The Role of Energy Substrates in Astrocyte Calcium Activity of Rat Hippocampus in Early Postnatal Ontogenesis

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The aim of the investigation was to study the astroglia functional activity in hippocampal slices of the rat at different postnatal development stages by modeling metabolic changes in the brain.

Materials and Methods. The study was carried out on hippocampal slices of Wistar rats of three age groups: postnatal days 5–10, 10–20, and more than 20 days of animal postnatal development using a functional calcium imaging method.

Results. In early developmental stages, normal astroglial calcium activity was found to require additional energy substrates. Changing the brain metabolic state at low temperature had no significant effect on astrocyte calcium activity in all postnatal periods under study.

Conclusion. The study of astrocyte calcium dynamics in different periods of postnatal development can be used as a method to assess functional activity of glial systems when modeling brain metabolic disorders.

Key words: astrocyte; calcium responses; specific energy substrates.

Astroglia is the most numerous glial cells performing a number of critical functions in brain. Astrocytes participate in potassium ion buffering, and regulate local blood flow causing vasoconstriction or vasodilatation. Trophic factors expressing in astrocytes have an effect on neuron growth and the formation of new synapses. Astrocytes have great importance in neuron nourishment by supplying them with glucose and other substances that can serve as energy substrate for neurons [1]. They can also be the main glycogen depot in brain [2].

Astrocytes are electrically inactive cells, though they have their own signal system presented by the generation of calcium responses, the duration of which can be up to several seconds [3]. Recent studies [4] have shown astrocytes to be of importance in memory formation by lactate delivered to neurons. In active neuronal work, as well in some nervous system pathologies, lactate is synthesized in astrocytes, lactate being the source of energy for neurons. Since neuronal activity depends on availability of energy substrates, the process can also be considered as a mechanism used by astrocytes to regulate neuronal functioning [5].

Lactate and pyruvate are basic energy substrates in a developing brain, as well as in adult brain, if glucose level is low [6]. Moreover, ketone bodies [7] have been shown to act as an energy substrate in the brain of young animals. Ketone bodies represent a group of organic compounds, which are intermediate products of fat, carbohydrate and protein metabolism, namely: acetoacetic acid (acetoacetate), β-hydroxy-butanoic acid (β-oxybutyrate or D-3-hydroxybutyrate) and acetone. Rat milk contains a great number of fatty acids, their cleavage resulting in ketone bodies formation. They are likely to have an effect on neuronal activity in an early postnatal period [8]. However age-related changes of calcium activity in astrocytes, as well as the effect of brain metabolic changes are still understudied.

The aim of the investigation was to study the effect of brain metabolic changes such as the age of animal postnatal development, temperature conditions, and the presence of specific energy substrates, on functional (calcium) astrocyte activity in rat hippocampus.

Materials and Methods. In the study we used acute hippocampal slices of Wistar rats of three age groups: postnatal days 5–10, 10–20, and over 20 days of postnatal development. Basic experimental animal management regulations conformed to the standards as defined in Order of Ministry of Health of the Russian Federation No.267 dated 19.06.03 “Concerning approval of the rules for laboratory practice in Russian Federation”. The study was carried out in accordance with the European Convention for Protection of Vertebrate Animals used

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with Experimental and other Scientific Purposes (the Convention took place in Strasbourg on March, 18, 1986 and was confirmed in Strasbourg on June, 15, 2006) and approved by Ethics Committee of Lobachevsky State University of Nizhni Novgorod.

