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EFFECT OF LACTATE ON THE SYNAPTIC POTENTIAL, ENERGY METABOLISM, CALCIUM HOMEOSTASIS AND EXTRACELLULAR GLUTAMATE CONCENTRATION IN THE DENTATE GYRUS OF THE HIPPOCAMPUS FROM GUINEA-PIG

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Abstract—Towards understanding the role of glycolysis on synaptic function, we examined the effect of lactate on synaptic potential, energy metabolism, Ca^{2+} homeostasis and extracellular glutamate in the dentate gyrus of guinea-pig hippocampus. Postsynaptic population spikes were recorded from the granule cell layer of the dentate gyrus in guinea-pig hippocampal slices after replacing glucose with lactate in the perfusion medium. Population spikes were not maintained and spontaneously recovered around 35 min after the replacement of glucose with lactate. However, ATP levels of the dentate gyrus remained unchanged while those during the glucose-free condition decreased to 73% of the initial levels at 60 min. Intracellular Ca^{2+} was measured with the calcium indicator dye fura-2 AM, and the population spike was recorded simultaneously. Ca^{2+} levels increased concomitantly with the early decay of synaptic potentials, and recovered partially with the spontaneous recovery of synaptic potentials. The time course of decay of population spikes and the increase of Ca^{2+} levels during lactate replacement were similar to those during glucose deprivation. Increase in Ca^{2+} levels during lactate replacement was completely blocked by the ryanodine receptor/calcium release channel antagonist dantrolene. Glutamate was released more significantly in the medium during lactate replacement than with normal Ringer solution, and less than that during glucose deprivation. Addition of the *N*-methyl-D-aspartate blocker, D-(−)-2-amino-5-phosphonovaleric acid, and the L-type calcium channel blocker, nimodipine, but not dantrolene blocked spontaneous recovery of population spikes.

The results indicate that lactate can maintain energy levels in hippocampal slices, but cannot maintain ion homeostasis in granule cells of the dentate gyrus. Glycolysis plays an important role in maintaining ion homeostasis, and activation of *N*-methyl-D-aspartate and L-type calcium channels is necessary for support of synaptic function by lactate utilization. © 2001 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: ATP, glycolysis, calcium pump, NMDA receptor, voltage-sensitive calcium channel.

Oxygen and glucose are essential for neural function, and hypoxia and hypoglycemia cause depression of synaptic transmission. Lactate can be utilized as an energy substrate instead of glucose in the immature brain.[28] Lactate can also fuel neurons and be used to maintain the energy level of the mature brain.[7,22,29] However, the ability of lactate to maintain neural activity in the mature brain is controversial. Schurr[23] and Fowler[3] reported that field potential was maintained by lactate in CA1 neurons, whereas Saitoh *et al.*,[22] Kanatani *et al.*[7] and Yoshioka *et al.*[33] observed that lactate could not support synaptic potential in granule cells of the dentate gyrus. Moreover, Takata and Okada,[25] using an intracellular recording technique, observed that lactate could not support the synaptic potential in CA3 neurons, though ATP levels remained constant. Therefore, lactate alone is not likely to be an absolute alternative substrate to glucose for the maintenance of synaptic function in adult neurons.

Previous reports have shown that ATP derived from anerobic glycolysis preferentially supports membrane function in the heart,[30] erythrocytes,[15] vascular smooth muscle[11] and the brain.[9] This suggests that cells sequester ATP from glycolysis, and glycolysis not only provides a minor amount of ATP for energy, but also may play an additional, as yet undefined, role in neural function. Furthermore, several investigators[13,27] have reported that glycolytic enzymes bound to sodium pump or internal calcium pump proteins, which were regulated by ATP derived from anerobic glycolysis.[24] Therefore, ATP produced from lactate through aerobic glycolysis cannot substitute for ATP from anerobic glycolysis.

To more clearly understand the role of anerobic glycolysis in neural function and to determine whether lactate serves as an alternative energy source for neurons, we investigated the effect of lactate on synaptic potential, ATP levels, intracellular calcium concentration ($[Ca^{2+}]_i$)

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Abbreviations: APV, D-(−)-2-amino-5-phosphonovaleric acid; $[Ca^{2+}]_i$, intracellular calcium concentration; 8-CPT, 8-cyclopentyl-1,3-dipropylxanthine; CrP, creatine phosphate; DG, dentate gyrus; EGTA, ethylenediaminetetraaetic acid; MCPG, methyl-4-carboxyphenylglycine; NADPH, nicotinamide adenine dinucleotide phosphate hydride; NMDA, *N*-methyl-D-aspartate; PS, population spike; TCA, tricarboxylic acid; VSCC, voltage-sensitive calcium channel.

and extracellular glutamate concentration in guinea-pig hippocampal slices.

