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Cerebral Energy State of Neonatal Rats During Seizures Induced by Homocysteine

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Summary

Seizures were induced in 7-day-old rats by intraperitoneal injection of DL-homocysteine thiolactone. Phosphocreatine (PCr), ATP, glucose, glycogen and lactate were determined in the cerebral cortex during various intervals after injection, corresponding to the early, as well as long periods of seizure activity. The unchanged levels of ATP, a very mild PCr decline and a pronounced accumulation of lactate (in the face of modest changes in brain glucose and glycogen) were observed. These results suggest that the immature rat brain is able to compensate energy expenditure associated with seizure activity by increased energy production, mainly due to increased anaerobic glycolysis. It remains to be determined whether a similar conclusion is also valid for other brain regions, e.g. subcortical structures.

Key words

Homocysteine-induced seizures – Neonatal rat – Cerebral cortex – Energy metabolism

Introduction

Homocysteine is a naturally occurring amino acid and its abnormal accumulation in the body of patients (homocystinuria) is known to be accompanied by convulsive episodes (Perry 1974, Mudd and Levy 1983). Homocysteine has also been shown to induce seizures in adult experimental animals (Sprince *et al.* 1969, Folbergrová 1974, Blennow *et al.* 1979, Freed 1985, Allen *et al.* 1986), whereas its effect on immature animals has not so far been reported.

As to the mechanism involved in inducing seizure activity, it is not yet clear whether the convulsant agent is homocysteine itself (Wuerthele *et al.* 1982) or whether some of its metabolic products, such as homocysteic acid, may play a role (Watkins and Evans 1981, Mewett *et al.* 1983, Turski 1989). It has been suggested that homocysteine could interact through one of the excitatory amino acid receptors, namely of the quisqualate (AMPA) type (Wuerthele *et al.* 1982, Freed 1985).

Cerebral metabolic changes, including the energy state, associated with homocysteine-induced seizures, have been studied and characterized in adult animals, i.e. in freely moving mice (Folbergrová 1974), as well as during sustained seizure activity (status epilepticus) induced in immobilized artificially ventilated rats (Blennow *et al.* 1979). The character of metabolic changes was similar to that reported for other seizure models. Thus, the results obtained in freely-breathing animals suggest that energy production cannot keep pace with the increased energy expenditure (Folbergrová 1974). Much less information is available concerning the metabolic alterations that occur in the brain of immature animals during seizures.

The aim of the present study was to induce seizures by homocysteine in 7-day-old rats and to investigate how the energy reserves in the immature cerebral cortex are influenced by seizure activity.

Material and Methods

Seven-day-old male Wistar albino rats of a SPF ("specific pathogen free") breed were used for these experiments. Rat pups were placed on a pad to maintain their body temperature by electrical heating at 35 °C (i.e. the temperature of the nest). DL-homocysteine thiolactone HCl (Sigma, St. Louis, Mo) was dissolved in saline and after adjusting the pH to about 7.0, 0.1 ml of the solutions were given intraperitoneally in the doses 5.5, 11 and 16.5 mmol/kg, respectively. Control animals were given the same

Table 1
Metabolite levels in the cerebral cortex of 7-day-old rats following administration of subthreshold dose of homocysteine (5.5 mmol/kg)

Treatment	PCr	ATP	Glucose	Glycogen	Lactate
			μmol/g wet wt.		
Controls	3.37 ± 0.03 (10)	2.52 ± 0.03 (10)	1.29 ± 0.07 (10)	0.97 ± 0.03 (10)	0.77 ± 0.05 (10)
DL-homocysteine (no seizures)	3.59 ± 0.06* (6)	2.59 ± 0.04 (6)	1.30 ± 0.15 (6)	0.94 ± 0.03 (6)	0.84 ± 0 05 (6)

Results are given as mean values (calculated from values obtained during various time intervals (9-75 min) after injection of homocysteine) ± S.E.M. Statistically significant differences from the controls are indicated by * for $P < 0.01$.

