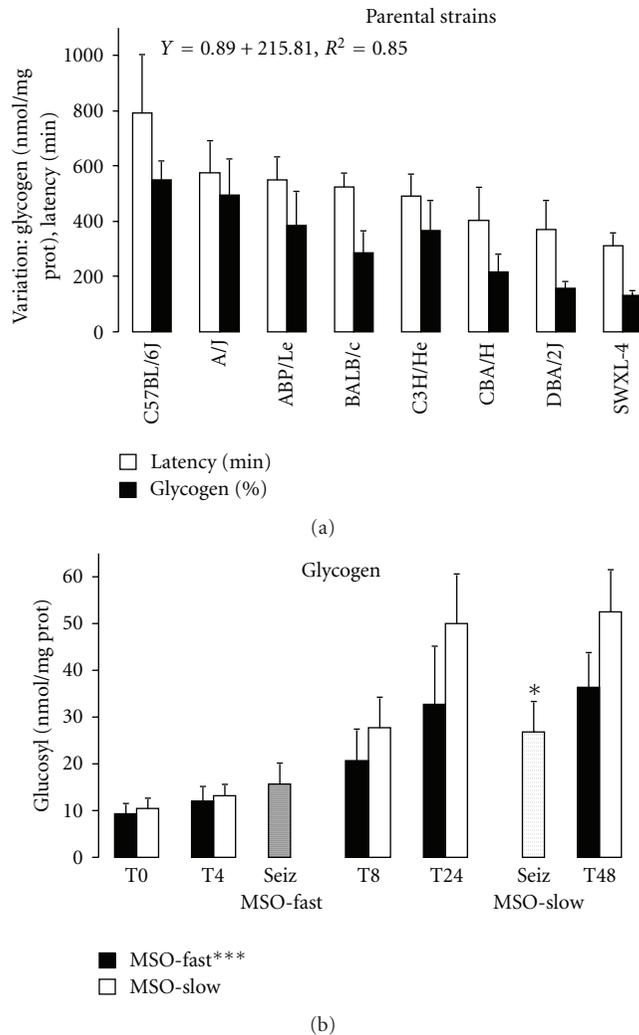


FIGURE 11: MSO effects on glycogen content determined on mouse cerebral cortex of various mice. (a): Various parental inbreed mouse strains are sacrificed 8 hours after receiving a single MSO dose (75 mg/kg). Variation in cerebral cortex induced by MSO was determined as percentage of glycogen increase observed 8 h after MSO administration and vehicle for the eight parental strains, $n = 8$. (b): MSO-Fast and MSO-Slow from G6 were given a single MSO dose (75 mg/kg) and were sacrificed either at various times after dosing or at the onset of seizures (Seiz). Significance was given for the total variation of brain glycogen content between the two lines and for value of MSO-Slow mice at the onset of seizures compared to before and after seizures. * $P < 0.05$, *** $P < 0.001$. Values are expressed as mean \pm SEM of at least 20 mice, and statistical analysis was made using Student test for (a) and using ANOVA test for (b). Data adapted from Cloix et al. [112].

9.3. Involvement of Brain Glycogen in MSO-Fast and MSO-Slow Mouse Lines. We had already suggested, based upon data obtained with C57BL/6J and CBA/J (Figure 10) [82, 90], that glycogen might be involved in the control of MSO-dependent seizures. Indeed, our hypothesis is that mice that can increase their brain glycogen content and mobilize it at the onset of seizures are able to postpone the onset of

seizures induced by MSO, conversely to mice that cannot modify their brain glycogen content. Among the various possibilities to validate such a hypothesis, we privilege the analysis of brain glycogen content in various mouse inbred strains submitted to MSO and the selection of MSO-sensitive and MSO-resistant mouse lines. Data obtained using this latter are summarized in Figure 11.

The 8 parental inbred strains, used for the production of G1, present an excellent parallelism between increase in brain glycogen content determined 8 hours after a single 75 mg/kg dose of MSO and the latency to MSO-dependent seizures (Figure 11(a)) [113]. When these data are analyzed by plotting the MSO-dependent latency as a function of MSO-dependent increase in brain glycogen content, a positive correlation was demonstrable ($Y = 0.89 + 215.81, R^2 = 0.85$). In addition, in MSO-Fast and MSO-Slow lines, brain glycogen content increases as a function of time after MSO dosing. This increase is significantly higher in MSO-Slow mice compared to that in MSO-Fast ones (Figure 11(b)). Only the MSO-Slow mice are able to decrease their brain glycogen content at the onset of seizures (Figure 11(b)). Overall, these data confirm and validate based on experimental results our previous hypothesis [82, 90] that suggested an involvement of brain glycogen content in MSO-dependent seizures.

9.4. Involvement of Brain Monoamines in MSO-Fast and MSO-Slow Mouse Lines. Variations of cerebral cortex content in monoamines were analyzed in MSO-Fast and MSO-Slow lines after a single 75 mg/kg dose of MSO. Among the indolamines and catecholamines we determined, the most relevant and significant changes were obtained for 5-HT and dopamine [113]. This data is represented in Figure 12.

Brain 5-HT content is always significantly higher in MSO-Fast mice than that in MSO-Slow ones, and this is true for any period of time before and after MSO dosing (Figure 12(a)). Particularly, the basal level is about 4 times higher in MSO-Fast brain as it is in MSO-Slow brain. Moreover, in both lines 5-HT content significantly decreases at the onset of seizures; however, at onset of seizures brain 5-HT content is still significantly higher in MSO-Fast mice than that in MSO-Slow. In the MSO-dependent seizure model it was reported that 5-HT was a potent antagonist of seizures induced by MSO [115]. MSO-dependent seizures and 5-HT increases were abolished by treatment with either 5-HT precursor, 5-hydroxytryptophan, or benserazide, a decarboxylase inhibitor [116]. The present data on the variations of 5-HT in cerebral cortices of the two lines of mice are therefore in agreement with the involvement of these compounds in the control of seizures. We found that 5-HT is involved in the epileptogenicity of MSO-Fast mice because of (i) changes in its concentration during convulsive and postconvulsive periods and (ii) the high basal level of 5-HT in MSO-Fast as compared with that of the MSO-Slow. In the MSO-Slow no variation of brain 5-HT could be observed. It is known that 5-HT has an antiepileptic action in humans and is of greater relevance in MSO-dependent seizures. The decrease of 5-HT content in MSO-Fast, and especially in