EXPERIMENTAL PROCEDURES

Preparation of hippocampal slices

Adult guinea-pigs (Hartley, SLC, Japan), weighing 200–300 g, were used for these experiments. Animals were killed according to the guidelines for animal experimentation at the Kobe University School of Medicine. Hippocampus slices (300–400 μm) were prepared by cutting transversely along the long axis of the hippocampus. Details of slice preparation have been reported elsewhere.[17] Each slice was preincubated for at least 20 min in standard medium (in mM: NaCl 125, KCl 4, KH_2PO_4 1.24, $MgSO_4$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 26, glucose 10) bubbled with 95% O_2/5% CO_2 at 35°C.

Electrical activity recording

After preincubation, each slice was transferred to the observation chamber under a stereoscope. The chamber was perfused continuously with the standard medium at a flow rate of 4 ml/min. The temperature was kept at 35°C throughout the experiment with an incubator and temperature controller (PDMI-2, Medical Systems Corp., New York, USA). As an index of neural activity, the perforant path was stimulated at 0.1 Hz with constant-current pulses (0.1 ms) and population spikes (PSs) were recorded from the granule cell layer of the dentate gyrus (DG) with glass microelectrodes filled with 2 M NaCl. The stimulation intensity was adjusted to obtain a PS amplitude at 60–70% of maximum elicited by supramaximal stimulation. After recording steady potentials for at least 20 min, the slices were perfused with conditioned medium. To test the effects of lactate during deprivation of glucose, glucose in the medium was replaced with 10 mM sodium lactate (lactate medium). Adding sodium lactate did not influence the pH of the medium.

ATP and creatine phosphate determination

Regions of the DG were dissected from hippocampal slices under a stereoscope. After preincubation for 20 min in oxygenated standard medium at 35°C, the dissected slices were incubated with standard medium, glucose-free medium or lactate medium for 60 min. At the end of the incubation, the DG regions of three or four slices were immediately homogenized in 0.5 N perchloric acid with 1 mM EGTA and centrifuged for 15 min at 2000 r.p.m. The supernatant was neutralized with 2 M $KHCO_3$, recentrifuged, and stored at −30°C until assay of ATP and creatine phosphate (CrP). ATP and CrP were quantitated enzymatically and fluorometrically by measuring the production of NADPH.[18] Protein content of the slices was determined by the method of Lowry and Passonneau.[10]

Calcium measurement

The Ca^{2+} indicator was prepared as follows: 20 μl of 2 mM fura-2 AM (Molecular Probes, Eugene, OR, USA) was dissolved in 4 ml of the standard medium, 5 μl of dimethyl sulfoxide (DMSO) containing 10% cremophor EL was added and the mixture was sonicated for 5 min. Hippocampal slices were incubated with 5 μM fura-2 solution for 40 min at 35°C. The slices were then transferred to a chamber mounted on an inverted microscope (DIAPHOT TMD, Nikon, Tokyo), submerged and superfused at 4 ml/min at 35°C. Fluorescence was measured with a ×10 objective, and the slices were excited alternately with 340 and 380 nm UV light every 30 s by a filter changer (C4312, Hamamatsu). Dye fluorescence was captured with a high-performance video camera (C2400, Hamamatsu), processed using an image processor (DVS3000, Hamamatsu), and then transferred to a personal computer (PC9801-BA, NEC, Tokyo, Japan) and TV monitor. Three small areas in the granule cell layer were selected manually on the TV monitor. Fluorescence intensity of fura-2 after excitation of the sample with 340 and 380 nm light was measured, and the calculated ratio of these fluorescence values was displayed on the monitor throughout the experiments. Slices which showed a spontaneous fall of the ratio were not recorded. The measured ratio was averaged and normalized to the initial ratio, which was assigned as 1.0.

Determination of glutamate release

After preincubation, five DG slices were incubated in 300 μl of standard medium bubbled with 95% O_2/5% CO_2. Glucose deprivation experiments were performed with standard medium that contained no glucose. Lactate replacement experiments were performed with glucose-free standard medium supplemented with 10 mM lactate. After incubation with each medium, the quantity of glutamate released from the slices was determined by a high-performance liquid chromatography system. Prior to chromatography, medium was filtered with 0.22-μm millipore filters. Aliquoted medium (60 μl) was injected into vials and mounted on a refrigerated automicrosampler (CMA/200, CMA, Stockholm, Sweden). Samples were mixed with reagents and transferred to the reverse-phase column (BAS, Tokyo, Japan), and the eluate was subsequently monitored with a fluorescence detector (CMA/280). Analyzed data were printed out with a chromatographic recorder (D-2500, Hitachi, Tokyo, Japan). Sample concentrations were calculated from known concentrations of amino acid standards.