The animals were anesthetized with ether and decapitated, their brain being placed in a special chamber filled with Ringer's solution at temperature close to 0°C. The solution was keeping saturated with carbogene (5% CO_2 + 95% O_2). The solution composition was as follows (mmol): NaCl — 124.0; KCl — 2.5; $MgSO_4$ — 8.48; NaH_2PO_4 — 1.24; $NaHCO_3$ — 26.2; $CaCl_2$ — 0.5; D-Glucose — 11.0. Hippocampal slices, 350 μm thick, were prepared using a vibratome Microm (Germany). Before the slices were put into the chamber, they had been stored in high magnesium ion solution to reduce the sectioning effects, the solution containing (mmol): NaCl — 124.0; KCl — 2.5; $MgSO_4$ — 1.3; NaH_2PO_4 — 1.0; $NaHCO_3$ — 26.2; $CaCl_2$ — 1.0; $MgCl_2$ — 1.6; D-Glucose — 11.0. The solution temperature was set in accordance with the experiment conditions ("room temperature" — 22–24°C, or "temperature close to physiological" — 32–34°C). Astrocyte calcium dynamics was recorded using a confocal laser scanning microscope Zeiss LSM 510 (Germany). Calcium indicator Oregon Green 488 (0.7 μmol) and specific astrocyte marker Sulforhodamine 101 (5 μmol) were used as fluorescent stains. Fluorescent intensity changes of calcium indicator was registered using step-frame recording (1 frame per second within 20 min) to measure calcium activity in astrocytes. For quantitative assessment of calcium dynamics two parameters were taken into consideration: the duration and frequency of calcium signals. The parameters were analyzed using a specifically developed software package Astroman. Since the amplitude of fluorescent signals is a complex parameter depending both on calcium concentration and stain concentration, as well as on laser radiation power that reaches the cells in tissue depth, the parameter was not taken into account in calcium activity assessment in astrocytes. The values are represented as mean ± a standard error of mean, n is the number of slices. The significance of statistical discrepancy of samplings was checked by a nonparametric Mann–Whitney test.

Results

Astrocyte calcium activity in postnatal development. We studied the frequency and duration of calcium signals of three age rat groups (Figure 1 (a)). A test group of animals aged 10 days showed the lowest frequency of calcium signals: 0.153±0.009 events/min (Figure 1 (b)).

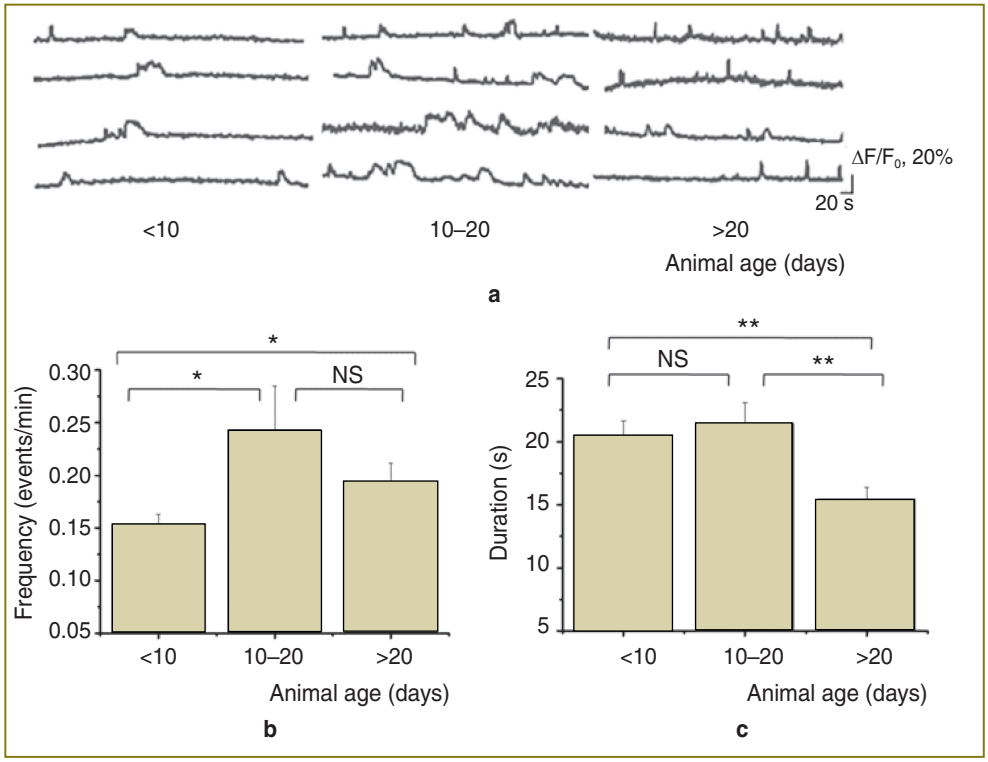

Figure 1. Spontaneous calcium activity in astrocytes during a postnatal period: (a) original recording of calcium events of four separate astrocytes in different slices in three age groups of animals; the animals aged 10 postnatal days showed the lowest frequency of calcium events; (b) mean frequency of calcium events per minute in rat hippocampal astrocytes; (c) mean duration of calcium events, by day 20 of postnatal period there is significant duration decrease of calcium responses in astrocytes. * p<0.05; ** p<0.001; NS: not significant