Table 2
Metabolite levels in the cerebral cortex of 7-day-old rats following administration of convulsive doses of homocysteine

Treatment	PCr	ATP	Glucose	Glycogen	Lactate
			μmol/g wet wt.		
Controls	3.38 ± 0.04 (7)	2.55 ± 0.03 (7)	1.26 ± 0.09 (7)	0.97 ± 0.03 (7)	0.77 ± 0.02 (7)
DL-homocysteine 11.0 mmol/kg (seizures)	3.33 ± 0.08 (9)	2.58 ± 0.02 (9)	0.76 ± 0.15* (9)	0.60 ± 0.05*** (9)	1.91 ± 0.34** (9)
Controls	3.37 ± 0.05 (9)	2.46 ± 0.03 (9)	1.47 ± 0.06 (9)	0.96 ± 0.04 (9)	0.71 ± 0.08 (9)
DL-homocysteine 16.5 mmol/kg (seizures)	3.00 ± 0.15* (10)	2.47 ± 0.04 (10)	1.03 ± 0.11** (10)	0.66 ± 0.08** (10)	3.96 ± 0.81*** (10)

Results are given as mean values (calculated from values obtained during various time intervals (9-77 min) after injection of homocysteine) ± S.E.M. Statistically significant differences from the appropriate controls are indicated by * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$.

volume of saline. At the desired time intervals the rat pups were quickly frozen in liquid nitrogen and their brains were dissected out in a glove box at −22 °C. Samples of the cerebral cortex (weighing approximately 25 mg) were extracted at −25 °C with HCl/methanol and subsequently at 0 °C with perchloric acid, as described in detail previously (Folbergrová *et al.* 1969, 1972). All metabolites studied (phosphocreatine (PCr), ATP, glucose, glycogen and lactate) were determined by enzymatic fluorimetric methods according to Lowry and Passonneau (1972). Statistical differences were evaluated by Student's t-test.

Results

The 5.5 mmol/kg dose of homocysteine which induced seizures in adult mice (Folbergrová 1974) appeared to be subthreshold for 7-day-old rats. The pups according to their behaviour during the whole period studied (i.e. until 75 min) did not differ from the controls. Homocysteine at concentrations 11 and 16.5

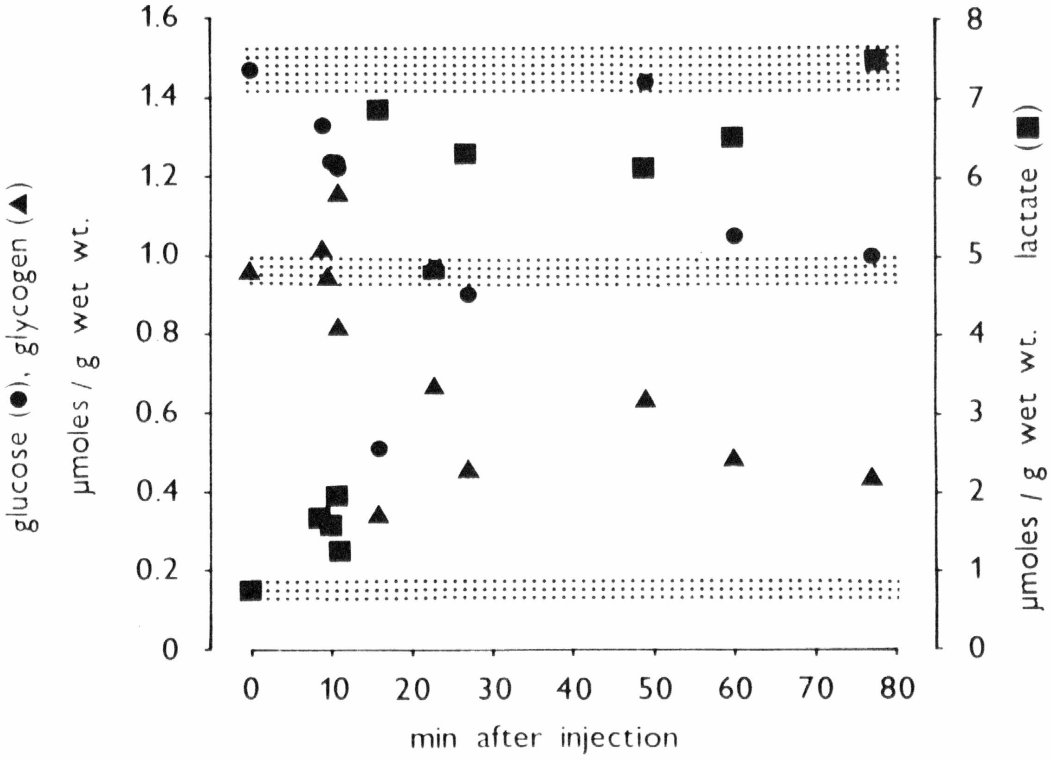

Fig. 1
Changes of glucose, glycogen and lactate in the cerebral cortex of 7-day-old rats during various periods of homocysteine-induced seizures. DL-homocysteine thiolactone HCl was administered intraperitoneally in a dose of 16.5 mmol/kg. With this dose, the first signs of seizure activity appeared approximately 4-5 min after injection. The rat pups were sacrificed during the seizures lasting for various time intervals (i.e. at the times indicated on the abcissa minus approximately 5 min). The dotted areas represent the mean values for 9 control animals ±S.E.M. Glucose (black dots) 1.47±0.06; glycogen (black triangles) 0.96±0.04; lactate (black squares) 0.71± 0.08 µmol/g wet wt.

