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Research Report

Pentylenetetrazol-induced status epilepticus up-regulates the expression of glucose transporter mRNAs but not proteins in the immature rat brain

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LCMRglc(s), local cerebral metabolic rate(s) for glucose
PTZ, pentylenetetrazol
SE, status epilepticus

ABSTRACT

Prolonged pentylenetetrazol (PTZ)-induced seizures increase cerebral energy demands in a region-specific manner. During PTZ seizures, cerebral glucose utilization increases over control levels in all brain regions at 10 days while 21-day-old rats exhibit increases, decreases or no change. To explore the effects of such acute changes in metabolic demand on the expression of glucose transporter proteins mediating glucose delivery to brain, we studied the consequences of PTZ seizures on GLUT1 and GLUT3 mRNAs and proteins between 1 and 72 h after seizure induction. At both ages, seizures induced a rapid up-regulation of GLUT1 and GLUT3 mRNAs which was prominent at 1 and 4 h, and was greater at 10 than at 21 days. By 24 h and 72 h, the levels of the mRNAs of the two transporter returned to control levels or were slightly down-regulated. The levels of GLUT1 and GLUT3 proteins were not affected by the seizures and only scattered decreases in GLUT3 protein were recorded, mainly in midbrain–brainstem areas. These data show that acute pentylenetetrazol seizures induce a rapid up-regulation of the GLUT1 and GLUT3 mRNAs, but do not result in measurable increases in protein levels, suggesting translational regulation.

1. Introduction

Glucose is the primary energy substrate for the adult brain. The immature rat brain uses ketone bodies together with glucose but even at that age, the brain cannot function without glucose (Nehlig and Pereira de Vasconcelos, 1993). Adequate glucose delivery to the brain takes on increased importance during prolonged seizure activity, when cerebral energy and glycolytic demand can increase up to 6-fold (Fernandes et al., 1999; Pardridge, 1983). A central question has traditionally focused on the extent to which the capacity to transport glucose to the brain may become limiting to the duration of seizure activity, or contribute to cellular energy failure and dysfunction. Much of the debate on this topic occurred prior to the identification of the family of proteins responsible for glucose transport, the facilitative glucose

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transporters or GLUTs. During the past decade, it has been demonstrated that cerebral glucose uptake and utilization are facilitated primarily by the glucose transporter proteins, GLUT1 and GLUT3. GLUT1 is localized in the blood–brain barrier (BBB) endothelium as a highly glycosylated protein (55 kDa isoform); a less glycosylated product (45 kDa) of the same gene is expressed in non-vascular cells, primarily glia, and the choroid plexus (Gerhart et al., 1989; Maher et al., 1992, 1993; Vannucci et al., 1998b). GLUT3 in the brain is expressed exclusively in neurons (Maher et al., 1992, 1993, 1994; Vannucci et al., 1998b). All of these proteins are developmentally regulated and increase their mRNA and protein expression postnatally in direct relation to cerebral maturation and increase in glucose utilization (Bondy et al., 1992; Devaskar et al., 1991; Kahn et al., 1999; Nehlig et al., 1988; Vannucci, 1994; Vannucci et al., 1997, 1998b; Zeller et al., 1996).

Under normal physiological conditions, the major sites of regulation in glucose metabolism are glucose phosphorylation by hexokinase and fructose-6P phosphorylation by phosphofructokinase (Clarke and Sokoloff, 1999). However, during hypermetabolic states, such as seizures, the rate of glycolytic demand may approach or even exceed the rate of glucose transport (Nehlig and Pereira de Vasconcelos, 1996) and glucose transport itself may become rate-limiting. This was reported to occur during status epilepticus (SE) in the adult brain (Chapman et al., 1977; Meldrum et al., 1983; Siesjö et al., 1983) and the immature rat brain (Fujikawa et al., 1988; Wasterlain et al., 1989), considering the depletion of the energy reserves of the seizing brain. The effects of seizures on brain glucose transporters have only been studied in adult animals or humans, and these studies suggest an increased expression of both GLUT1 and GLUT3 (Cornford et al., 2000; Gronlund et al., 1996; Ho et al., 1993; Lawrence et al., 1995). Cerebral glucose metabolism, as well as the expression of GLUT1 and GLUT3, undergo active changes in the developing brain, mainly between 10 and 21 days after birth (Bondy et al., 1992; Devaskar et al., 1991; Kahn et al., 1999; Nehlig and Pereira de Vasconcelos, 1993, Nehlig et al., 1988; Vannucci, 1994; Vannucci et al., 1994, 1997; Zeller et al., 1996). Likewise, the cerebral metabolic response to PTZ-induced SE is quite different between 10- (P10) and 21-day-old rats (P21). Indeed, in a model of SE based on repetitive low dose injections of pentylenetetrazol (PTZ) in the immature rat, we showed that local cerebral metabolic rates for glucose (LCMRglcs) largely increase over control levels (200–400%) in P10 rats, while they increase, decrease or do not change compared to control animals in P21 rats (Pereira de Vasconcelos et al., 1992). Therefore, we proposed that PTZ-induced SE would differently affect GLUT1 and GLUT3 expression at the two ages.