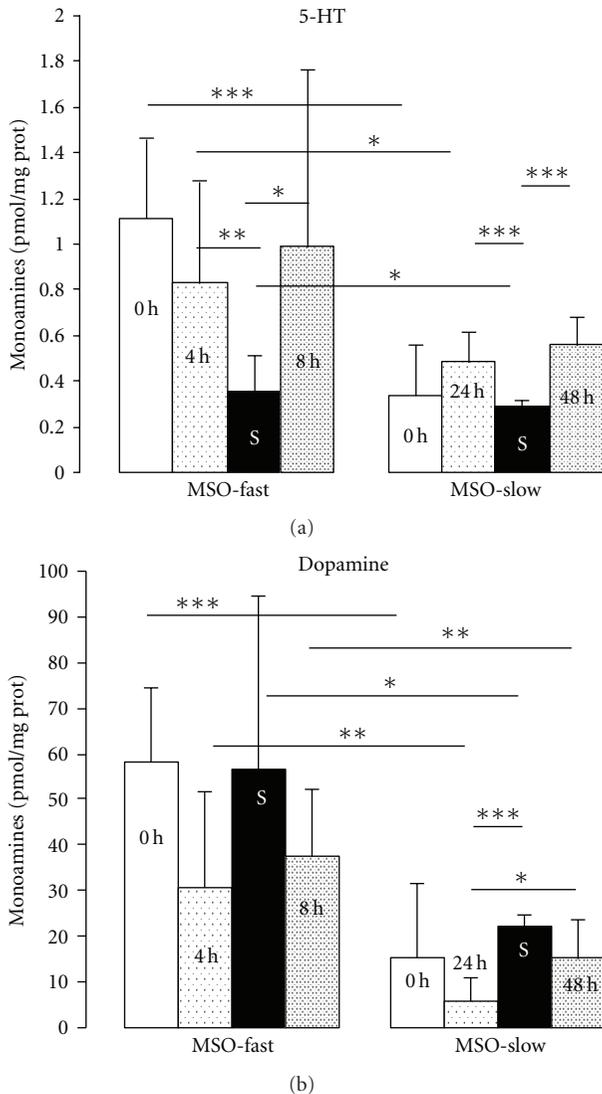


FIGURE 12: MSO effects on monoamine content determined on cerebral cortex of MSO-Fast and MSO-Slow mice. They were given a single MSO dose (75 mg/kg) and were sacrificed thereafter either at various predetermined times or at the onset of seizures (S). (a): Variation of cerebral cortex 5-HT content. (b): Variation of cerebral cortex dopamine content. Significance was given for the total variation of brain monoamine content between the two lines and for value of MSO-Slow mice at the onset of seizures compared to before and after seizures. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean \pm SEM of 20 mice. Data adapted from Cloix et al. [113].

MSO-Slow at the onset of convulsions, is in agreement with previous results [84, 115]. The decrease induced by MSO could be the reflection of a release of 5-HT in the cerebral cortex of the mice to struggle epileptogeny.

Very similar data are obtained on brain dopamine content (Figure 12(b)), except that dopamine content increases at the onset of seizures. Involvement of both 5-HT and dopamine in the control of glycogenesis is well known. However, while a good parallelism is observable between the variation

of both 5-HT and dopamine induced by MSO in MSO-Fast and MSO-Slow lines, no correlation could be observed between dopamine and glycogen content. Conversely, an excellent and positive correlation is demonstrable between glycogen and 5-HT brain content in both MSO-Fast and MSO-Slow lines (Figures 13(a) and 13(b)). Both 5-HT and dopamine have glycogenolytic effect [84, 117, 118], both *in vivo* and in cell culture; so a decrease was expected in glycogen content when monoamine contents increase. In contrast our results showed a clear increase in glycogen content in the two selected lines. The group of Magistretti [103, 119–121] showed that exposure of cultures of astrocytes to norepinephrine resulted in a short period of time in a small decrease in their glycogen content, which was followed by a large increase in this content for several hours. So monoamines may exert a dissymmetric biphasic action on brain glycogen, that is, a short-term, short-duration, and a small decrease followed by a large and long-term increase. In both selected lines, although a short time action of MSO was not evaluated in our experiments, the convulsant enhanced the metabolism of at least one monoamine, and this may explain the observed increase in glycogen content.

9.5. Conclusion on MSO-Fast and MSO-Slow Mouse Lines. We have demonstrated that brain glycogen content is a relevant marker and target for controlling the MSO-dependent seizures. Whether this is also valid for other animal models of epilepsies and moreover in human, the brain glycogen content will have to take into account in the management of this disease. We hope that this model of chemical-induced seizures will be helpful to study the relationship between seizures induction and control, and the brain glycogen content. When it will be possible to follow the *in vivo* variations of brain glycogen content in patients and in animal models of epilepsies, a new age will be opened to the diagnostic and therapeutic approaches of epilepsies.

10. Conclusion

In addition to the above-exemplified relevance of brain glycogen content in epilepsy and cancer, it is described as involved in other brain diseases and in neuropathologies. For example, it has been reported that traumatic brain injury triggers a marked accumulation of glycogen that may protect the brain during ischemia by serving as an endogenous source of metabolic energy [122]. Moreover, it is claimed about the important roles of brain glycogen metabolism and inhibition of glycogenolysis as a therapeutic approach to cerebral ischemia [123]. Using a specific inhibitor of GP in rat, the brain glycogen content increases. These rats maintained brain electrical activity longer than rats with normal brain glycogen levels and showed markedly reduced neuronal death [124].