Materials

ATP, CrP, protein assay reagents, fura-2 AM, cremophor EL, nimodipine, D-(−)-2-amino-5-phosphonovaleric acid (APV) and α-methyl-4-carboxyphenylglycine (MCPG) were purchased from Nacalai.Co. (Japan). Hexokinase, glucose-6-phosphate dehydrogenase and all other enzymes were obtained from Boehringer Mannheim (Germany). Sodium lactate, sodium dantrolene and 8-cyclopentyl-1,3-dipropylxanthine (8-CPT) were obtained from Sigma (St Louis, MO, USA).

Statistical analysis

Values are shown as mean ± S.E.M. Statistical analysis was performed by ANOVA and Bonferroni post hoc test. Treatment differences were considered significant at $P < 0.05$.

RESULTS

Simultaneous recording of field potential and Ca^{2+} mobilization

The time course for simultaneous recording of PS and Ca^{2+} mobilization from the granule cell layer of the DG is shown in Fig. 1. Standard medium was replaced with glucose-free medium (Fig. 1A) or lactate medium (Fig. 1B). The amplitude of PSs decreased gradually during glucose deprivation, and was completely depressed after 35 min (Fig. 1A). Standard medium was recirculated 40 min after deprivation of glucose, and the PS amplitude recovered by 50% after 15 min. Ten minutes after incubation with glucose-free medium, the fura-2 fluorescence ratio increased gradually and reached the maximum levels when the PS could not be evoked further (Fig. 1A). After re-introduction of standard medium, the fluorescence ratio decreased partially concomitant with PS recovery. Incubation of hippocampal slices in lactate-supplemented, glucose-free medium also resulted in a gradual decrease in PS amplitude, which reached minimum amplitude after 35 min. However, PS amplitude increased spontaneously and recovered to 80% of the initial level 60 min after lactate medium treatment.

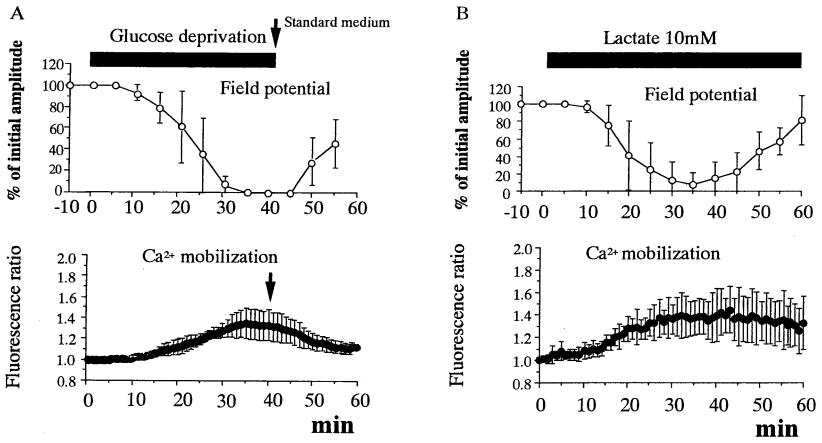

Fig. 1. Effects of glucose deprivation (A) and replacement of glucose with lactate (B) on the field potentials (PSs) and simultaneous intracellular Ca^{2+} mobilization. Each plot indicates the average PS amplitude as a percentage of the original level of seven slices. Amplitudes of PSs before exposure to glucose deprivation or lactate replacement correspond to 100%. Arrows in A indicate the start of reperfusion with the standard medium. As for the Ca^{2+} mobilization, the ordinate is the ratio of fura-2 fluorescence evoked at 340/380 nm. Vertical bars indicate S.E.M.