This study investigated the regional and temporal changes of GLUT1 and GLUT3 mRNA and protein expression occurring between 1 and 72 h post-SE and compared them with the previously observed changes in neuronal activation and LCMRglcs induced by PTZ. The results demonstrate a relationship between increased neuronal activity, metabolic demand, and transcriptional activation of GLUT1 and GLUT3, but further suggest an additional level of translational regulation as corresponding changes in protein were not detected.

2. Results

2.1. *Seizure expression and behavior*

In response to PTZ, rats expressed clinical signs of seizures similar to those previously described (El Hamdi et al., 1992). Briefly, P10 rats exhibited prostration, hyperventilation, scratching and tremor followed by tonic–clonic seizures. At P21, the first signs were hyperkinesia, running phases, scratching, wet-dog shakes, and rearings. Whole body tremors, clonic seizures of the face and chewing were followed by wild running and tonic–clonic seizures similar to but more intense than those occurring at P10. The tonic phase characterizing the onset of SE usually started after a mean dose of 60–70 and 80–90 mg/kg PTZ at P10 and P21, respectively. It was characterized by the loss of quadruped posture followed by flexions and extensions of the limbs and poorly coordinated running movements. The mean duration of seizure activity reached approximately 60 min in P10 rats and 80 min in P21 rats, as previously reported (El Hamdi et al., 1992).

2.2. *Effects of seizures on GLUT1 and GLUT3 mRNA expression and on LCMRglcs*

The distribution of GLUT1 mRNA expression was relatively homogeneous throughout the brain, characterized by intense punctate signal in microvessels and in ependyma, with less intense and more diffuse signal throughout the parenchyma, as can be seen in P21 rats in Fig. 1 and as previously shown (Bondy et al., 1992; Vannucci et al., 1998b). The distribution of GLUT3 mRNA expression, reflected its neuronal localization, as seen in Fig. 1, and as previously shown (Vannucci et al., 1998b). At P10, the distribution of GLUT1 and GLUT3 mRNAs was similar to that observed at P21, with lower levels of expression (data not shown), in agreement with previous studies (Vannucci et al., 1998b).

Alterations in GLUT1 and GLUT3 mRNA expression at 1, 4, 24 and 72 h following SE in P10 rats are shown in Figs. 2, 3 Corresponding changes in LCMRglc, as previously measured between 10 and 55 min after the onset of PTZ-induced status epilepticus (Pereira de Vasconcelos et al., 1992) are included for comparison. In P10 rats, PTZ seizures induced a 58–178% increase in the levels of GLUT1 mRNA as early as 1 h after the onset of SE (Figs. 2, 3). This increase was significant in all regions studied and persisted at 4 h in the piriform cortex (+132%) and in the brainstem, mainly the inferior colliculus and medullary reticular formation (+45–49%). At 24 h after SE, GLUT1 mRNA levels returned to normal in the cerebral cortex, but were 30–48% lower than control levels in all other regions; at 72 h after SE GLUT1 mRNAs were lower than control levels in most regions studied. In P10 rats, PTZ seizures induced large increases in GLUT3 mRNA levels at 1 h after the onset of SE, primarily in the cerebral cortex, forebrain, and hypothalamus (+122–200%); more moderate increases were observed in the CA3 area, thalamic, and brainstem regions (Figs. 2, 3). At 4 h after SE induction, GLUT3 mRNA levels remained higher than controls in the parietal cortex, amygdala, hippocampal CA3 area, medial geniculate body and the entire brainstem. At 24