In older animals (10–20 days of postnatal development) frequency grew up to 0.244±0.04 events/min (p=0.05, compared to test group animals aged up to 10 days of postnatal period, Mann–Whitney test) and underwent no further significant changes. In a group of animals aged over 20 days, mean frequency of calcium events was 0.194±0.017 events/min (p=0.01, compared to test group animals aged up to 10 days, and p=0.007 compared to those aged 10–20 days, Mann–Whitney test).

Mean duration of calcium events was 20.48±1.15 s in animals aged up to 10 postnatal days, and 21.45±1.66 s in animals aged from 10 to 20 days (p=0.1, compared to the animals aged up to 10 postnatal days, Mann–Whitney test), and by the age over 20 days the duration decreased up to 15.43±0.91 s (p=0.008, compared to the animals aged up to 10 days, and p=0.008, compared to those aged 10–20 days of postnatal period, Mann–Whitney test) (Figure 1 (c)).

The effect of temperature conditions on spontaneous calcium dynamics in astrocytes. One of possible calcium event changing mechanisms in astrocytes in brain development is cell metabolic activity alteration. Metabolic activity level in slices can be altered by temperature change. We compared calcium dynamics in astrocytes at temperature close to physiological (32–34°C) with that at room temperature (22–24°C). Such temperature change results in significant decrease of spontaneous synaptic events. However temperature conditions appeared to have no effect neither on the frequency nor duration of calcium responses in astrocytes in none of the age groups (Figure 2). The findings turned out to be unexpected, since the neuronal activity change has been conventionally considered to be the astrocyte activation signal. Astrocyte activity baseline is likely to be spontaneous in slices and be independent of neuronal activity, since temperature decrease in neuronal activity has no effect on calcium dynamics in astrocytes.

The effect of specific energy substrates on frequency of calcium events in astrocytes of animals in an early postnatal period. This part of the experiment was carried out by changing energy substrates in an extracellular solution. A glucose solution was used as control. Moreover, glucose is not a basic substrate in the developing brain [9]. We supposed energy substrates used by brain during breast milk feeding to be able to have an effect on calcium activity in astrocytes. To study frequency and duration of calcium events in astrocytes we added various energy substrates to glucose: glucose-control (10 mmol); glucose (5 mmol) + lactate (5 mmol); glucose (5 mmol) + pyruvate (5 mmol); glucose (5 mmol) + β-hydroxybutyrate (10 mmol) (Figure 3). The findings suggest specific energy substrates to increase significantly the frequency of calcium events in astrocytes of animals aged up to 10 postnatal days, though have no significant effect on the parameters of calcium dynamics in two succeeding test groups of animals.

Discussion. In the developing brain many processes can have an impact on astrocyte activity. The changes of astrocyte calcium activity can be due to different expression levels of receptors and transporters in postnatal development, and changed morphology of an astrocyte itself [10]. Our findings suggest that in animals aged up to 10 postnatal days, the frequency of calcium events is determined not only by the period of animal development but also by specific energy substrates entering the brain from breast milk (pyruvate, lactate, β-hydroxybutyrate). When the slices of such animals are perfused by glucose-based solution (energy substrate uncharacteristic of this age), the frequency of calcium events in astrocytes decreases. Specific energy substrates added increase the frequency of calcium events up to the level typical of older animals. Thus, normal brain functioning, astrocytes in particular, at early postnatal stages requires energy substrates from breast milk. It makes a great difference, since it is astrocytes that deliver nutrients to neurons, and as a result, regulate their activity. Therefore, breast milk feeding is essential in this period of development to maintain the necessary level of astrocyte activity, and accordingly, deliver nutrients to neurons. The findings can explain why breast milk is necessary for normal brain development at early postnatal stages.

