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Effects of glycolytic metabolites on preservation of high energy phosphate level and synaptic transmission in the granule cells of guinea pig hippocampal slices

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Abstract. The present study was undertaken to investigate whether neural activity of hippocampal slices can be preserved after replacing D-glucose with glycolytic intermediate metabolites such as lactate, pyruvate and citrate or with other sugars such as fructose, mannose, maltose, glucosamine, sucrose and galactose. As an index of neural activity, population spikes (PS) were recorded in the granule cell layers after electrical stimulation to the perforant path of guinea pig hippocampal slices. In addition, we determined the levels of ATP and creatine phosphate (CrP) in each slice after the replacement of D-glucose with these substrates, and correlated it with the neural activity. Substrates other than D-glucose could not maintain the PS for even 20 min although the slices perfused with medium containing lactate, pyruvate, galactose, fructose and maltose maintained similar levels of ATP and CrP as in slices incubated in the D-glucose-containing medium. These results indicate that D-glucose is essential for the preservation of synaptic activity in addition to its main role as the substrate for energy production to maintain the levels of high energy phosphates.
Key words. Hippocampal slices; population spikes; glucose deprivation; lactate; pyruvate; sugars; neural activity; high energy phosphates.

Glucose has generally been considered to be the main substrate for energy production to maintain neural activity and synaptic transmission in the central nervous system. Long lasting hypoglycemia leads to coma and causes functional derangement of the brain[1]. During deprivation of D-glucose, high energy phosphates such as ATP and creatine phosphate (CrP) decreased in the central tissue[2]. Pyruvate, lactate and other sugars such as galactose, fructose and mannose can also be substrates for energy metabolism in cortical slices[3], although there have been few reports studying the ability of these substrates to preserve neural activity in the nervous system. Cox and Bachelard[4] reported that pyruvate together with malate was able to maintain the levels of high-energy phosphates in hippocampal slices whereas it failed to maintain the population spikes (PS). They reported also that replacing glucose in the perfusion medium with pyruvate or lactate failed to maintain the PS and could not maintain neural activity[5]. On the other hand, Schurr et al.[6] and Fowler et al.[7], recording the PS in the CA1 pyramidal cell layer of hippocampal slices, showed that replacement of glucose and lactate could preserve the PS, although they did not measure the levels of high energy phosphates in the slices. Thus, there have been no systematic studies to show the correlation of the effect of substrates such as sugars and glycolytic intermediate metabolites on the energy metabolism and the preservation of neural activity. The purpose of this experiment is to investigate the effect of replacing D-glucose with other sugars and intermediate metabolites on the levels of ATP and CrP and on the preservation of neural activity of guinea pig hippocampal slices.

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Materials and methods

Guinea pigs (250–300 g) were decapitated and the brains removed from the skull. Isolated hippocampi were sliced transversely 300–400 μm thick along the long axis with a razor blade under a stereomicroscope. Details of the slice preparation were reported elsewhere[8,9]. The resulting slices were preincubated for at least 20 min in a standard medium (in mM: glucose 10, NaCl 125, KCl 3, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 26) bubbled with 95% O_2 and 5% CO_2 at 36 °C. The slices were placed in the chamber perfused continuously with the standard medium at a flow rate of 5 ml/min. As an index for the neural activity, orthodromic synaptic field potentials (population spikes, PS) were recorded (Oscilloscope: Nihon Kohden VC10) in the granular cell layers of the dentate gyrus after electrical stimulation (Stimulator: Nihon Kohden 3101) to the perforant path. Stimulation parameters were current pulses of 0.1 msec duration applied every 5 sec. A schematic drawing of the experimental procedure is shown in figure 1. A glass microelectrode filled with 2 M NaCl (resistance: 1–5 MΩ) was used for recording the PS. In this experiment, after obtaining the maximal response of the PS elicited by supramaximal stimulation, the stimulation strength was adjusted and fixed to obtain PS at 60–70% of maximal amplitude. After recording stable PS for at least 20 min, the standard medium

