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Crossover effects of acidosis on the recovery of neuronal function following glucose-oxygen deprivation in rat hippocampal slices

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Abstract: The present study was designed to determine whether acidosis modifies the effect of simulated ischemia on neuronal function. Hippocampal evoked potentials were recorded in vitro from the CA1 region after stimulation of the Schaffer collaterals and the change in the evoked potentials was analyzed in response to glucose-oxygen deprivation under variable acid-base conditions ranging from pH 7.4 to pH 4.5. Population spike (PS) activity was almost abolished with glucose-oxygen deprivation except for pH 6.5, indicating that mild acidosis minimizes the depressant effect of glucose-oxygen deprivation on neuronal transmission. The recovery of PS amplitude during recovery from glucose-oxygen deprivation was not significantly inhibited by moderate acidosis of pH 6 and 5.5 but was significantly inhibited when the pH was 5 or lower. The results suggest that severe acidosis may depress PS amplitude and prevent their recovery after reversal of glucose-oxygen deprivation, and that moderate acidosis may have no significant effect on PS amplitudes on their recovery.

Key words: Cerebral ischemia, Acidosis, Hippocampal slice, Glucose-oxygen deprivation, Hypoxia

Introduction

Acidosis has been reported to compromise neuronal function after cerebral resuscitation. The deleterious effects were reported in several lines of study: Restoration of the reperfused cerebral energy state was more inhibited by accompanying acidosis after incomplete ischemia [1]. Histology revealed more edema, destruction of the blood-brain barrier, and necrosis when cerebral ischemia was accompanied by profound acidosis [2–6]. Acidosis in the periresuscitation period produced a higher incidence of seizure [3,6] and worse neurologic outcome [1,7] in animals subjected to cerebral ischemia.

However, it has recently been reported that moderate lactic acidosis of pH 5.5 or higher did not appear to have any deleterious effects on the recovery of the population spike amplitude in the rat hippocampal slices following hypoxia [8]. Moreover, it was reported that mild acidosis actually protected against *N*-methyl-D-aspartate(NMDA)-induced neurotoxicity and ischemic injury in hippocampal neurons [9,10] and cortical culture [11]. The paradoxical protection by mild acidosis against ischemia may partly be attributed to the reduction of NMDA receptor-activated calcium current [12].

The conflicting reports compelled us to address the effects of acidosis on impaired neuronal function by ischemia. We studied the depression and recovery of evoked PS amplitudes of hippocampal CA1 region following glucose-oxygen deprivation under different acid-base conditions.

Methods

The study protocol was approved by the Animal Care and Use Committee, Hokkaido University School of Medicine. A young Wistar rat (either sex, 50–60 g body weight) was anesthetized with 3% halothane in oxygen. After craniotomy, the left hemisphere of the brain was rapidly removed and placed in cold, oxygenated physiological solution. Slices of the hippocampus, 400 μm thick, were cut perpendicular to the septotemporal axis using a vibrating slicer (Dosaka EM, DTK-1000, Tokyo, Japan) and were incubated in physiological solutions for 2 to 3 h at 30°C. The medium had the following composition (mM): NaCl 145, KCl 4, NaH_2PO_4 1.25,

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$MgCl_2$ 1.5, $CaCl_2$ 2, HEPES 6, glucose 10; adjusted at pH 7.4. The solution was continuously bubbled with oxygen. Following incubation, a hippocampal slice was gently placed in a bathing chamber (capacity, 1.5 ml) and was continuously perfused with the solution at a rate of 2 to 3 ml/min. The temperature was kept at 30°C with a thermoelectric heating device.

A bipolar stimulating electrode was placed on the Schaffer collaterals of the stratum radiatum. The electrode consisted of a pair of teflon-coated platinumiridium wires with a diameter of 25 μm and a tip separation of 0.2–0.3 mm. Extracellular glass recording electrodes were pulled with a programmable micropipette puller (Sutter, P-87, Novato USA) and had a resistance of 2 to 5 MΩ when filled with 3M-NaCl. The electrode was connected to a unity gain high impedance electrometer (WPI, duo773, Sandsota USA) and positioned in the cell body region of the CA1 area. The dis-tance between the stimulating and the recording electrode was 1.2 to 1.7 mm. Supramaximal stimulation of 0.1 ms rectangular pulse was delivered and the PS amplitude was measured from the resting electrical potential prior to stimulation to the peak negativity (Fig. 1).