Nowadays, brain glycogen content becomes an important parameter in the process of controlling pathological processes. Moreover, this molecule and its variation will be in the near future a diagnostic tool and a therapeutic one as well. This will depend upon the feasibility of determination

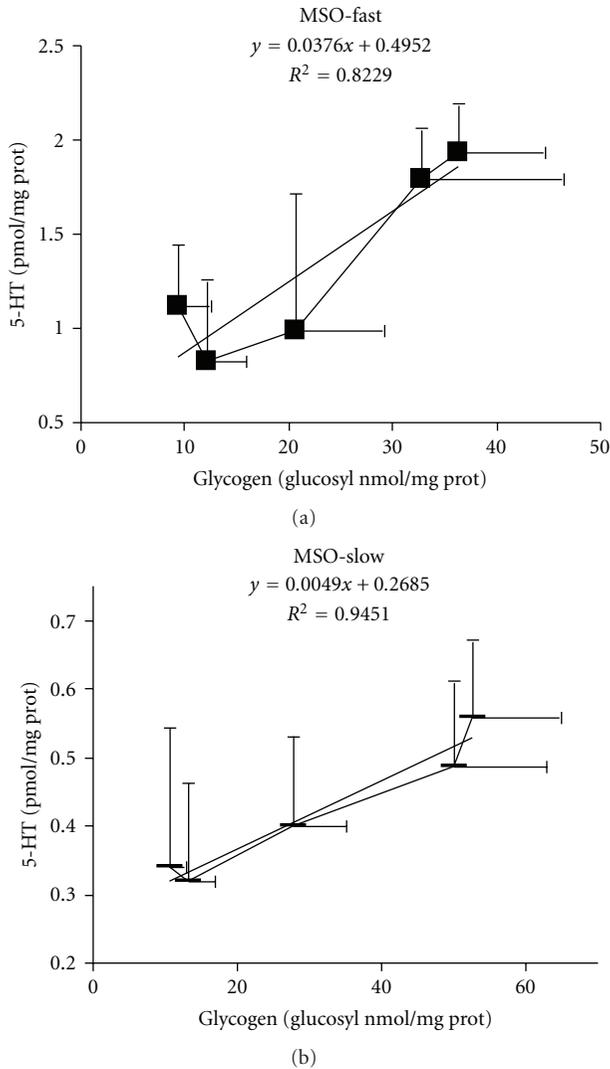


FIGURE 13: Correlation between monoamine content and glycogen content in cerebral cortices of MSO-Fast mice (a) and MSO-Slow mice (b). Data adapted from Cloix et al. [113].

of brain glycogen content and variation in both living human and animal. However, this very existing putative projection in the near future may open new understanding of brain pathologies in which glycogen may be involved. Moreover, new therapeutic agents may be discovered as targeting brain glycogen directly or not, with potential effects on various pathologies.

Abbreviations

ANLSH: Astrocyte-neuron lactate shuttle hypothesis
 BBB: Blood-brain barrier
 C8: Generation 8 of the inbreeding process of selected MSO-Fast and MSO-Slow mice
 cDNA: Complementary DNA
 CNS: Central nervous system
 DCA: Dichloroacetate

EEG: Electroencephalography
 FBPase: Fructose-1,6-bisphosphatase
 G1: Generation 1 obtained by three intercrossing of the eight parental stains of mice
 G-1-P: Glucose-1-phosphate
 G-6-P: Glucose-6-phosphate
 G6: Generation 6 of the selection process of MSO-Fast and MSO-Slow mice
 GABA: γ -aminobutyric acid
 Gln: Glutamine
 Glu: Glutamate
 GLUT: Glucose transporter, facilitative type
 GP: Glycogen phosphorylase
 GS: Glycogen synthase
 KA: Kainic acid
 kDa: Kilo Dalton
 MCT: Monocarbohydrate transporter
 MK-801: 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten 5-10-imine maleate (dizocilpine)
 MSO: Methionine sulfoximine
 MSO-Fast: Mouse line with fast latency to MSO
 MSO-Slow: Mouse line with no, or long, latency to MSO
 NMDA: N-methyl-D-aspartate
 PCR: Polymerase Chain Reaction
 PP1a: Protein phosphatase 1a
 pRK5-Neo: Mammalian expression plasmid
 pRK5-GS: Mammalian expression plasmid containing the full sense of GS cDNA
 pRK5-PBF: Mammalian expression plasmid containing the antisense of FBPase cDNA
 pRK5-SG: Mammalian expression plasmid containing the antisense of GS cDNA
 PTG: Protein targeting to glycogen
 PTZ: Pentylentetrazol
 SEM: Standard error on the mean
 VPA: Valproic acid
 WHO: World Health Organization.

References

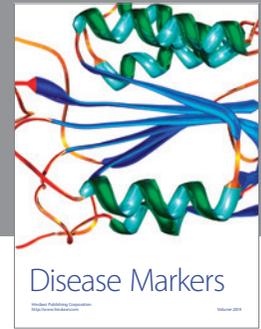
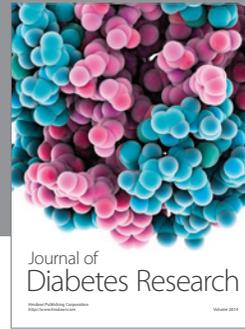
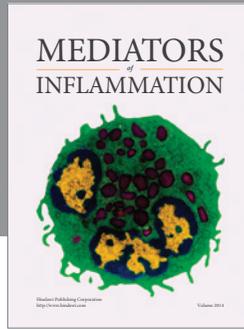
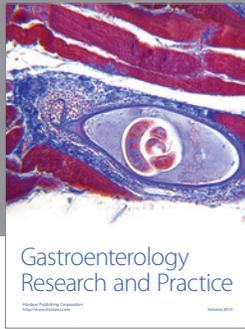
- [1] L. Netchiporouk, N. Shram, D. Salvert, and R. Cespuglio, "Brain extracellular glucose assessed by voltammetry throughout the rat sleep-wake cycle," *European Journal of Neuroscience*, vol. 13, no. 7, pp. 1429–1434, 2001.
- [2] M. Fillenz and J. P. Lowry, "The relation between local cerebral blood flow and extracellular glucose concentration in rat striatum," *Experimental Physiology*, vol. 83, no. 2, pp. 233–238, 1998.
- [3] J. P. Lowry, M. Demestre, and M. Fillenz, "Relation between cerebral blood flow and extracellular glucose in rat striatum during mild hypoxia and hyperoxia," *Developmental Neuroscience*, vol. 20, no. 1, pp. 52–58, 1998.
- [4] M. Fillenz and J. P. Lowry, "Studies of the source of glucose in the extracellular compartment of the rat brain," *Developmental Neuroscience*, vol. 20, no. 4-5, pp. 365–368, 1998.
- [5] J. P. Lowry, M. Miele, R. D. O'Neill, M. G. Boutelle,