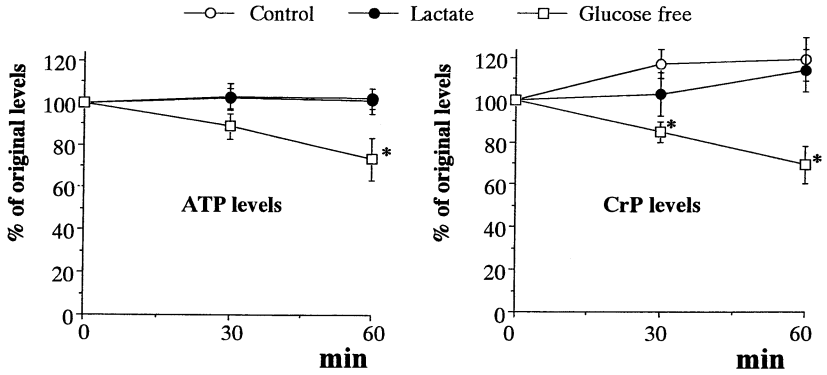

Fig. 2. Concentrations of ATP and CrP in the DG of hippocampal slices before, and 30 and 60 min after, initiation of glucose deprivation or lactate replacement. The ordinate is the concentration of ATP or CrP expressed as a percentage of the levels prior to treatment. Each plot indicates the average value of four DG samples. Vertical bars indicate S.E.M. Asterisks indicate a significant difference between control and glucose-free treatment at 30 and 60 min ($P < 0.05$). The original levels of ATP and CrP were 15.8 ± 1.31 and 32.1 ± 2.53 mmol/mg protein, respectively.

ATP and creatine phosphate levels in the dentate gyrus region

To correlate neural activity with ATP and CrP in the DG region, the DG region of each slice was selectively dissected, and incubated with standard medium, glucose-free medium or lactate-supplemented, glucose-free medium bubbled with 95% O_2/5% CO_2 gas at 35°C. The ATP and CrP concentrations of each dissected sample were determined by a sensitive microassay method. Both ATP and CrP levels remained unchanged throughout the lactate replacement period (Fig. 2). During glucose deprivation, CrP levels decreased to 84% of the initial level after 30 min ($P < 0.05$). After 60 min, ATP decreased to 73% of the initial level and CrP decreased to 69% of the initial level ($P < 0.05$).

Glutamate release from the dentate gyrus region

Energy deprivation such as hypoxia or hypoglycemia induces extracellular glutamate accumulation. To investigate the effect of lactate replacement on glutamate homeostasis, the concentration of glutamate released from the DG region was measured. The glutamate concentrations in the medium increased 40 and 60 min after incubation with lactate and glucose-free medium (Fig. 3). No significant change in glutamate concentration occurred after incubation of DG fragments in control medium for 60 min at 35°C.

The adenosine antagonist 8-CPT attenuates the decay of synaptic potential

Adenosine causes depression of synaptic potential, at least in the early phase of energy deprivation.[2,3,34] The time course of the PS amplitude during glucose deprivation and lactate replacement in the presence of the adenosine antagonist, 8-CPT (10 μM), is shown in Fig. 4. The rate of decay of PS amplitude was similarly attenuated under both conditions. The PS was maintained at 20% of the initial amplitude 60 min after glucose deprivation and 45% of the initial amplitude 60 min after lactate replacement. Recirculation with standard medium resulted in

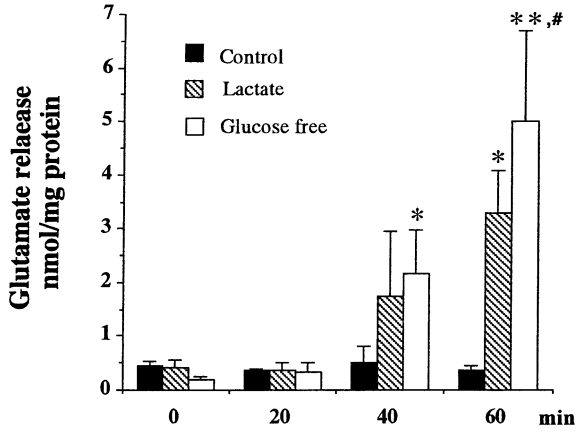

Fig. 3. Release of glutamate from the DG of hippocampal slices during glucose deprivation or lactate replacement. Each histogram represents the mean glutamate release from the dissected DG slices into the medium, and the results are mean values obtained from four experiments. Filled columns indicate the results of the control treatment, hatched columns show the results of lactate replacement, and open columns show the results of glucose deprivation. Vertical bars indicate S.E.M. Asterisks show significant differences between control treatment and glucose deprivation or lactate replacement ($^{*}P < 0.05$, $^{**}P < 0.001$). $^{\#}P < 0.05$, significant difference between glucose deprivation and lactate replacement conditions.

85–90% of recovery of the initial PS amplitude. Spontaneous recovery of PS amplitude was less evident in DG samples incubated in lactate medium supplemented with 8-CPT than in samples incubated in unsupplemented lactate medium (Fig. 1B).