It should be noted that by day 20 of postnatal period, the duration of calcium events decreases. The main calcium ion disposal systems are certain to have been formed by this period that can result in such decrease. Changed parameters of calcium responses in astrocytes can be due to metabolism alteration in them. In this case, the decrease of total metabolism in brain under lowered temperature is to have an effect on calcium activity in astrocytes. However, the temperature of slices decreased from that close to physiological to room temperature appeared to have no impact on calcium dynamics in astrocytes. Kinetics of many biochemical reactions changes under temperature increase or decrease. For example, enzymes catalyzing most biochemical reactions exhibit thermolability: they change their activity at temperature variations. Certain optimal temperature conditions can influence the formation rate of enzyme-substrate complex causing its increase. This example is one among many other temperature-dependent reactions that have an effect on metabolic reactions in a cell. However such changes occur within the range of microseconds. Duration of calcium signals is recorded within the range of seconds, and it is may be due to significant difference in time scales that there are no significant changes in astrocyte calcium dynamics.

The study of brain metabolic processes controlling astrocyte activity is one of important problems of neurobiology that is still uninvestigated. Now it is clear that specific energy substrates at early postnatal stages necessary for brain cells should be present in nutrient media, therefore it is of great importance to control the presence of such substrates in infant food. If infants instead of breast-feeding are given artificial feeding

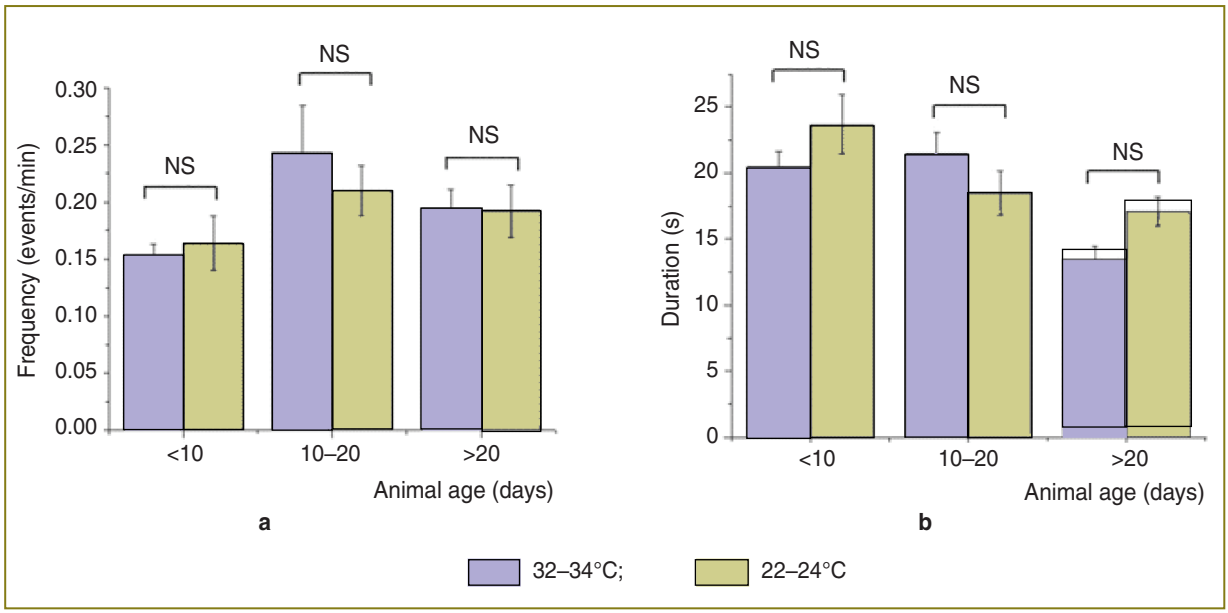

Figure 2. Temperature conditions have no effect on spontaneous calcium dynamics of astrocytes. Frequency (a) and duration (b) calcium activity in astrocytes under two temperature conditions. Neither of parameters changed, as evidenced by no sampling accuracy (NS)