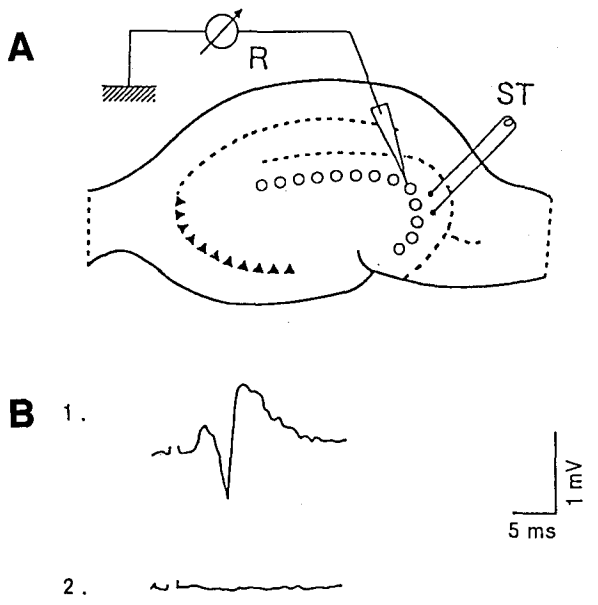

Figure 1. Effect of deprivation of D-glucose on the amplitude of population spikes (PS) evoked in the granule cell layer in the dentate gyrus. *A* Schematic illustration of the electrophysiological study. R, recording of the PS; ST, stimulation electrode. *B* indicates typical examples of PS before (1) and 30 min after (2) deprivation of D-glucose in the perfusion medium.

containing D-glucose (at the concentration of 10 mM) was replaced with medium containing 5 mM, 2 mM, 1 mM, 0.5 mM or 0 mM D-glucose or with medium containing other sugars such as mannose, fructose, galactose, glucosamine, maltose and sucrose (5 mM each) or glycolytic intermediate metabolites such as lactate, pyruvate or citrate (5 mM each). The addition of these substrates to the medium did not change the pH of the medium. The changes in PS amplitudes after the treatment were observed for 30 min. To exclude irreparable damage to the slices, the standard medium containing 10 mM D-glucose was reintroduced after the treatment of 30 min. The recovery of the PS amplitude was observed, which indicated that the slices could recover after the treatment. Each experimental medium was also bubbled with 95% O_2 and 5% CO_2. The temperature of the medium was maintained at 36 °C throughout the experiment. ATP and CrP in each slice was determined 0 and 30 min after incubation of the experimental medium. For the determination of ATP and CrP, each slice was homogenized in ice-cold 0.5 M perchloric acid with 1 mM ethylenediaminetetraacetic acid (EDTA) and centrifuged for 10 min at 2000 rpm. The supernatant was neutralized with 2 M $KHCO_3$. The slices were recentrifuged and the supernatants were stored at −30 °C until ATP and CrP levels were assayed. The precipitate was homogenized with 0.5 N NaOH and used for the determination of protein. ATP and CrP were measured enzymatically and fluorometrically according to the production of NADPH[10]. The protein content of the slice was determined by the method of Lowry et al.[11].

Results

To investigate the effects of different concentrations of D-glucose on synaptic activity, the slices were perfused with medium containing D-glucose concentrations of 0, 0.5, 1, 2, 5 and 10 mM. Slices perfused with medium containing 2, 5, and 10 mM D-glucose maintained the normal evoked activity (fig. 2A). Removal of D-glucose from the standard medium abolished the PS completely in 30 min (fig. 2A). The media containing 5, 10 and 20 mM lactate instead of D-glucose did not preserve the PS which were abolished within 30 min in a similar manner to D-glucose-free medium (fig. 2A). Replacing D-glucose with pyruvate (5, 10 and 20 mM) showed a decay in the PS with a similar time course observed in the absence of D-glucose (fig. 2B). Citrate (5 mM) or