- and M. Fillenz, "An amperometric glucose-oxidase/poly(o-phenylenediamine) biosensor for monitoring brain extracellular glucose: in vivo characterisation in the striatum of freely-moving rats," *Journal of Neuroscience Methods*, vol. 79, no. 1, pp. 65–74, 1998.
- [6] J. P. Lowry, R. D. O'Neill, M. G. Boutelle, and M. Fillenz, "Continuous monitoring of extracellular glucose concentrations in the striatum of freely moving rats with an implanted glucose biosensor," *Journal of Neurochemistry*, vol. 70, no. 1, pp. 391–396, 1998.
- [7] M. J. Birnbaum, H. C. Haspel, and O. M. Rosen, "Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 83, no. 16, pp. 5784–5788, 1986.
- [8] W. Sivitz, S. DeSautel, P. S. Walker, and J. E. Pessin, "Regulation of the glucose transporter in developing rat brain," *Endocrinology*, vol. 124, no. 4, pp. 1875–1880, 1989.
- [9] F. Maher, S. Vannucci, J. Takeda, and I. A. Simpson, "Expression of mouse-GLUT3 and human-GLUT3 glucose transporter proteins in brain," *Biochemical and Biophysical Research Communications*, vol. 182, no. 2, pp. 703–711, 1992.
- [10] S. I. Harik, R. N. Kalaria, L. Andersson, P. Lundahl, and G. Perry, "Immunocytochemical localization of the erythroid glucose transporter: abundance in tissues with barrier functions," *Journal of Neuroscience*, vol. 10, no. 12, pp. 3862–3872, 1990.
- [11] C. L. Farrell and W. M. Pardridge, "Blood-brain barrier glucose transporter is asymmetrically distributed on brain capillary endothelial luminal and abluminal membranes: an electron microscopic immunogold study," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 13, pp. 5779–5783, 1991.
- [12] F. Maher, S. J. Vannucci, and I. A. Simpson, "Glucose transporter proteins in brain," *FASEB Journal*, vol. 8, no. 13, pp. 1003–1011, 1994.
- [13] F. Maher and I. A. Simpson, "The GLUT3 glucose transporter is the predominant isoform in primary cultured neurons: assessment by biosynthetic and photoaffinity labelling," *Biochemical Journal*, vol. 301, no. 2, pp. 379–384, 1994.
- [14] T. J. Wheeler, I. A. Simpson, and D. C. Sogin, "Detection of the rat adipose cell glucose transporter with antibody against the human red cell glucose transporter," *Biochemical and Biophysical Research Communications*, vol. 105, no. 1, pp. 89–116, 1982.
- [15] F. Maher and I. A. Simpson, "Modulation of expression of glucose transporters GLUT3 and GLUT1 by potassium and N-methyl-D-aspartate in cultured cerebellar granule neurons," *Molecular and Cellular Neurosciences*, vol. 5, no. 4, pp. 369–375, 1994.
- [16] W. H. Lee and C. A. Bondy, "Ischemic injury induces brain glucose transporter gene expression," *Endocrinology*, vol. 133, no. 6, pp. 2540–2544, 1993.
- [17] D. Z. Gerhart, R. L. Leino, W. E. Taylor, N. D. Borson, and L. R. Drewes, "GLUT1 and GLUT3 gene expression in gerbil brain following brief ischemia: an in situ hybridization study," *Molecular Brain Research*, vol. 25, no. 3-4, pp. 313–322, 1994.
- [18] S. Nagamatsu, H. Sawa, K. Kamada, Y. Nakamichi, K. Yoshimoto, and T. Hoshino, "Neuron-specific glucose transporter (NSGT): CNS distribution of GLUT3 rat glucose transporter (RGT3) in rat central neurons," *FEBS Letters*, vol. 334, no. 3, pp. 289–295, 1993.
- [19] A. L. McCall, A. M. Van Bueren, M. Moholt-Siebert, N. J. Cherry, and W. R. Woodward, "Immunohistochemical localization of the neuron-specific glucose transporter (GLUT3) to neuropil in adult rat brain," *Brain Research*, vol. 659, no. 1-2, pp. 292–297, 1994.
- [20] D. Z. Gerhart, M. A. Broderius, N. D. Borson, and L. R. Drewes, "Neurons and microvessels express the brain glucose transporter protein GLUT3," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 2, pp. 733–737, 1992.
- [21] D. Janigro, "Blood-brain barrier, ion homeostasis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms," *Epilepsy Research*, vol. 37, no. 3, pp. 223–232, 1999.
- [22] I. A. Simpson, A. Carruthers, and S. J. Vannucci, "Supply and demand in cerebral energy metabolism: the role of nutrient transporters," *Journal of Cerebral Blood Flow and Metabolism*, vol. 27, no. 11, pp. 1766–1791, 2007.
- [23] E. Oby and D. Janigro, "The blood-brain barrier and epilepsy," *Epilepsia*, vol. 47, no. 11, pp. 1761–1774, 2006.
- [24] J. Rao, G. Oz, and E. R. Seaquist, "Regulation of cerebral glucose metabolism," *Minerva Endocrinologica*, vol. 31, no. 2, pp. 149–158, 2006.
- [25] A. M. Brown and B. R. Ransom, "Astrocyte glycogen and brain energy metabolism," *Glia*, vol. 55, no. 12, pp. 1263–1271, 2007.
- [26] L. Hertz, "The astrocyte-neuron lactate shuttle: a challenge of a challenge," *Journal of Cerebral Blood Flow and Metabolism*, vol. 24, no. 11, pp. 1241–1248, 2004.
- [27] L. Pellerin, G. Bonvento, J.-Y. Chatton, K. Pierre, and P. J. Magistretti, "Role of neuron-glia interaction in the regulation of brain glucose utilization," *Diabetes, Nutrition and Metabolism*, vol. 15, no. 5, pp. 268–273, 2002.
- [28] L. Pellerin, A. K. Bouzier-Sore, A. Aubert et al., "Activity-dependent regulation of energy metabolism by astrocytes: an update," *Glia*, vol. 55, no. 12, pp. 1251–1262, 2007.
- [29] P. J. Magistretti and L. Pellerin, "The contribution of astrocytes to the 18F-2-deoxyglucose signal in PET activation studies," *Molecular Psychiatry*, vol. 1, no. 6, pp. 445–452, 1996.
- [30] I. Y. Choi, E. R. Seaquist, and R. Gruetter, "Effect of hypoglycemia on brain glycogen metabolism in vivo," *Journal of Neuroscience Research*, vol. 72, no. 1, pp. 25–32, 2003.
- [31] P. J. Roach, A. V. Skurat, and R. A. Harris, "Regulation of glycogen metabolism," in *Handbook of Physiology*, L. S. Jefferson and A. D. Cherrington, Eds., pp. 609–647, Oxford University Press, New York, NY, USA, 2001.
- [32] P. J. Roach, "Glycogen and its metabolism," *Current Molecular Medicine*, vol. 2, no. 2, pp. 101–120, 2002.
- [33] J. Lomako, W. M. Lomako, and W. J. Whelan, "A self-glycosylating protein is the primer for rabbit muscle glycogen biosynthesis," *FASEB Journal*, vol. 2, no. 15, pp. 3097–3103, 1988.
- [34] J. Pitcher, C. Smythe, and P. Cohen, "Glycogenin is the priming glucosyltransferase required for the initiation of glycogen biogenesis in rabbit skeletal muscle," *European Journal of Biochemistry*, vol. 176, no. 2, pp. 391–395, 1988.
- [35] C. Smythe and P. Cohen, "The discovery of glycogenin and the priming mechanism for glycogen biogenesis," *European Journal of Biochemistry*, vol. 200, no. 3, pp. 625–631, 1991.
- [36] M. D. Alonso, J. Lomako, W. M. Lomako, and W. J. Whelan, "A new look at the biogenesis of glycogen," *FASEB Journal*, vol. 9, no. 12, pp. 1126–1137, 1995.
- [37] L. F. Leloir, J. M. Olavarria, S. H. Goldemberg, and H. Carmignatti, "Biosynthesis of glycogen from uridine diphosphate