After control PS amplitudes were obtained, the slices were then perfused randomly with one of the solutions (pH adjusted to 4.5, 5, 5.5, 6, 6.5, or 7.4) for 15 min. pH was adjusted by titration of 1N NaOH or 1N HCl using a pH meter (Radiometer, PHM-83, Copenhagen, Denmark). Thereafter, combined glucose-oxygen deprivation was induced for 15 min while pH was maintained unchanged. Ten mM mannitol and N_2 were substituted for glucose and O_2, respectively [13,14]. Mannitol was used for the adjustment of osmolarity. The measured osmolarity was the same value (308 $mOsm/kgH_2O$) as that of glucose-contained medium. At the termination of the test period, PS was evaluated as the percent of the control amplitude. The bathing medium was then returned to normal oxygenated solution with pH 7.4; percent recovery of PS amplitudes was measured 60 min later and the slice was discarded.

As a control, the effects of acidosis without glucose-oxygen deprivation was evaluated. Slices were perfused with each of the pH adjusted solutions for 30 min and then switched back to a normal oxygenated solution with pH 7.4. Data were collected at the corresponding points of the first experiments.

Signals were collected and processed by a Hewlett-Packard (Mountain View, CA, USA) 340CH computer system linked with a 14-bit, high speed analog-to-digital converter (TEAC, PS-9351, Tokyo, Japan). The number of the slices in each subgroup was from 5 to 11. Values are expressed as mean + SEM. Student's *t*-test was utilized for statistical analysis and $P < 0.05$ was considered significant.

Results

Effects of acidosis and combined glucose-oxygen deprivation on PS amplitudes

PS amplitudes were almost abolished by glucose-oxygen deprivation in all acid-base conditions tested except for pH 6.5 where PS activity persisted and the percent amplitude was 31.5% ($P < 0.05$ *vs* pH 7.4, Fig. 2; upper graph). Following perfusion with normal solution, PS amplitudes recovered to the baseline values within 60 min for the slices which were tested with pH 5.5 or higher. Recovery of PS amplitudes was significantly inhibited for the slices tested with pH 5 and 4.5 ($P < 0.01$ *vs* pH 7.4), indicating enhanced deterioration of the glucose-oxygen-deprived impairment by profound acidosis (Fig. 2; lower graph).

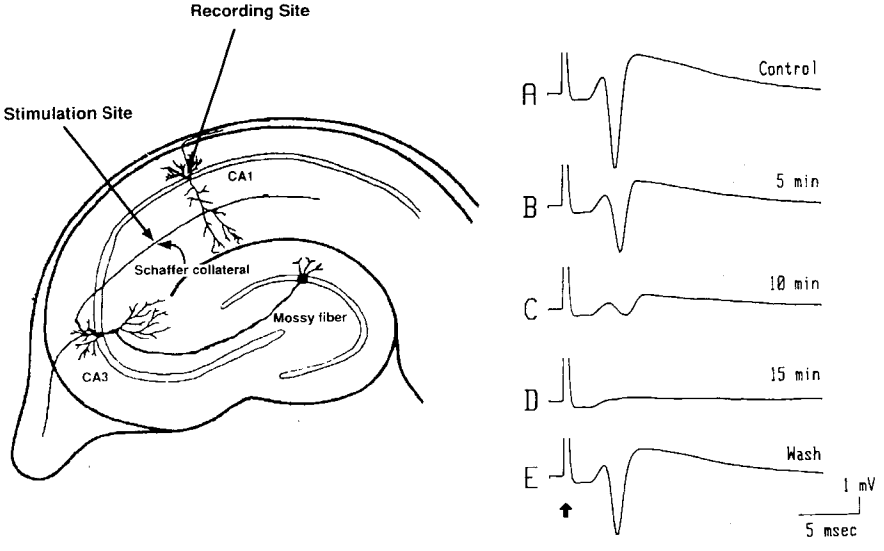

Fig. 1. An illustration of recording hippocampal evoked potentials and changes of population spike (PS) activity with glucose-oxygen deprivation. *Left*, sites of stimulation and recording; *right*, an example of changes of PS activity with glucose-oxygen deprivation. Negativity is indicated by downward deflection. An *arrow* indicates stimulus artifact. *A*, control; *B-D*, time dependent depression of PS amplitude with glucose-oxygen deprivation; *E*, recovery of PS amplitude 60 min after perfusion with control solution

