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Effects of lactate/pyruvate on synaptic plasticity in the hippocampal dentate gyrus

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【１２７】

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【 学位論文題目 】

Effects of Lactate/Pyruvate on Synaptic Plasticity in the Hippocampal Dentate Gyrus
（海馬歯状回における乳酸とピルビン酸のシナプス可塑性に及ぼす作用）

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Introduction

Although glucose is a principal metabolic fuel in the central nervous system (CNS), brain can utilize alternative metabolic substrates to sustain brain function, such as monocarboxylates, citric acid cycle intermediates, free amino acids, and free fatty acids, when glucose is unavailable. It has been proposed that impaired glucose utilization in the CNS could be a possible cause of cognitive dysfunction. Diabetic subjects undergoing hypoglycemia often exhibit the deficiencies in learning, attention and memory. Protective effect of lactate on the cerebral function during hypoglycemia has been demonstrated. In Alzheimer' disease (AD), impaired glucose utilization and oxidation of energy substrates other than glucose have been reported. Increased expression of monocarboxylate transporter 1 and decreased immunoreactivity of glucose transporter 3 in AD brain suggest that elevated utilization of monocarboxylates may influence the cognitive decline. Based on these observations, it should be investigated whether monocarboxylates could function to support neural plasticity. However, there is little information if higher synaptic function can be supported by monocarboxylates.

Hippocampal long-term potentiation (LTP) and depression (LTD) are two forms of synaptic plasticity, which have attracted considerable attention in the search for the mechanisms of learning and memory. We have been investigating the effects of monocarboxylates on synaptic function and neural energy metabolism in the hippocampal dentate gyrus. Monocarboxylates can be utilized to preserve the high-energy phosphates levels. We have found that substitution of lactate for glucose in the perfusion medium results in a decay of field population spike (PS), followed by spontaneous recovery to normal levels. In this respect, however, Schurr, Fowler, and Izumi have reported that PS is maintained consistently after replacement of glucose with lactate in CA1 pyramidal cell layer of rat hippocampal slices. This discrepancy may result from the differences in temperature and/or technical procedures for slice preparation.

Izumi et al. have reported that pyruvate and lactate are effective alternative energy substrates for the induction of LTP. However, their experiments are carried out at 30℃, at which temperature neural metabolism is changed. In addition, differences in the experimental procedures for slice preparation could be crucial to analyze the relationship between neuronal metabolism and synaptic plasticity. Therefore, in the present experiment, we re-evaluated the effects of monocarboxylates on paired-pulse facilitation (PPF), LTP and LTD of granule cell population responses in hippocampal slices.

Methods

Hippocampal slices were prepared from guinea pig brains (Hartley, SLC, Shizuoka, Japan) weighing 200-300 g by a standard technique. All animals were treated according to the guidelines for animal experimentation at the Kobe University School of Medicine. The hippocampi were quickly dissected and sliced into 300-400-μm-thick transverse sections. The slices were incubated for at least 20 min in standard medium (in mM: 125 NaCl, 4 KCl, 1.24 KH_2PO_4, 1.3 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, 10 Glucose) oxygenated with 95% O_2/5% CO_2 at 35-36 ℃. At the time of study, slices were transferred to the recording chamber which was perfused continuously with the standard medium at a flow rate of 3 ml/min and was kept at 35-36 ℃. PS were recorded from the granule cell layer of the hippocampal dentate gyrus with glass microelectrodes filled with 2 M NaCl. Evoked synaptic responses were elicited with 0.1-msec constant current pulses through a bipolar electrode placed in the perforant pathway. In each experiment, a full input-output curve was determined. The stimulus

intensity that evoked a half-maximal granule cell population spike amplitude was selected for subsequent test stimuli. Test pulses were then given at 0.05 Hz throughout the experiments. The synaptic responses were estimated by the PS amplitude, which was measured by averaging the distance from the negative peak to the midpoint of the preceding and following positive peaks. After checking the stable baseline for 20 min, condition stimuli for PPF, LTP and LTD were delivered. PPF was evaluated by systematic variation of the inter-pulse intervals ranging from 5 to 1000 msec using pulses of the same intensity. To induce LTP in the hippocampal dentate gyrus, three sets of high-frequency stimulus (HFS) trains were delivered, each consisting of 8 pulses at 500 Hz, at a frequency of one train per 10 sec. In the other set of experiments, LTD was induced by applying low-frequency stimulation (LFS) consisting of 900 pulses delivered at 1 Hz. Testing with single stimuli was continued for at least 60 min. Adding Na-lactate or Na-pyruvate, accompanied by reduction of NaCl did not influence the pH and the osmolarity of circulation medium. Values are presented as means ± s.e.m. Statistical analysis was performed by non paired t-test.