- glucose," *Archives of Biochemistry and Biophysics*, vol. 81, no. 2, pp. 508–520, 1959.
- [38] S. M. Crosson, A. Khan, J. Printen, J. E. Pessin, and A. R. Saltiel, "PTG gene deletion causes impaired glycogen synthesis and developmental insulin resistance," *Journal of Clinical Investigation*, vol. 111, no. 9, pp. 1423–1432, 2003.
- [39] C. Lerín, E. Montell, H. K. Berman, C. B. Newgard, and A. M. Gómez-Foix, "Overexpression of protein targeting to glycogen in cultured human muscle cells stimulates glycogen synthesis independent of glycogen and glucose 6-phosphate levels," *Journal of Biological Chemistry*, vol. 275, no. 51, pp. 39991–39995, 2000.
- [40] J. A. Printen, M. J. Brady, and A. R. Saltiel, "PTG, a protein phosphate 1-binding protein with a role in glycogen metabolism," *Science*, vol. 275, no. 5305, pp. 1475–1478, 1997.
- [41] J. F. Cloix, M. Y. Ardourel, and T. Hévor, "Glycogen metabolism and brain pathologies," *Central Nervous System Agents in Medicinal Chemistry*, vol. 8, no. 3, pp. 187–197, 2008.
- [42] A. M. Brown, R. Wender, and B. R. Ransom, "Metabolic substrates other than glucose support axon function in central white matter," *Journal of Neuroscience Research*, vol. 66, no. 5, pp. 839–843, 2001.
- [43] J. M. Medina and A. Taberero, "Lactate utilization by brain cells and its role in CNS development," *Journal of Neuroscience Research*, vol. 79, no. 1–2, pp. 2–10, 2005.
- [44] L. Pellerin, G. Pellegrini, J. L. Martin, and P. J. Magistretti, "Expression of monocarboxylate transporter mRNAs in mouse brain: support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 7, pp. 3990–3995, 1998.
- [45] H. Wiesinger, B. Hamprecht, and R. Dringen, "Metabolic pathways for glucose in astrocytes," *Glia*, vol. 21, no. 1, pp. 22–34, 1997.
- [46] P. J. Magistretti, "Neuron-glia metabolic coupling and plasticity," *Journal of Experimental Biology*, vol. 209, no. 12, pp. 2304–2311, 2006.
- [47] P. J. Magistretti, L. Pellerin, D. L. Rothman, and R. G. Shulman, "Energy on demand," *Science*, vol. 283, no. 5401, pp. 496–497, 1999.
- [48] D. L. Rothman, N. R. Sibson, F. Hyder, J. Shen, K. L. Behar, and R. G. Shulman, "In vivo nuclear magnetic resonance spectroscopy studies of the relationship between the glutamate-glutamine neurotransmitter cycle and functional neuroenergetics," *Philosophical Transactions of the Royal Society B*, vol. 354, no. 1387, pp. 1165–1177, 1999.
- [49] N. R. Sibson, A. Dhankhar, G. F. Mason, D. L. Rothman, K. L. Behar, and R. G. Shulman, "Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 1, pp. 316–321, 1998.
- [50] A. M. Brown, "Brain glycogen re-awakened," *Journal of Neurochemistry*, vol. 89, no. 3, pp. 537–552, 2004.
- [51] A. M. Brown, S. B. Tekkök, and B. R. Ransom, "Energy transfer from astrocytes to axons: the role of CNS glycogen," *Neurochemistry International*, vol. 45, no. 4, pp. 529–536, 2004.
- [52] A. M. Brown, H. M. Sickmann, K. Fosgerau et al., "Astrocyte glycogen metabolism is required for neural activity during aglycemia or intense stimulation in mouse white matter," *Journal of Neuroscience Research*, vol. 79, no. 1–2, pp. 74–80, 2005.
- [53] K. Pierre and L. Pellerin, "Monocarboxylate transporters in the central nervous system: distribution, regulation and function," *Journal of Neurochemistry*, vol. 94, no. 1, pp. 1–14, 2005.
- [54] D. Govender, P. K. Ramdial, B. Clarke, and R. Chetty, "Clear cell (glycogen-rich) gastric adenocarcinoma," *Annals of Diagnostic Pathology*, vol. 8, no. 2, pp. 69–73, 2004.
- [55] J. Albores-Saavedra, X. Gu, and M. A. Luna, "Clear cells and thyroid transcription factor I reactivity in sclerosing mucoepidermoid carcinoma of the thyroid gland," *Annals of Diagnostic Pathology*, vol. 7, no. 6, pp. 348–353, 2003.
- [56] F. Gorin, W. Harley, J. Schmier, B. Lyeth, and T. Jue, "Perinecrotic glioma proliferation and metabolic profile within an intracerebral tumor xenograft," *Acta Neuropathologica*, vol. 107, no. 3, pp. 235–244, 2004.
- [57] O. H. Lowry, S. J. Berger, and J. G. Carter, "Diversity of metabolic patterns in human brain tumors: enzymes of energy metabolism and related metabolites and cofactors," *Journal of Neurochemistry*, vol. 41, no. 4, pp. 994–1010, 1983.
- [58] M. Rousset, A. Zweibaum, and J. Fogh, "Presence of glycogen and growth-related variations in 58 cultured human tumor cell lines of various tissue origins," *Cancer Research*, vol. 41, no. 3, pp. 1165–1170, 1981.
- [59] T. Sato, A. Nakamura, H. Shibuya, W. Shirai, and H. Koie, "Cerebral high-grade astrocytoma (glioblastoma) in a cat," *Journal of Veterinary Medicine Series A*, vol. 50, no. 5, pp. 269–271, 2003.
- [60] M. Wrensch, T. Rice, R. Miike et al., "Diagnostic, treatment, and demographic factors influencing survival in a population-based study of adult glioma patients in the San Francisco Bay Area," *Neuro-Oncology*, vol. 8, no. 1, pp. 12–26, 2006.
- [61] M. E. Beckner, G. T. Gobbel, R. Abounader et al., "Glycolytic glioma cells with active glycogen synthase are sensitive to PTEN and inhibitors of PI3K and gluconeogenesis," *Laboratory Investigation*, vol. 85, no. 12, pp. 1457–1470, 2005.
- [62] O. Mbarek, V. Vergé, and T. Hevor, "Direct cloning of astrocytes from primary culture without previous immortalization," *In Vitro Cellular and Developmental Biology—Animal*, vol. 34, no. 5, pp. 401–411, 1998.
- [63] R. A. Gatenby and R. J. Gillies, "Why do cancers have high aerobic glycolysis?" *Nature Reviews Cancer*, vol. 4, no. 11, pp. 891–899, 2004.
- [64] R. J. Shaw, "Glucose metabolism and cancer," *Current Opinion in Cell Biology*, vol. 18, no. 6, pp. 598–608, 2006.
- [65] E. D. Michelakis, G. Sutendra, P. Dromparis et al., "Metabolic modulation of glioblastoma with dichloroacetate," *Science Translational Medicine*, vol. 2, no. 31, p. 31ra34, 2010.
- [66] F. E. Dreifuss, M. Martinez-Lage, and J. Roger, "Proposal for classification of epilepsies and epileptic syndromes," *Epilepsia*, vol. 26, no. 3, pp. 268–278, 1985.
- [67] F. E. Dreifuss, "Classification of the epilepsies: influence on management," *Revue Neurologique*, vol. 143, no. 5, pp. 375–380, 1987.
- [68] H. G. Wieser, W. T. Blume, D. Fish et al., "Proposal for a new classification of outcome with respect to epileptic seizures following epilepsy surgery," *Epilepsia*, vol. 42, no. 2, pp. 282–286, 2001.
- [69] J. Roger, F. E. Dreifuss, M. Martinez-Lage et al., "Proposal for revised classification of epilepsies and epileptic syndromes," *Epilepsia*, vol. 30, no. 4, pp. 389–399, 1989.
- [70] F. E. Dreifuss, J. Bancaud, and O. Henriksen, "Proposal for revised clinical and electroencephalographic classification