Source of $[Ca^{2+}]_i$ increase during lactate replacement

To determine the source of the increase in $[Ca^{2+}]_i$ during lactate replacement, DG samples were first incubated in medium containing no supplemental calcium but with 200 μM EGTA (Ca^{2+}-free medium). The PS disappeared 2 min after incubation of DG with Ca^{2+}-free medium (data not shown). DG samples were incubated in Ca^{2+}-free standard medium for 30 min and then in lactate-supplemented, glucose-free, Ca^{2+}-free medium (Fig. 5). After 10 min, the fura-2 fluorescence ratio gradually increased. This result is similar to that observed for the DG incubated in lactate-supplemented, glucose-free medium (Fig. 1B). However, the increase in $[Ca^{2+}]_i$ reached a plateau after 60 min, and $[Ca^{2+}]_i$ gradually recovered to near initial levels. Thus, during the early phase, extracellular calcium had little influence on the increase of $[Ca^{2+}]_i$ during lactate replacement. The later spontaneous decrease of $[Ca^{2+}]_i$ indicated that lactate provided a source of energy for the calcium pump after the 30- to 40-min latency period.

Calcium release from internal Ca^{2+} stores was blocked by the ryanodine receptor/calcium release channel antagonist dantrolene. In the presence of dantrolene, $[Ca^{2+}]_i$ decreased gradually throughout incubation of the DG with lactate replacement medium. In contrast, simultaneous PS recordings in the presence of dantrolene were similar to PS recordings in the absence of dantrolene. Moreover, PS amplitude recovered to 70% of the initial levels at 60 min (Fig. 6).

When the non-selective metabotropic glutamate receptor blocker, (*S*)-MCPG (250 μM), was added to the perfusion medium, PS amplitudes during lactate replacement recovered to 80% of the initial levels, and $[Ca^{2+}]_i$ mobilization was not modified ($n = 3$; data not shown).

These results indicate that the increase in $[Ca^{2+}]_i$ originated from internal stores and that the changes in $[Ca^{2+}]_i$ were not causally related to PS recovery.

Effects of N-methyl-D-aspartate receptors and voltage-sensitive calcium channels on the recovery of population spikes during lactate replacement

Glutamate was released during lactate replacement, even though ATP or CrP levels in the slices were

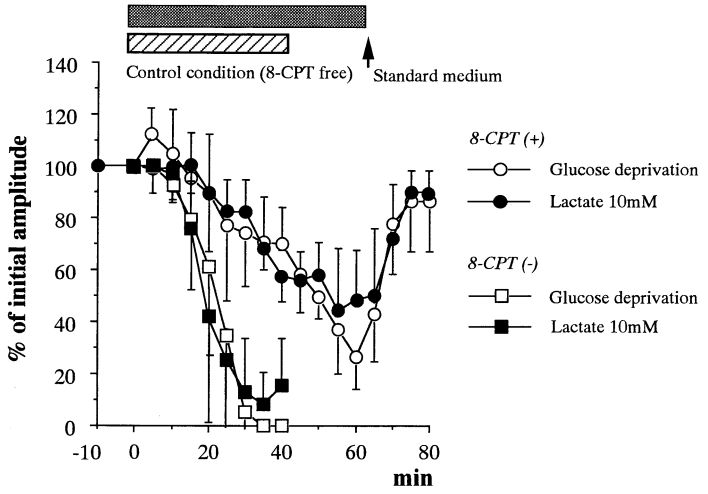

Fig. 4. Effects of 8-CPT (10 μM) on synaptic potentials (PSs) during glucose deprivation (open circles) or lactate replacement (filled circles). The perforant path was electrically stimulated and PSs were recorded from the granule cell layer of the DG. Glucose deprivation or lactate replacement medium were circulated for 60 min, shown as a horizontal hatched bar. The standard medium was recirculated after 60 min. The control curves (8-CPT free) are also shown (square symbols) for comparison. The ordinate indicates the percentage of the initial PS amplitudes. Each plot is the average value of seven experiments. Arrow indicates the start of reperfusion with the standard medium. Vertical bars indicate S.E.M.

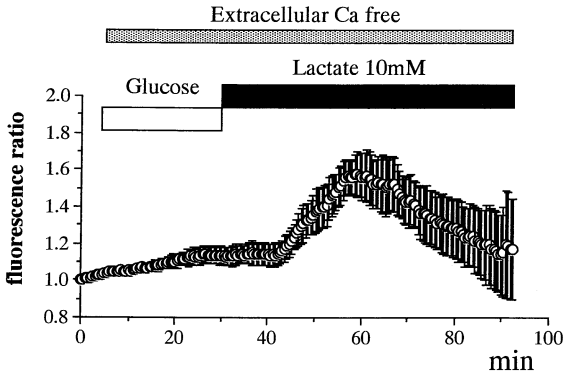

Fig. 5. Calcium mobilization during incubation of tissue samples in calcium deprivation or lactate replacement media. Tissue samples were first incubated in calcium-free medium supplemented with 200 μM EGTA. To allow the calcium levels in the tissue samples and medium to stabilize, incubation proceeded for 30 min, after which lactate replacement (filled bar) was initiated. The fura-2 fluorescence ratio of samples incubated in glucose and EGTA increased for a short time and then stabilized. The reason for this phenomenon is not known. The ratio levels in the presence of glucose and EGTA reached stable levels after the slight increases; the reason for this is unknown. The ordinate is the fura-2 fluorescence ratio after excitation with light at 340 and 380 nm every 30 s. Each plot is the average of five experiments. Vertical bars indicate S.E.M.