Results

Consistent with our previous reports, removal of glucose from the perfusion medium produced a complete elimination of the PS within 30 min. replacement of exogenous glucose with 10 mM Na-lactate during glucose deprivation transiently depressed the PS amplitude, followed by spontaneous recovery to 118.7 ± 9.4 % of the control PS after 60 min. 10 mM Na-pyruvate in the absence of glucose also resulted in transient depression of PS and spontaneous recovery to 124.3 ± 12.9 % of the control levels with a similar time course.

To test the effects of monocarboxylates on the synaptic plasticity, PPF, LTP and LTD were induced in the hippocampal slices that were pretreated with 10 mM lactate or 10 mM pyruvate. Similarly, PPF of 10 mM glucose, lactate or pyruvate-supported PS was elicited by paired-pulses of 10, 20, 40 msec interval and a maximal facilitation was obtained at 10 msec interval pulses. By stimulation with 10 msec interval pulses, the averages of a maximal facilitation were 189.8 ± 11.2%, 195.7 ± 14.7% and 169.6 ± 14.3% of glucose-supported, lactate-supported and pyruvate-supported PS, respectively. There were not any significant differences among all groups.

Next, we studied the effects of monocarboxylates on LTP and LTD. At 60 min after HFS, the amplitude of 10 mM glucose-supported PS increased to 183.8 ± 9.3% of baseline PS amplitude (control LTP). LTP were induced of 10 mM lactate- and 10 mM pyruvate-supported PS (138.2 ± 4.8% and 135.0 ± 3.6% of the initial baseline, respectively), which were significantly suppressed, compared with control LTP ($p < 0.01$). The amplitude of PS in slices, which were pre-incubated with lactate, increased to 140.6 ± 10.8% of baseline, which was also significantly depressed than control LTP.

when 1 Hz LFS applied for 15 min, a substantial depression of synaptic responses was observed in slices incubated with 10 mM glucose (80.3 ± 4.9% of baseline at 60 min after LFS, control LTD). In contrast, LFS failed to induce LTD in slices treated with 10 mM lactate and 10 mM pyruvate (99.9 ± 7.3% and 101.6 ± 5.9% of the original PS amplitude at 60 min after LFS, respectively). Then, we re-introduced the control glucose medium to hippocampal slices pre-incubated with lactate, resulting in the failure of LTD induction (95.1 ± 5.8% of the initial baseline PS, $p < 0.05$ compared with control LTD).

Discussion

In the present study, we have found that 1) monocarboxylates (lactate and pyruvate) can sustain granule cell population responses after transient depression; 2)

substitution of monocarboxylates for glucose results in a similar degree of PPF; 3) HFS can induce LTP of monocarboxylates-supported PS, but the degree of enhancement is less than that of glucose-supported PS; and 4) LFS can not induce LTD of monocarboxylates-supported synaptic responses.

It is not likely that altered energy homeostasis in monocarboxylates-treated slices is responsible for the suppression of LTP and LTD by the following reasons. 1) Re-application of control glucose medium to hippocampal slices that were pretreated with lactate or pyruvate failed to induce the maximal LTP and LTD, and 2) In the previous experiments, we have observed the preserved levels of high-energy phosphates (ATP and creatine phosphate) in dentate gyrus after substitution of lactate for glucose. Based on these observations, impaired LTP and LTD induction of monocarboxylates-supported synaptic responses is apparently due to some metabolic alteration, occurred during the transient synaptic depression after the replacement of glucose with monocarboxylates.

The molecular mechanisms that underlie the induction of LTP and LTD have been investigated extensively. In hippocampal granular cells, most of the available evidence speaks in favor of a postsynaptic induction of LTP and LTD by a rise in the intracellular calcium concentration. In this respect, we have found that intracellular calcium concentration increases concomitantly with the early decay of synaptic potentials and recovers partially with the spontaneous recovery of PS after the replacement of glucose with lactate. Addition of blockers for N-methyl-D-aspartate (NMDA) glutamate receptor and L-type voltage-dependent calcium channel inhibits spontaneous recovery of PS. Activation of NMDA receptors by weak tetanus markedly diminishes the ability of a strong tetanus to generate LTP, which is presumably due to pre-activated NMDA receptors mediated by elevated intracellular calcium concentration during the weak tetanic stimulation. After incomplete ischemia not accompanied with morphological changes, but with alteration in intracellular calcium homeostasis, LTP in perforant path-dentate gyrus synapses has also been reported to be suppressed. Taken together, it seems plausible that monocarboxylate-induced alteration of intracellular calcium homeostasis during transient PS depression affects on the subsequent induction of LTP and LTD.

Finally, our results suggest that continuous presence of glucose is essential to induce maximal long-term synaptic plasticity in the brain. Impaired induction of LTP and LTD of monocarboxylates-supported synaptic function may partially explain the neural dysfunction caused by problems with glucose utilization. Molecular insight for the impaired induction of long-term synaptic plasticity of monocarboxylates-supported synaptic responses will be investigated in future studies.