- of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy," *Epilepsia*, vol. 22, no. 4, pp. 489–501, 1981.
- [71] A. D. Everitt and J. W.A.S. Sande, "Classification of the epilepsies: time for a change?" *European Neurology*, vol. 42, no. 1, pp. 1–10, 1999.
- [72] S. Rampp and H. Stefan, "Fast activity as a surrogate marker of epileptic network function?" *Clinical Neurophysiology*, vol. 117, no. 10, pp. 2111–2117, 2006.
- [73] M. V. Johnston, "Developmental aspects of epileptogenesis," *Epilepsia*, vol. 37, no. 1, pp. S2–S9, 1996.
- [74] F. E. Dudek, P. R. Patrylo, and J. P. Wuarin, "Mechanisms of neuronal synchronization during epileptiform activity," *Advances in Neurology*, vol. 79, pp. 699–708, 1999.
- [75] D. A. McCormick and D. Contreras, "On the cellular and network bases of epileptic seizures," *Annual Review of Physiology*, vol. 63, pp. 815–846, 2001.
- [76] P. J. Uhlhaas and W. Singer, "Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology," *Neuron*, vol. 52, no. 1, pp. 155–168, 2006.
- [77] H. E. Scharfman, "The neurobiology of epilepsy," *Current Neurology and Neuroscience Reports*, vol. 7, no. 4, pp. 348–354, 2007.
- [78] J. Engel Jr., W. J. Brown, and D. E. Kuhl, "Pathological findings underlying focal temporal lobe hypometabolism in partial epilepsy," *Annals of Neurology*, vol. 12, no. 6, pp. 518–528, 1982.
- [79] J. Engel Jr., D. E. Kuhl, and M. E. Phelps, "Local cerebral metabolism during partial seizures," *Neurology*, vol. 33, no. 4, pp. 400–413, 1983.
- [80] Y. Y. Sitoh and R. D. Tien, "Neuroimaging in epilepsy," *Journal of Magnetic Resonance Imaging*, vol. 8, no. 2, pp. 277–288, 1998.
- [81] M. K. Dalsgaard, F. F. Madsen, N. H. Secher, H. Laursen, and B. Quistorff, "High glycogen levels in the hippocampus of patients with epilepsy," *Journal of Cerebral Blood Flow and Metabolism*, vol. 27, no. 6, pp. 1137–1141, 2007.
- [82] K. Bernard-Hélary, M. Y. Ardourel, T. Hévor, and J. F. Cloix, "In vivo and in vitro glycolytic effects of methionine sulfoximine are different in two inbred strains of mice," *Brain Research*, vol. 929, no. 2, pp. 147–155, 2002.
- [83] P. Delorme and T. K. Hevor, "Glycogen particles in methionine sulfoximine epileptogenic rodent brain and liver after the administration of methionine and actinomycin D," *Neuropathology and Applied Neurobiology*, vol. 11, no. 2, pp. 117–128, 1985.
- [84] T. K. Hevor, "Some aspects of carbohydrate metabolism in the brain," *Biochimie*, vol. 76, no. 2, pp. 111–120, 1994.
- [85] T. K. Hevor and P. Delorme, "Biochemical and ultrastructural study of glycogen in cultured astrocytes submitted to the convulsant methionine sulfoximine," *Glia*, vol. 4, no. 1, pp. 64–69, 1991.
- [86] T. K. Hevor, P. Delorme, and J. C. Beauvillain, "Glycogen synthesis and immunocytochemical study of fructose-1,6-bisphosphatase in methionine sulfoximine epileptogenic rodent brain," *Journal of Cerebral Blood Flow and Metabolism*, vol. 6, no. 3, pp. 292–297, 1986.
- [87] C. H. Phelps, "An ultrastructural study of methionine sulfoximine induced glycogen accumulation in astrocytes of the mouse cerebral cortex," *Journal of Neurocytology*, vol. 4, no. 4, pp. 479–490, 1975.
- [88] J. Folbergrová, "Glycogen and glycogen phosphorylase in the cerebral cortex of mice under the influence of methionine sulfoximine," *Journal of Neurochemistry*, vol. 20, no. 2, pp. 547–557, 1973.