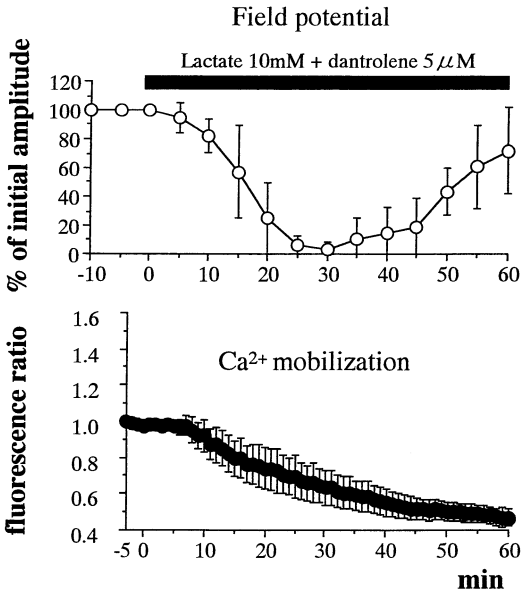

Fig. 6. Simultaneous recording of field potentials (PSs) and Ca^{2+} mobilization during lactate replacement in the presence of 5 μM sodium dantrolene. Lactate replacement was started at time zero, indicated as a filled bar. Each plot indicates the average of seven experiments, and the values are percentages of initial amplitudes. For Ca^{2+} mobilization measurements, each plot is the average of seven experiments. The ordinate indicates the fura-2 fluorescence ratio after excitation light at 340 and 380 nm every 30 s. Vertical bars indicate S.E.M.

maintained at original levels (Figs. 2 and 3). To investigate the role of glutamate receptors or voltage-sensitive calcium channels (VSCCs) in the recovery of synaptic potential during lactate replacement, the *N*-methyl-D-aspartate (NMDA) blocker APV and L-type VSCC blocker nimodipine (50 μM each) were added to the lactate medium. Incubation of hippocampal slices in lactate replacement medium supplemented with both APV and nimodipine blocked recovery ($P < 0.001$) of DG granule cell synaptic potential (Fig. 7). A slight but statistically insignificant attenuation of synaptic potential was detected in DG granule cells of hippocampal slices treated with lactate replacement medium supplemented with either APV or nimodipine (Fig. 7A, B). Treatment of hippocampal slices with lactate replacement medium with dantrolene did not affect the PS recovery of DG granule cells (Fig. 7B). The PS amplitude of DG granule cells remained unchanged after treatment of hippocampal slices with standard medium supplemented with APV and nimodipine.

DISCUSSION

We reported previously that synaptic potential maintenance was lost upon treatment of hippocampal slices with medium lacking glucose but supplemented with glycolytic intermediates such as lactate, fructose or pyruvate.[7,22,25] This loss occurred despite maintenance of high-energy phosphate (ATP and CrP) content in the overall slice. These results indicate that glucose plays an essential role in the maintenance of synaptic function, which is not simply explained by its role as the substrate for energy production. In this experiment, we demonstrated that replacement of glucose with lactate neither supported synaptic potential nor controlled glutamate and calcium homeostasis, and activation of NMDA receptors and VSCCs was necessary for recovery of synaptic potential when lactate was the only source of energy.

Synaptic potential and Ca^{2+} homeostasis during glucose deprivation and lactate replacement

Intracellular calcium increases rapidly during glucose deprivation,[25] and elevation of $[Ca^{2+}]_i$ occurs 10 min after deprivation in the CA3 region of the hippocampus.[25] Increase in $[Ca^{2+}]_i$ was also observed when lactate was added to glucose-free medium, and the time-course of PS decay and the increase of $[Ca^{2+}]_i$ were almost identical under glucose-free and lactate replacement conditions in the DG (Fig. 1), as well as the CA3 region.[25] The time required for $[Ca^{2+}]_i$ increase after lactate replacement was similar in both the CA3 and the DG regions, and the levels of ATP and CrP remained unchanged throughout lactate replacement. Therefore, it is not likely that the decay of PS and breakdown of Ca^{2+} homeostasis are the result of ATP depletion.