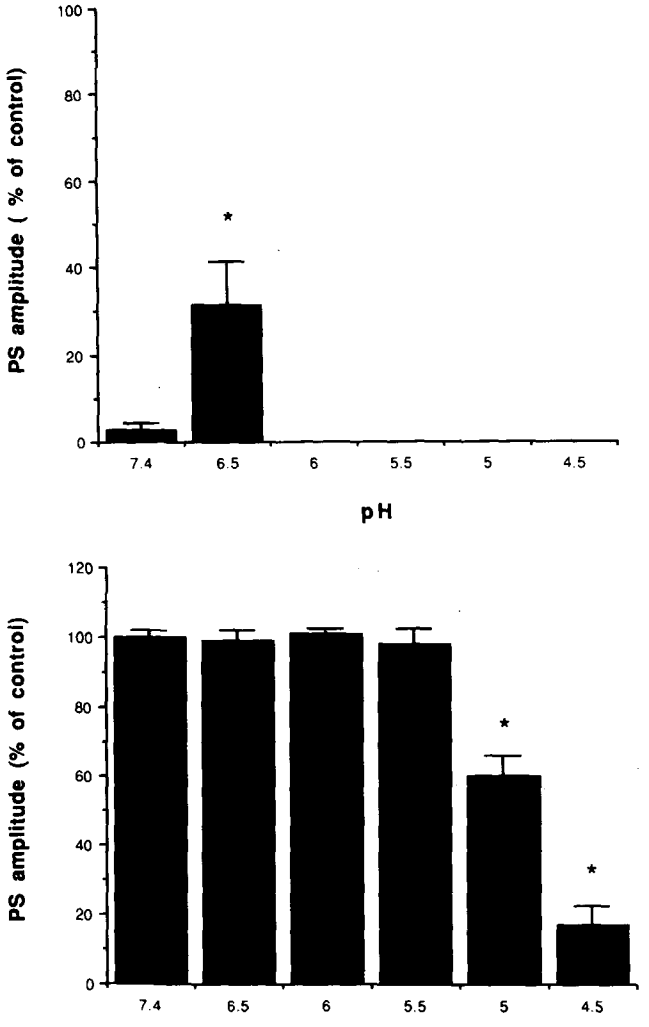

Fig. 2. Effects of acidosis and combined glucose-oxygen deprivation on PS amplitudes. *Upper graph*, depression of PS amplitudes by 15 min glucose-oxygen deprivation. Glucose and oxygen were replaced by mannitol and nitrogen in the perfused solution while pH was adjusted to either 4.5, 5, 5.5, 6, 6.5, or 7.4. Fifteen min after the withdrawal of glucose and oxygen, PS amplitude was plotted as a percent of the control. PS activity was persistent for slices tested with pH 6.5. *Lower graph*, recovery of the PS amplitudes 60 min after perfusion with normal oxygenated solution following glucose-oxygen deprivation. The test slices were perfused with normal oxygenated solution for 60 min following glucose-oxygen deprivation. Recovery of PS amplitudes was significantly inhibited for slices tested with pH 5 and 4.5 significant *vs* pH 7.4. Values are expressed by mean + SEM

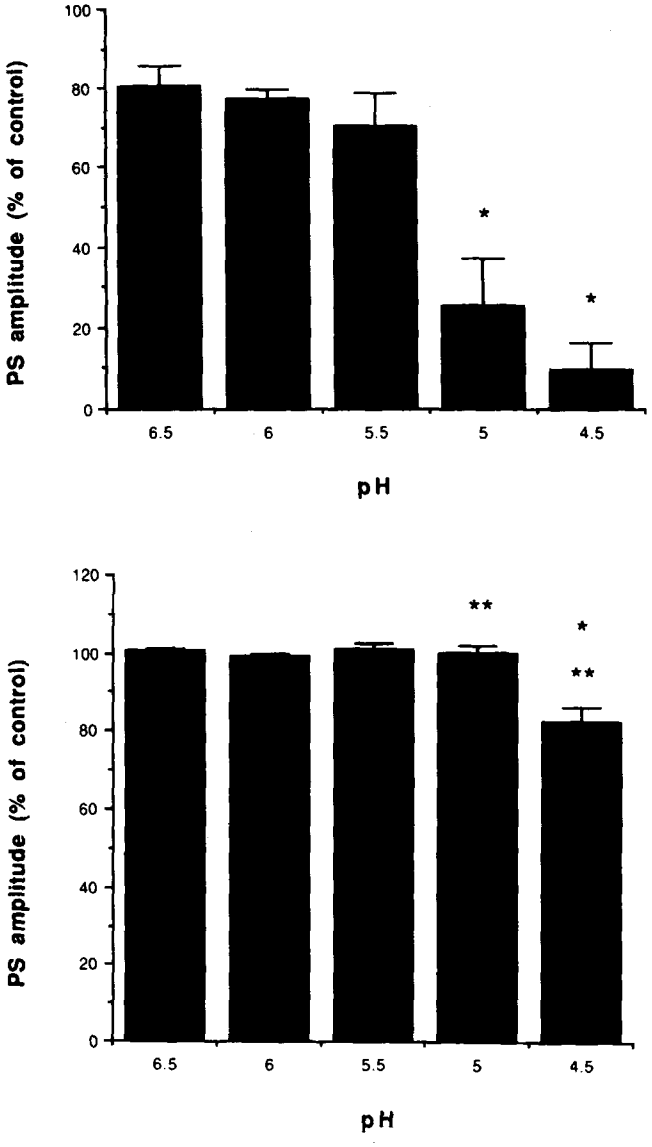

Fig. 3. Effects of acidosis on PS amplitudes without glucose-oxygen deprivation. *Upper graph*, depression of PS amplitudes by 30 min perfusion with acidotic test solution without glucose-oxygen deprivation. *Lower graph*, recovery of PS amplitudes 60 min after perfusion with normal oxygenated solution following acidotic challenge. The slices were perfused with normal oxygenated solution for 60 min following perfusion with acidotic test solution. The recovery in pH 5 and 4.5 was significantly greater than that in the corresponding pH groups with glucose-oxygen deprivation. *significant *vs* pH 7.4; **significant *vs* the same pH groups with glucose-oxygen deprivation; values are expressed by mean + SEM