- [89] J. Folbergrová, J. V. Passonneau, O. H. Lowry, and D. W. Schulz, "Glycogen, ammonia and related metabolites in the brain during seizures evoked by methionine sulfoximine," *Journal of Neurochemistry*, vol. 16, no. 2, pp. 191–203, 1969.
- [90] K. Bernard-Hélary, E. Lapouble, M. Ardourel, T. Hévor, and J. F. Cloix, "Correlation between brain glycogen and convulsive state in mice submitted to methionine sulfoximine," *Life Sciences*, vol. 67, no. 14, pp. 1773–1781, 2000.
- [91] T. K. Hevor, P. Delorme, and J. Gayet, "Glycogen content and fructose-1, 6-bisphosphatase activity in methionine sulfoximine epileptogenic mouse brain and liver after protein synthesis inhibition," *Neuropathology and Applied Neurobiology*, vol. 11, no. 2, pp. 129–139, 1985.
- [92] J. Folbergrová, R. Druga, R. Haugvicová, P. Mareš, and J. Otáhal, "Anticonvulsant and neuroprotective effect of (S)-3,4-dicarboxyphenylglycine against seizures induced in immature rats by homocysteic acid," *Neuropharmacology*, vol. 54, no. 4, pp. 665–675, 2008.
- [93] W. Wang, H. Lohi, A. V. Skurat, A. A. DePaoli-Roach, B. A. Minassian, and P. J. Roach, "Glycogen metabolism in tissues from a mouse model of Lafora disease," *Archives of Biochemistry and Biophysics*, vol. 457, no. 2, pp. 264–269, 2007.
- [94] D. Vilchez, S. Ros, D. Cifuentes et al., "Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy," *Nature Neuroscience*, vol. 10, no. 11, pp. 1407–1413, 2007.
- [95] E. L. Peters and D. B. Tower, "Glutamic acid and glutamine metabolism in cerebral cortex after seizures induced by methionine sulfoximine," *Journal of Neurochemistry*, vol. 5, pp. 80–90, 1959.
- [96] A. Meister, "Inhibition of glutamine synthase and gamma glutamylcysteine synthetase by methionine sulfoximine and related compounds," in *Enzyme Activated Irreversible Inhibitors*, N. Seiler, M. J. Jung, and J. Koch-Weser, Eds., pp. 187–210, Elsevier, Amsterdam, The Netherlands, 1978.
- [97] D. L. Somers and R. M. Beckstead, "Chronic methionine sulfoximine administration reduces synaptosomal aspartate and glutamate in rat striatum," *Neuroscience Letters*, vol. 115, no. 2-3, pp. 335–340, 1990.
- [98] F. Fonnum and R. E. Paulsen, "Comparison of transmitter amino acid levels in rat globus pallidus and neostriatum during hypoglycemia or after treatment with methionine sulfoximine or γ -vinyl γ -aminobutyric acid," *Journal of Neurochemistry*, vol. 54, no. 4, pp. 1253–1257, 1990.
- [99] B. Engelsens and F. Fonnum, "The effect of methioninesulfoximine, an inhibitor of glutamine synthetase, on the levels of amino acids in the intact and decorticated rat neostriatum," *Brain Research*, vol. 338, no. 1, pp. 165–168, 1985.
- [100] M. Blin, W. E. Crusio, T. Hévor, and J. F. Cloix, "Chronic inhibition of glutamine synthetase is not associated with impairment of learning and memory in mice," *Brain Research Bulletin*, vol. 57, no. 1, pp. 11–15, 2002.
- [101] J. F. Cloix, E. Beaulieu, and T. Hévor, "Various fructose-1,6-bisphosphatase mRNAs in mouse brain, liver, kidney and heart," *NeuroReport*, vol. 8, no. 3, pp. 617–622, 1997.
- [102] K. Bernard-Hélary, M. Ardourel, P. Magistretti, T. Hévor, and J. F. Cloix, "Stable transfection of cDNAs targeting specific steps of glycogen metabolism supports the existence of active gluconeogenesis in mouse cultured astrocytes," *Glia*, vol. 37, no. 4, pp. 379–382, 2002.
- [103] G. Pellegrini, C. Rossier, P. J. Magistretti, and J. L. Martin,