mmol/kg induced generalized clonic-tonic seizures, reccurring frequently during the whole period studied, i.e. from about 5 min until 100 min after the injections. The seizures were of greater intensity and occurred more frequently following the dose of 16.5 mmol/kg. A detailed description of the behavioural pattern of homocysteine-induced seizures in rats during ontogenesis will be presented in a separate publication (Kubová *et al.* in preparation).

Changes in metabolite levels

Metabolites were determined during various time intervals after the homocysteine injection and the data given in Tables 1 and 2 represent means of all values obtained with each dose of homocysteine.

a) Changes occurring with the subthreshold dose of homocysteine

As can be seen in Table 1, all metabolites remained unchanged with the subthreshold dose of homocysteine as compared to the controls, with the exception of a modest elevation of PCr.

b) Changes occurring during convulsions

During homocysteine-induced seizures, ATP levels did not change at any time interval studied (Table 2). PCr remained unaffected during seizures induced with a lower dose of homocysteine and there was only a slight decrease with the higher dose (−11 %).

The present results indicate that during homocysteine-induced seizures in 7-day-old rats, an increase in glycolytic flux occurred, as is reflected by a decrease in brain glucose and glycogen, and especially by an increase in brain lactate (Table 2, Fig. 1). In order to evaluate the early metabolic changes as compared to those associated with long-lasting seizure activity, individual values for glucose, glycogen and lactate are given in Fig. 1, i.e. values obtained at different time intervals after homocysteine injection (16.5 mmol/kg), corresponding to various durations of seizure activity.

The average brain glucose concentrations decreased by only 30-40 % as compared to control values (Table 2). As the data in Fig. 1 demonstrate, during the early period of seizures (approximately 4-6 min) brain glucose levels decreased by about 13 %. This decline became more pronounced during 11–22 min of seizure activity (–46 %), whereas after 45–73 min of continuous seizure activity brain glucose levels were reduced to only approximately 80 % of control values (Fig. 1), indicating that no depletion had occurred even after prolonged seizure activity.

As can be seen in Fig. 1, glycogen levels did not change during the first 4–6 min. The decrease was observed only later, after 11–22 min of seizure activity, corresponding to about 50 % of control values, and it remained at approximately same level during the longer periods of seizures (Fig. 1).

It is evident (Table 2, Fig. 1) that there is a pronounced accumulation of lactate associated with homocysteine-induced seizures in 7-day-old rats. The lactate levels are already more than doubled during the early period of seizures; during later periods of seizure activity there is an 8 to 9.5-fold increase (Fig. 1).

Discussion

The present results have demonstrated that not only in adult animals, but also in neonatal rats, the administration of homocysteine can induce seizures. These seizures have, however, a different behavioural pattern as compared to that in adult freely-breathing animals (Folbergrová 1974) and they reoccur frequently for a long period (at least for 120 min) (Kubová *et al.* in preparation). The present data indicate that in the cerebral cortex of the immature rat, in spite of intensive and long-lasting seizure activity, there are unchanged ATP levels and only a very moderate PCr decline. These findings are in marked contrast to the changes concerning these metabolites in the cerebral cortex of adult freely moving mice during homocysteine seizures, when both PCr and ATP already decreased markedly during the first clonic seizure (Folbergrová 1974). As far as freely-breathing immature animals during convulsions are concerned, a significant depletion of energy reserves was reported in the cerebral cortex of newborn marmoset monkeys during bicuculline-induced seizures (Fujikawa *et al.* 1988) and also in immature mice during fluorothyl-induced seizures (Sacktor *et al.* 1966). On the other hand, in agreement with our results, PCr and ATP levels remained unchanged in the brain of 4-day-old rats during seizures induced by bicuculline or fluorothyl (Wasterlain and Duffy 1976). It is not clear whether the apparent discrepancy may be due to species differences, intensity of seizure activity, degree of local cerebral blood flow increase and/or a different metabolic rate. Nevertheless, the results suggest that in the neonatal rat cerebral cortex during seizures induced by various convulsant agents, the increased energy utilization can be sufficiently compensated by increased energy production. It remains to be determined whether a similar conclusion can also be drawn as far as other CNS regions are concerned (e.g. subcortical structures).