Extracellular accumulation of glutamate and adenosine during lactate replacement

Energy deprivation in the brain leads to suppression of sodium pump activity and membrane depolarization of neurons. Consequently, the rise in intracellular sodium is likely to reverse the action of glial glutamate uptake and increase extracellular glutamate.[12,21] We measured the release of glutamate into the medium, which was bubbled with O_2/CO_2. We observed elevation of glutamate

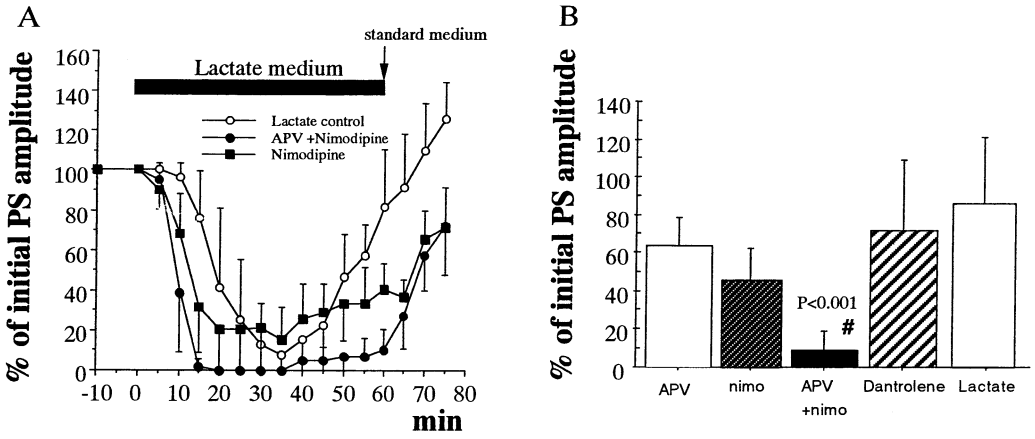

Fig. 7. Effect of APV or nimodipine (50 μM each) on recovery of PS amplitude during lactate replacement. (A) The effect of these agents on PS amplitude over 60 min. Open circles indicate values from samples incubated under lactate replacement conditions. Filled squares indicate addition of nimodipine, and filled circles indicate addition of APV and nimodipine. Each plot is the average of seven experiments. (B) The PS amplitude of DG specimens 60 min after incubation with lactate replacement medium supplemented with nimodipine (nimo) and/or APV. The circulation of lactate with each condition is also shown, as is the effect of dantrolene supplementation (Fig. 5). The PS amplitude of DG specimens incubated in lactate medium supplemented with APV and nimodipine differed significantly from that of DG specimens incubated in lactate replacement medium containing no supplementation (ANOVA with post hoc test, $P < 0.001$).

concentration in the medium 40–60 min after glucose deprivation. Lactate replacement also resulted in statistically greater glutamate concentration after 60 min but not after 40 min. After 60 min of incubation, the glutamate concentration in the lactate replacement medium was lower than that in the glucose deprivation medium. This observation suggested that glutamate uptake in tissue incubation in lactate replacement medium was re-initiated (Fig. 1B). Thus, glutamate homeostasis is not supported during the early phase of lactate replacement, but is supported during the later phase of lactate replacement. One possible explanation for this phenomenon is reactivation of the sodium pump, and subsequent re-uptake of extracellular glutamate. This recovery phenomenon after the transient blockade of function is completely coincident with that of PS and $[Ca^{2+}]_i$ during perfusion of lactate replacement medium.

The time-course of PS decay in the granule cell layer of the DG during glucose deprivation was the same as that during lactate replacement (Fig. 4). The mechanism by which decay occurs under both conditions is likely to be similar. Suppression of transmitter release by adenosine is likely to be considered as the cause of decay of synaptic function in the early phase of energy deprivation.[2,3,34] Adenosine is generated from the degradation of ATP or (*S*)-adenosylhomocysteine[14,19] and transported to the interstitial space by facilitated transport.[19] The protective effect of 8-CPT on the amplitude of PS decay during lactate replacement and glucose deprivation indicates that adenosine accumulates extracellularly. The accumulation of extracellular adenosine during lactate replacement suggests that ATP derived from lactate cannot support the local energy demands for maintenance of sodium and calcium pump activity, and this local energy shortage may lead to facilitated production of adenosine.