- “Cloning, localization and induction of mouse brain glycogen synthase,” *Molecular Brain Research*, vol. 38, no. 2, pp. 191–199, 1996.
- [104] M. Ardourel, M. Blin, J. L. Moret et al., “A new putative target for antisense gene therapy of glioma: glycogen synthase,” *Cancer Biology and Therapy*, vol. 6, no. 5, pp. 719–723, 2007.
- [105] V. Vergé, A. Legrand, and T. Hévor, “Isolation of carbohydrate metabolic clones from cultured astrocytes,” *Glia*, vol. 18, no. 3, pp. 244–254, 1996.
- [106] P. Benda, J. Lightbody, G. Sato, L. Levine, and W. Sweet, “Differentiated rat glial cell strain in tissue culture,” *Science*, vol. 161, no. 3839, pp. 370–371, 1968.
- [107] R. Gruetter, “In vivo ^{13}C NMR studies of compartmentalized cerebral carbohydrate metabolism,” *Neurochemistry International*, vol. 41, no. 2-3, pp. 143–154, 2002.
- [108] R. Gruetter, G. Adriany, I. Y. Choi, P. G. Henry, H. Lei, and G. Öz, “Localized in vivo ^{13}C NMR spectroscopy of the brain,” *NMR in Biomedicine*, vol. 16, no. 6-7, pp. 313–338, 2003.
- [109] G. Öz, P. G. Henry, E. R. Seaquist, and R. Gruetter, “Direct, noninvasive measurement of brain glycogen metabolism in humans,” *Neurochemistry International*, vol. 43, no. 4-5, pp. 323–329, 2003.
- [110] G. Öz, E. R. Seaquist, A. Kumar et al., “Human brain glycogen content and metabolism: implications on its role in brain energy metabolism,” *American Journal of Physiology*, vol. 292, no. 3, pp. E946–E951, 2007.
- [111] V. Verge and T. K. Hevor, “Regulation of fructose-1,6-bisphosphatase activity in primary cultured astrocytes,” *Neurochemical Research*, vol. 20, no. 9, pp. 1049–1056, 1995.
- [112] J. F. Cloix, Z. Tahí, B. Martin, and T. Hévor, “Selection of two lines of mice based on latency to onset of methionine sulfoximine seizures,” *Epilepsia*, vol. 51, no. 1, pp. 118–126, 2010.
- [113] J.-F. Cloix, Z. Tahí, A. Boissonnet, and T. Hévor, “Brain glycogen and neurotransmitter levels in fast and slow methionine sulfoximine-selected mice,” *Experimental Neurology*, vol. 225, no. 2, pp. 274–283, 2010.
- [114] A. Boissonnet, *Biological and biochemical analyses of mice selected based upon their seizures latency toward methionine sulfoximine*, Thesis defence, Université de Orléans, 2011.
- [115] D. A. Blizard and V. Balkoski, “Tryptophan availability, central serotonergic function and methionine sulphoximine-induced convulsions,” *Neuropharmacology*, vol. 21, no. 1, pp. 27–30, 1982.
- [116] T. K. Hevor and P. Delorme, “Possible involvement of indolamines in the glycolytic effect of the convulsant methionine sulfoximine in rat brain,” *Experientia*, vol. 46, no. 7, pp. 710–713, 1990.
- [117] A. J. Pennington and V. W. Pentreath, “Transmitter-induced glycogenolysis and gluconeogenesis in leech segmental ganglia,” *Journal de Physiologie*, vol. 82, no. 4, pp. 218–228, 1987.
- [118] S. Verleysdonk, S. Kistner, B. Pfeiffer-Guglielmi et al., “Glycogen metabolism in rat ependymal primary cultures: regulation by serotonin,” *Brain Research*, vol. 1060, no. 1-2, pp. 89–99, 2005.
- [119] I. Allaman, L. Pellerin, and P. J. Magistretti, “Glucocorticoids modulate neurotransmitter-induced glycogen metabolism in cultured cortical astrocytes,” *Journal of Neurochemistry*, vol. 88, no. 4, pp. 900–908, 2004.
- [120] P. J. Magistretti, O. Sorg, N. Yu, J. L. Martin, and L. Pellerin, “Neurotransmitters regulate energy metabolism in astrocytes: implications for the metabolic trafficking between neural cells,” *Developmental Neuroscience*, vol. 15, no. 3-5, pp. 306–312, 1993.
- [121] L. Pellerin, M. Stolz, O. Sorg, J. L. Martin, C. F. Deschepper, and P. J. Magistretti, “Regulation of energy metabolism by neurotransmitters in astrocytes in primary culture and in an immortalized cell line,” *Glia*, vol. 21, no. 1, pp. 74–83, 1997.
- [122] T. Otori, J. C. Friedland, G. Sinson, T. K. McIntosh, R. Raghupathi, and F. A. Welsh, “Traumatic brain injury elevates glycogen and induces tolerance to ischemia in rat brain,” *Journal of Neurotrauma*, vol. 21, no. 6, pp. 707–718, 2004.
- [123] L. Xu and H. Sun, “Pharmacological manipulation of brain glycogenolysis as a therapeutic approach to cerebral ischemia,” *Mimi-Reviews in Medicinal Chemistry*, vol. 10, no. 12, pp. 1188–1193, 2010.
- [124] W. S. Sang, J. P. Bergher, C. M. Anderson, J. L. Treadway, K. Fosgerau, and R. A. Swanson, “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),” *Journal of Pharmacology and Experimental Therapeutics*, vol. 321, no. 1, pp. 45–50, 2007.



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