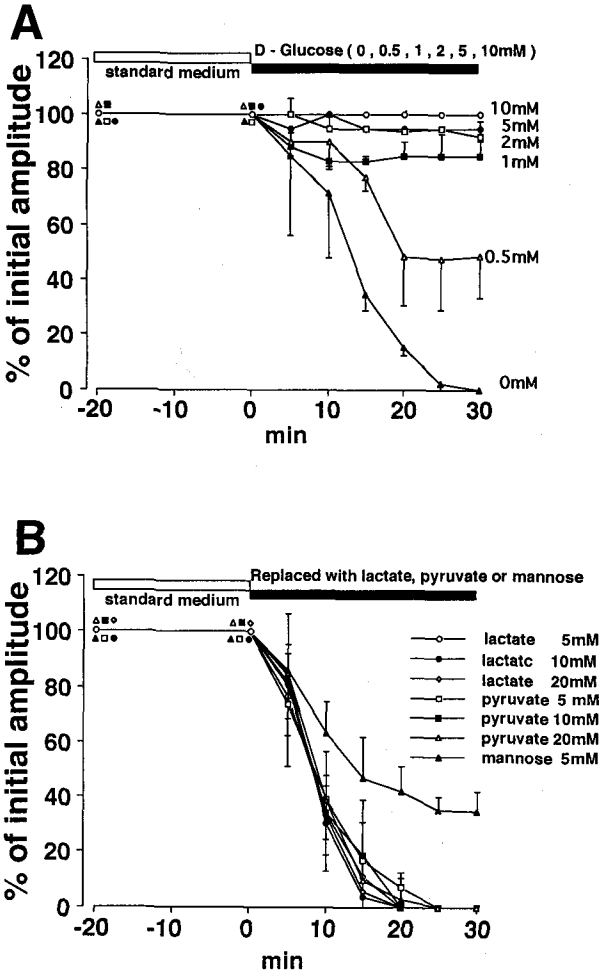

Figure 2. *A* Effect of D-glucose at different concentrations (the concentrations are indicated as different symbols in the figure) in the perfusion medium on the amplitude of PS. The amplitude of PS just before treating with D-glucose at different concentrations was taken as 100%. Each plot shows the average from 4 slices. Each point represents the average ±S.E.M. *B* Effect of replacement with lactate (5, 10 and 20 mM), pyruvate (5, 10 and 20 mM) and mannose (5 mM) on the amplitude of PS. The amplitude of PS before the deprivation of D-glucose or addition of lactate, mannose or pyruvate was taken as 100%. Each plot indicates the average ±S.E.M. from 4 slices.

other sugars such as fructose (5 mM), maltose (5 mM), glucosamine (5 mM), sucrose (5 mM) and galactose (5 mM) could not preserve the PS, which diminished with a time course similar to that where lactate or pyruvate were used (fig. 2B). In the presence of mannose (5 mM), the time course of the PS decay was slower than that obtained with the other sugars, although the PS could not be maintained (fig. 2B). All slices with reduced PS amplitude showed good recovery after reperfusion of the standard medium containing 10 mM D-glucose.

In the slices deprived of D-glucose for 30 min, ATP concentration decreased to 58% and CrP to 50% of the original levels. The original concentration of ATP was 15.1 ± 1.6 mmol/kg protein and CrP was 29.3 ± 2.5 mmol/kg protein, respectively. Although lactate and pyruvate failed to preserve synaptic transmission, they maintained the levels of ATP and CrP at over 80% of the original level (table). In the citrate-containing medium, ATP and CrP levels in the slices decreased to 66% and 83% of original concentration. In medium containing sugars such as fructose, galactose and maltose, the levels of ATP and CrP were preserved at over 80% of the control values. The levels of ATP and CrP of the slices incubated in mannose-, glucosamine-, or sucrose-containing medium decreased to below 80% of the original concentration. These results indicate that glycolytic intermediate metabolites and sugars other than D-glucose could not support synaptic transmission in hippocampal slices, even though substrates such as lactate, pyruvate, fructose, galactose or maltose maintained the levels of ATP in the tissue. The blockade of synaptic activity during application of substrates in the replacement of D-glucose thus cannot be explained only by reduction in levels of the high energy phosphates.