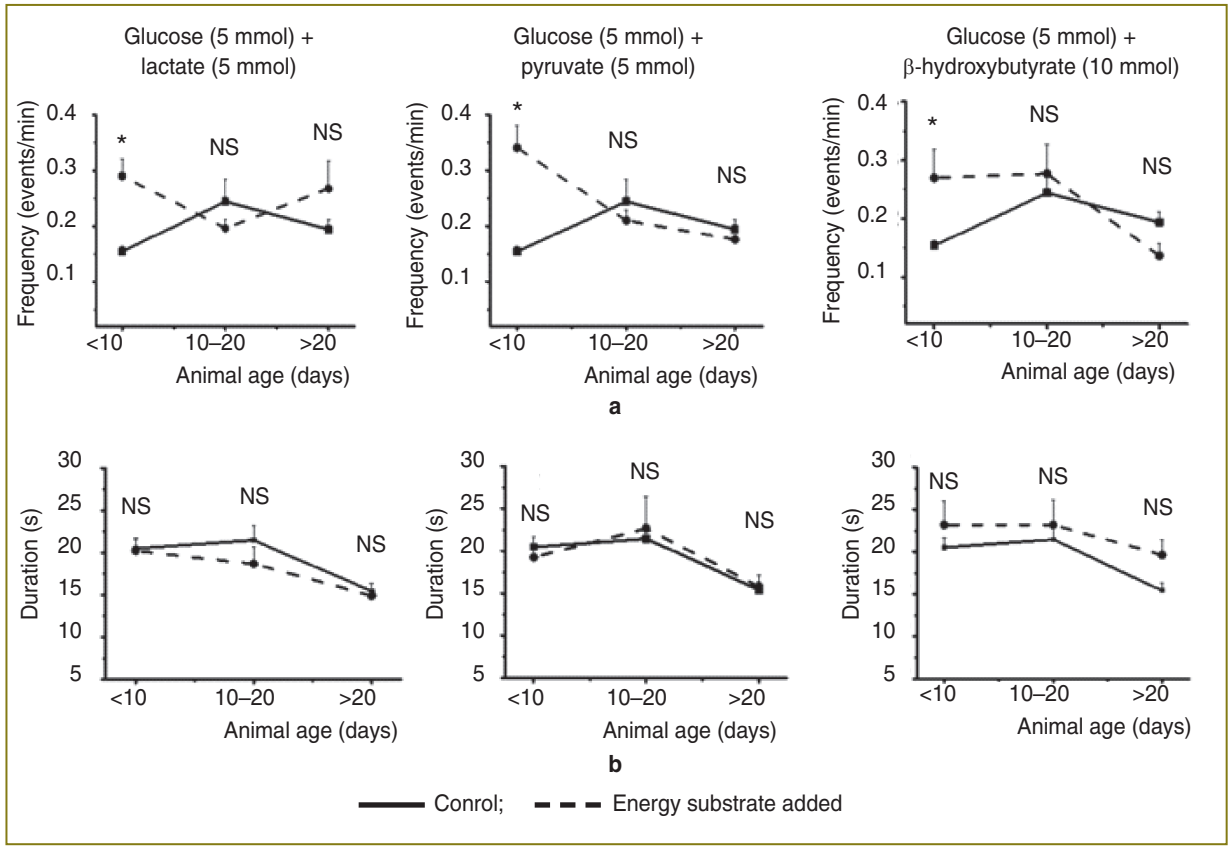

Figure 3. Specific energy substrates increase the frequency of calcium events in astrocytes in animals in early postnatal development: (a) frequency of calcium events in astrocytes with specific energy substrates added (lactate, pyruvate, β-hydroxybutyrate); in animals aged 10 postnatal days there is significant frequency increase of calcium events in astrocytes; (b) duration of calcium responses in astrocytes with specific energy substrates added does not change significantly, though there being age-related decrease of response duration by day 20 of postnatal period. NS: not significant; * $p<0.05$

too early, astrocyte activity can decrease resulting in retarded brain development.

Brain metabolism at later postnatal stages (over 10 days) depends slightly on energy substrates added. Therefore, when fasting, or on physical exertion that can also result in the increase of ketone bodies in brain, astrocyte activity should not change. Our findings can also throw light on the events occurring in brain under temperature decrease. Astrocytes also act as regulators of normal brain functioning leaving unchanged the parameters of the main signaling system. This has an important bearing on the study of human hypothermia processes, and in transplantology.

Conclusion. The study of astrocyte calcium dynamics in different periods of postnatal development can be used as a method to assess functional activity of glial systems when modeling brain metabolic disorders.

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Conflicts of Interest. The authors have no conflicts of interest related to the present study.

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