Source of $[Ca^{2+}]_i$ increase, and the relation between $[Ca^{2+}]_i$ increase and recovery of population spikes during lactate replacement

Removal of extracellular calcium from the medium had no effect on the increase of $[Ca^{2+}]_i$ during lactate replacement (Fig. 5). Within 2 min after incubation of hippocampal slices with calcium-free medium supplemented with EGTA, the PS was no longer detected. These results showed that extracellular calcium did not participate in $[Ca^{2+}]_i$ increase during lactate replacement. Calcium-induced Ca^{2+} release is part of the origin of $[Ca^{2+}]_i$ increase during ischemia,[16] and it is sensitive to dantrolene.[8,16] Intracellular calcium gradually decreased after treatment with dantrolene and could not be elevated after lactate replacement. The change in PS amplitude during lactate replacement after dantrolene treatment was the same as that with no dantrolene treatment. For both treatments, the decrease in PS amplitude occurred once and recovered gradually (Fig. 1B). The involvement of metabotropic glutamate receptor-induced Ca^{2+} release from internal calcium stores is unlikely because the non-selective metabotropic glutamate receptor antagonist (*S*)-MCPG had no effect on Ca^{2+} mobilization or PS recovery. Therefore, the source of $[Ca^{2+}]_i$ release during lactate replacement is likely to be an internal calcium store that is sensitive to dantrolene, and $[Ca^{2+}]_i$ release has no apparent effect on the mechanism of PS recovery.

The role of N-methyl-D-aspartate receptors and voltage-sensitive calcium channels in the recovery of population spikes during lactate replacement

Increases in $[Ca^{2+}]_i$ activates the tricarboxylic acid (TCA) cycle.[5] Since PS recovery during lactate replacement does not depend on increase in $[Ca^{2+}]_i$, it is not likely that this recovery is a consequence of ATP

generated by the TCA cycle. Since extracellular glutamate levels increase during lactate replacement, we hypothesized that NMDA receptors and VSCCs may be activated under these conditions. As shown in Fig. 7B, PS amplitude at 60 min was attenuated during treatment with the NMDA receptor blocker APV and the L-type calcium channel blocker nimodipine. NMDA receptors are critical mediators of activity-dependent synaptic changes, including the formation of long-term potentiation.[31] Activation of NMDA receptors leads to Ca^{2+} influx and activation of immediate early genes, and Ca^{2+} influx through L-type calcium channels also triggers the gene expression.[6,31] The dependency of PS recovery on NMDA receptor and L-type calcium channel activation may suggest involvement of gene transcription and protein synthesis, which in turn shift ATP synthesis to anerobic glycolysis and the TCA cycle to aerobic glycolysis.

The role of anerobic glycolysis for maintenance of synaptic function

Several investigators have suggested that anerobic glycolysis supports membrane functions in a variety of tissues.[9,11,15,30] Silver and Erencinska[24] reported that glycolytic generation of ATP was required for sodium pump activity in cultured murine cortical neurons. Martinez-Zaguilan and Wesson[13] suggested that glucose or its metabolites regulated Ca^{2+} homeostasis, and Xu *et al.*[32] showed that ATP generated from glycolysis played an important role in regulating Ca^{2+} homeostasis via the calcium pump in the sarcoplasmic reticulum. These reports suggest that ATP generated by oxidative phosphorylation in mitochondria makes up the vast majority of the total ATP in neurons, but does not necessarily support focal demand of ATP. This focal demand must be met by the mechanisms involving glycolysis. Therefore, lactate is an efficient substrate for production of energy via mitochondria, but cannot support the local energy demand of the sodium and calcium pumps for maintenance of ion homeostasis. We further demonstrated that lactate replacement, after a short latent period, enabled maintenance of field potential and Ca^{2+} homeostasis. This implies that a mechanism must exist for access of mitochodrial ATP to glycolysis-dependent processes, such as ion homeostasis, by means of sodium and calcium pump activities, and that activation of this mechanism is dependent on NMDA receptor or VSCC activation (Fig. 7). We are the first to report this dynamic change in source of ATP for ion homeostasis.

Lactate from glial cells is likely to be a major substrate for energy production in neurons,[26] as evidenced by the presence of monocarboxylate transporters in glia and neurons and differences in lactate dehydrogenase isozymes between neurons and glia.[1,20] However, our results indicate that lactate cannot substitute for glucose as an energy source for maintenance of synaptic function, which includes adenosine, glutamate and Ca^{2+} homeostasis. Environmental conditions, such as ischemia or hypoglycemia, which lead to activation of NMDA receptors or VSCCs, trigger utilization of lactate for maintenance of synaptic function. *In vivo*, the relation between physiological activation of neural activity and rate of energy metabolism suggests that non-oxidative metabolism of glucose becomes dominant during neural activation.[4] This observation implies that anerobic glycolysis plays an important role in the physiological activity of the brain *in vivo*. Further investigation will be required in order to understand the mechanism by which the source of ATP supply is switched from anerobic glycolysis to mitochondria in neurons, and how energy production from these sources influences synaptic plasticity and other synaptic functions.

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