神戸大学大学院医学系研究科（博士課程）

論文審査の結果の要旨			
受付番号	甲第 1524 号	氏　名	楊　波
論文題目	Effects of Lactate/ Pyruvate on Synaptic Plasticity in the Hippocampal Dentate Gyrus 海馬歯状回における乳酸とピルビン酸のシナプス可塑性に及ぼす作用		
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（要旨は１，０００字～２，０００字程度）

脳はグルコースを主たるエネルギー源として利用しているが、グルコースの利用が低下した状況では monocarboxylate（乳酸／ピルビン酸）などのグルコース以外の基質を利用する。糖尿病患者の低血糖でみられる認知障害は、乳酸を投与することで一部改善される。またアルツハイマー病脳ではグルコース代謝率が減少しており、一方、乳酸／ピルビン酸の利用が亢進している可能性が報告されている。これらの知見は monocarboxylate が脳の可塑性を維持できる可能性を示している。しかしながら乳酸／ピルビン酸が高次のシナプス機能を維持できるかについては、ほとんど検討されていない。これまで私達は、海馬切片を用いた研究で、乳酸／ピルビン酸の神経活動に及ぼす作用について研究を行ってきた。そこで今回、乳酸／ピルビン酸がシナプスの可塑性、即ち paired-pulse facilitation (PPF), 長期増強現象（long-term potentiation : LTP)、長期抑制現象（long-term depression : LTD）に与える影響について検討を行った。

モルモット（250-300 g）から脳を摘出し、厚さ 300-400 μm の海馬切片を作成した。海馬切片は 95% O_2, 5% CO_2 で飽和した、グルコース（10mM）を含む標準リンゲル液でインクベートした。海馬の貫通線維を電気刺激し歯状回顆粒細胞層で誘発される場のシナプス電位（population spikes : PS）を記録し、その振幅をシナプス機能の指標とした。グルコースを除去したリンゲル液、グルコースを 10mM 乳酸や 10mM ピルビン酸に置換したリンゲル液で海馬切片を環流し、このときの PS の変化を記録した。また PPF, LTP, LTD を誘発し、グルコース標準液（対照）での変化と比較・検討した。

細胞外液からグルコースを除去すると、PS は 30 分以内に消失した。細胞外液のグルコースを 乳酸、ピルビン酸に置換すると、PS は一過性に抑制されたが、その後自然に回復した。PPF の発現について検討したところ、乳酸／ピルビン酸で維持されたシナプス活動では、10-40 msec 間隔の paired-pulse により PPF が観察された。これらの PPF は対照と比較しても有意差を認めなかった。次に、高頻度刺激により LTP を誘発したところ、乳酸／ピルビン酸で維持されたシナプス活動では 138%、135% の LTP が誘発されたが、対照（グルコース）での LTP（184%）と比較すると有意に抑制されていた。ここで乳酸／ピルビン酸で維持されたシナプス活動を、再び 10mM グルコースを含むリンゲル液で環流した。引続き高頻度刺激にて LTP を誘発したが、

141%までの増強に留まった。次にLTDについて検討した。グルコースにより環流されたPSでは、低頻度刺激により80%のLTDが発現した。しかし乳酸、ピルビン酸で維持されたシナプス活動ではLTDの発現が見られなかった。LTPでの実験と同様に、乳酸／ピルビン酸で維持されたPSに再びグルコースを環流して低頻度刺激を与えたが、やはりLTDは発現されなかった。

以上の結果より、乳酸／ピルビン酸は、短期のシナプス可塑性(PPF)については、グルコースと同程度の発現を支持できるが、長期のシナプス可塑性(LTP, LTD)の発現に対しては、十分ではないことが示された。乳酸／ピルビン酸で維持されたシナプス活動に再びグルコースを環流しても、LTP, LTDの発現が抑制されていた。このことはグルコースと乳酸／ピルビン酸のエネルギー代謝基質としての差は、原因として重要ではないこと示した。むしろ細胞外液のグルコースを乳酸／ピルビン酸に置換すると、一過性にシナプス活動が抑制されたが、この時に生じる何らかの代謝性変化が、その後のLTP, LTDの発現に作用している可能性が示された。この点について私達は、細胞外液のグルコースを乳酸／ピルビン酸に置換すると、カルシウム細胞内濃度が変動することを以前報告した。LTP, LTDは細胞内カルシウム濃度に依存する現象である。これらの結果は、乳酸／ピルビン酸により十分なLTP, LTDが発現されなかった原因として、カルシウムホメオスターシスの変化が重要である可能性を示唆していた。

本研究は、monocarboxylateの神経可塑性に及ぼす作用について研究したものであるが、従来ほとんど行われなかった海馬切片における短期、長期のシナプス可塑性(PPF, LTP, LTD）に対する乳酸／ピルビン酸の作用について重要な知見を得たものとして価値ある集積であると認める。よって、本研究は、博士（医学）の学位を得る資格があると認める。