Effects of acidosis on PS amplitudes without glucose-oxygen deprivation

The PS amplitudes were depressed by perfusion with acidotic solutions in a pH-dependent fashion (Fig. 3; upper graph). The PS amplitudes were significantly suppressed with pH 5 and 4.5 solutions ($P < 0.05$ *vs* pH 7.4). Sixty-minute perfusion with normal medium completely restored PS amplitudes to the baseline values for the slices tested with solutions pH 5 or higher. Profound acidosis of pH 4.5 slightly inhibited the recovery of PS amplitude (the recovery was 82.6%); however, the recovery in pH 5 and 4.5 was significantly greater than that in the same pH groups with glucose-oxygen deprivation.

Discussion

In general, lactate accumulates in the tissue accompanied by a decrease in pH during cerebral ischemia [3]. However, in cerebral ischemia following cardiac arrest, the amount of accumulated lactate may not be proportional to the degree of the pH reduction, because the level of lactic accumulation is different due to cardiac arrest [4]. To examine the effect of lactic acidosis on brain ischemia, we must understand the effect of the relationship between pH and lactate on brain ischemia. As the first step of this process, we evaluated the effect of pH changes alone.

The depression of PS amplitude by glucose-oxygen deprivation was significantly less at pH 6.5 than at pH 7.4. This finding supported the idea that mild acidosis of pH 6.5 minimizes the depressant effect of glucose-oxygen deprivation on neuronal transmission. The present study did not address whether the mechanism of this antagonistic effect was attributed to depression of NMDA receptor-activated calcium current [9–12].

During recovery from glucose-oxygen deprivation, PS amplitudes at pH 6 and 5.5 were not significantly different from those at pH 7.4 indicating that moderate acidosis of pH 6 and 5.5 did not impair recovery from the PS amplitude suppression caused by glucose-oxygen deprivation. These results confirmed the data reported by Schurr et al. that revealed the lack of difference in the recovery of PS amplitudes among various levels of lactic acidosis after hypoxic challenge, though in their study the lowest pH level used was 5.5 [8].

In the present study, the recovery of PS amplitudes following glucose-oxygen deprivation was markedly inhibited when the pH was 5 or lower. These results are consistent with in vivo data reported by Hurn et al. [15]. In their canine incomplete global ischemic model, the recovery of cerebral energy phosphate, intracellular pH (pHi) and somatosensory-evoked potentials were all inhibited after hyperglycemic ischemia when end-ischemic pHi fell to lower than 5.5.

The viability of neurons may be reduced by severe acidotic pH lower than 5 [16,17]. Inhibited recovery of PS amplitudes following glucose-oxygen deprivation at a pH of 5 and 4.5 may be attributed to the effects of profound acidosis itself. However, the complete recovery of PS amplitude in the slices exposed to pH 5 and 80% recovery by pH 4.5 suggests an additive or synergetic effect of profound acidosis over the damage induced by glucose-oxygen deprivation alone. The mechanism of the effect may be explained as follows. First, low pH may enhance cell swelling by activating Na^+/H^+ exchange [16,18] though the mechanism is also a safeguard against abnormal intracellular acid-base conditions [3,16,18]. In in vivo preparations, swelling of the cells may produce further neuronal damage by disturbing in situ circulation [3]. Second, severe intracellular acidosis may inhibit mitochondrial function which may deteriorate the regulation of calcium homeostasis [3]. Therefore, acidosis during ischemia may accelerate accumulation of intracellular calcium. Increased intracellular calcium may activate destructive enzymes such as lipases and proteases, which trigger cell death [3,19]. The third factor may be an enhancement of free radical reactions. Both ischemia and acidosis have been reported to generate free radicals [3,19,20].

In conclusion, mild acidosis of pH 6.5 minimized depression of PS amplitudes during glucose-oxygen deprivation. Profound acidosis of pH 5 or lower prevented recovery of PS amplitudes after reversal of glucose-oxygen deprivation. A "crossover point" was observed at moderate acidosis of pH 6 and 5.5 with no significant effect on depression of PS amplitudes during glucose-oxygen deprivation and recovery of PS amplitudes after reversal of glucose-oxygen deprivation.

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