Table. Effect of replacement of D-glucose with sugars and glycolytic intermediate metabolites for 30 min on the level of high energy phosphates (ATP and CrP) and the presence of the population spikes (PS) of hippocampal slices.

	ATP%	CrP%	PS
D-glucose (10 mM)	103.6 ± 5.35	110.4 ± 2.42	+
D-glucose (0 mM)	58.1 ± 4.21*	50.8 ± 4.52*	−
mannose (5 mM)	63.3 ± 4.45*	57.5 ± 6.80*	−
fructose (5 mM)	87.2 ± 4.58	94.4 ± 3.03	−
galactose (5 mM)	94.1 ± 11.51	81.4 ± 4.93	−
pyruvate (5 mM)	90.8 ± 9.08	82.3 ± 5.61	−
lactate (5 mM)	93.9 ± 10.93	103.9 ± 4.54	−
glucosamine (5 mM)	66.4 ± 1.27*	82.3 ± 9.63	−
citrate (5 mM)	66.0 ± 3.06*	82.8 ± 1.51*	−
maltose (5 mM)	86.8 ± 10.34	91.5 ± 6.57	−
sucrose (5 mM)	73.4 ± 2.95*	53.7 ± 2.15*	−

The results of ATP and CrP are average concentrations (mean ± S.E.M.) from 3 slices. The mean is expressed as percentage of control value before the replacement with sugars and metabolites. In the results of PS, (+) indicates the presence of PS whose amplitude was over 80% of original level and (−) indicates below 80% of original level. The concentrations (mM) of sugars and metabolites are shown in parentheses. Asterisks in the table show that the difference between each value and that obtained from control slices before treatment with other sugars and metabolites is statistically significant. (ANOVA with Fisher's least-significant test: *$p < 0.05$.

Discussion

There have been many reports showing the relationship between energy metabolism and neural activity in brain tissue [4,5,12–16]. Using the hippocampal slices, Lipton et al.[12] showed a relationship between the decrease in ATP level in the tissue and neural activity during hypoxia. However, Okada[9] found a discrepancy between the time course of the reduction of ATP levels and the decrease in the PS amplitude during deprivation of oxygen or glucose. ATP and CrP levels in the hippocampal slices decreased in a similar manner during either deprivation of O_2 or D-glucose whereas the PS amplitude diminished and disappeared much faster during glucose deprivation than during O_2 deprivation. Cox et al.[14] also reported that replacement of 10 mM D-glucose with 20 mM fructose maintained ATP level in the tissue but the amplitude of the PS decreased to 70% of the original level. They also showed[5,16] that neural activity in the dentate gyrus was attenuated by lactate although they did not determine the level of ATP in the hippocampal slices. On the other hand Shurr et al.[6] and Fowler [7] reported that lactate supported synaptic transmission of the Schaffer collateral-CA1 connection in the hippocampal slices, although they did not measure the levels of ATP and CrP in each slice. Thus, the correlation between ATP level and neural activity in the brain is still under discussion. Monitoring the synaptic function as PS amplitude and determining the levels of ATP and CrP, the present experiment showed that substrates such as galactose, fructose, maltose, lactate and pyruvate maintained the levels of ATP and CrP in the tissue slices, although they did not support the synaptic function over 20 min. Only D-glucose supported neural activity as well as maintaining the levels of ATP and CrP in the tissue. The discrepancy between our and Shurr or Fowler's results may be due to differences in the area of hippocampus and the species studied, temperature, or technical procedures such as the flow rate of the medium[4,5,7,14–17]. The reduction in the PS amplitude and blockade of transmission is thus unlikely to be due to lack of oxygen during treatment with lactate or other substrates which are metabolized under aerobic conditions. The result that substrates other than D-glucose failed to maintain synaptic activity even though they maintained the levels of high energy phosphates in the slices strongly indicates that D-glucose has an important role in the maintenance of synaptic activity in addition to maintaining the levels of ATP and CrP as substrates for energy production.

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