The present results have demonstrated a marked increase in glycolytic flux during homocysteine-induced seizures in 7-day-old rats. As far as glucose is concerned, a pronounced decrease in brain glucose concentration, despite normoglycaemia, has been reported during experimental neonatal seizures, induced by bicuculline or fluorothyl in various animal species (Wasterlain and Duffy 1976, Dwyer and Wasterlain 1981, Fujikawa *et al.* 1988, Vannucci and Fujikawa 1990). Especially in newborn marmoset monkeys , a marked depletion of brain glucose to only 1-4 % of control values was observed (Fujikawa *et al.* 1988). These findings were not unexpected in view of the limited glucose transport capacity across the blood-brain barrier of neonates as compared to adults (Moore *et al.* 1971, Cremer *et al.* 1979, Wasterlain and Dwyer 1983). The present results concerning brain glucose concentrations during homocysteine-induced seizures in 7-day-old rats, indicating that no depletion occurred even after prolonged seizure activity, seem to differ from the above mentioned findings. It should be mentioned in this connection that certain peculiarities concerning glucose concentrations were previously observed during homocysteine-induced seizures in the adult brain (Folbergrová 1974). Thus, during seizures, brain glucose levels remained unchanged, whereas they increased during the preparoxysmal period or under conditions when seizures were prevented. Blood glucose levels were significantly increased both during the preparoxysmal period, as well as during the seizures (Folbergrová 1974). These findings were interpreted as being due to some specific effect of homocysteine itself. It remains to be shown whether a similar situation may also exist in the brain of immature animals. Blood glucose levels were not measured in the present experiments and it is thus not possible to estimate the brain/blood glucose ratio. Since postnatal suckling rats have been shown to utilize alternate substrates as cerebral fuels, it remains to be determined whether utilization of these substrates (e.g. ketone bodies) could not be increased during homocysteine-induced seizures,which could then have a sparing effect on glucose metabolism. Further experiments are thus required in order to elucidate the mechanism underlying modest changes in brain glucose during homocysteine-induced seizures in immature animals.

Glycogen constitutes the large storage form of carbohydrates in the brain. During homocysteine-induced seizures in freely breathing adult mice, a rapid fall in glycogen already occurred at the onset of first clonic seizures (Folbergrová 1974), in accordance with

a rapid activation of glycogen phosphorylase (Folbergrová 1975a) and, during the late period of seizures, the level continued to fall to approximately 30 % of control values. In contrast to these findings, homocysteine-induced seizures in 7-day-old rats did not significantly change the glycogen levels during the early period of seizures. The slow rate of cerebral glycogenolysis during seizures in immature rats as compared to adults is presumably related to a low activity of enzymes involved in glycogen breakdown, namely glycogen phosphorylase and/or phosphoglucomutase (Shapiro and Wertheimer 1943, Vannucci and Vannucci 1980). The efficiency of the activation system of glycogen phosphorylase in the immature brain as compared to the adult one (Folbergrová 1975a,b) should also be considered and determined.

The present results have demonstrated that there is pronounced accumulation of lactate associated with homocysteine-induced seizures in 7-day-old rats. The observed values may even be underestimated since the rate of escape of the accumulated lactate across the blood-brain barrier in young animals can be much faster than in adults (Cremer *et al.* 1979). The pronounced lactate accumulation, in the face of rather modest changes of brain glucose and glycogen during homocysteine-induced seizures in immature animals, suggests that glucose transport from the blood was apparently able, to a large extent, to keep up with the rate of glucose utilization. This assumption concerns especially longer periods. Thus, after 1 h of seizure activity, only about 17 % of accumulated lactate can be accounted for by the degradation of glycogen and only about 10 % based on the glucose decline measured at that time.

In conclusion, the present results demonstrating unchanged levels of ATP and only a very mild decline of PCr in cerebral cortex of 7-day-old-rats during long-lasting seizure activity induced by homocysteine suggest that the immature rat brain is able to compensate energy expenditure associated with seizure activity by increased energy production, most likely due to a highly increased anaerobic glycolysis. It remains to be determined whether a similar conclusion is also valid for other brain regions (e.g. subcortical structures).

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