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Depletion of ATP and release of presynaptic inhibition in the CA1 region of hippocampal slices during hypoglycemic hypoxia

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Abstract

Transient recovery (TR) of evoked synaptic potentials and ATP depletion during the late stage of hypoxic hypoglycemic insults were investigated in rat hippocampal slices. TR was observed not only in the late stage of insult, but also during recovery. The concentration of ATP corresponded to the appearance (27% of control) and disappearance (15% of control) of TR. Paired pulse studies showed the presynaptic nature of the release of inhibition of synaptic transmission during TR. Both N- and P/Q-type voltage-dependent calcium channels were involved in the appearance of TR. This evidence suggests that underlying mechanisms of TR appearance during hypoxic hypoglycemic insult might be related to ATP depletion and release of A1 adenosine receptor mediated inhibition of presynaptic voltage-dependent calcium channels.

Keywords: A1 adenosine receptor; Hypoglycemic hypoxia; Synaptic depression; Transient recovery; Voltage-dependent calcium channels

In the central nervous system, metabolic stress such as oxygen–glucose deprivation and hypoxic hypoglycemia *in vitro* deplete intracellular ATP stores and lead to the development of irreversible neuronal tissue damage [14,18]. One of the earliest signs of neuronal responsiveness during a hypoxia-induced fall in ATP levels, coupled with extracellular accumulation of adenosine, is a depression of synaptic transmission, mainly due to presynaptic inhibition of L-glutamate release resulting from suppression of voltage-gated calcium currents [1,13]. However, during the later stage of synaptic depression, transient recovery (TR) of evoked synaptic potentials is recorded in the hippocampus [3,7,19]. When hypoxia ceases during this period, synaptic transmission recovers fully and no obvious structural changes are seen in the neurons [8]. Although the neuroprotective relevance of synaptic depression remains controversial [2,9], A1 adenosine-mediated synaptic depression limits Ca^{2+} entry and reduces the release of excitatory neurotransmitters and metabolic demand, which are considered to have neuroprotective roles against metabolic insults [4,6]. Additionally, blockade of neural transmission facilitates recovery after a hypoxic insult [11]. Thus, temporary release from synaptic depression has also been receiving specific attention. The mechanisms underlying the development of TR have not yet been fully elucidated. It has been suggested that TR might be linked to slow depolarization and hyperexcitability preceding the anoxic depolarization or to excessive release of glutamate through loss of the adenosine inhibitory action [7,8,19]. Recently, we showed that TR of evoked synaptic responses is related to rapid energy depletion, and we raised the possibility of ATP-dependent release of presynaptic inhibition [12]. This investigation was designed to further elucidate underlying mechanisms of the development of TR, especially on the site of action and the relation with ATP concentration.

All experiments were carried out with male Sprague Dawley rats (4–6 weeks old, weighing 100–150 g). All animal procedures were reviewed and approved by the Korea University Institutional Animal Care and Use Committee and complied with guidelines outlined in the Korean Academy of Medical Science's *Guide for the Care and Use of Laboratory Animals*. The basic preparations for experimental settings were similar to those described previously [12]. Briefly, rats were decapitated under ether anesthesia. The brains were rapidly removed and placed in 4 °C ice-cold oxygenated (95% O_2 and 5% CO_2) artificial cerebrospinal fluid (aCSF). Both hippocampi of each rat were dissected for removal and cut into 450 μm-thick slices perpendicular to the longitudinal axis, using a McIlwain tissue chopper.

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Slices were incubated in vials of continually oxygenated aCSF at 30 ± 1 °C before use. Normal aCSF (125 mM NaCl, 3.5 mM KCl, 4 mM NaH_2PO_4, 1.5 mM $MgCl_2$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, 10 mM D-glucose) was saturated with 95% O_2/5% CO_2 to a pH of 7.4 ± 0.05. After a 90 min recovery period, slices were individually transferred as needed to a submerged recording chamber (volume 0.12 ml). Within the recording chamber, slices were continuously perfused at a rate of 3 ml/min with fresh, oxygenated aCSF warmed to 30 ± 1 °C.

For extracellular recordings, after the slices had been immobilized in the recording chamber, two recording electrodes filled with 150 mM NaCl (resistance 1–5 M) were placed in the hippocampal CA1 area. One was positioned in the pyramidal cell layer to record population spikes (PSs), and the other in the apical dendritic layer, stratum radiatum, to measure dendritic field excitatory postsynaptic potential (fEPSPs 250 μm from the stratum pyramidale). The two recording electrodes were placed in a line perpendicular to the cell body layer so that each would tend to record responses from the same set of neurons. In each slice, a bipolar platinum–iridium electrode with a tip diameter of 25 μm was placed in the alveus, to elicit antidromic PSs. For the orthodromic stimulation, another bipolar electrode was placed in the stratum radiatum to stimulate the Schaffer collaterals/commissural bundle on the CA3 side. The inter-electrode distance was 500 μm. In some experiments, a third stimulating electrode was placed in the stratum radiatum on the subicular side of the recording electrodes. This electrode was used to deliver a different intensity stimulation. At the beginning of each experiment, a stimulus response curve was established by increasing the stimulus. Stimulation producing half-maximum responses of population spike was used as standard strength. Stimuli of duration 50 μs were delivered at 0.05 Hz. In some experiments, paired electrical stimuli were delivered to the Schaffer collaterals at interstimulus intervals ranging between 10 and 200 ms, and the slope of the second fEPSP expressed as a percentage of the first evoked response. Only slices those were able to produce single PS responses with stable amplitudes greater than 3 mV were used. If a slice satisfied this criterion, the basal PS was evoked for at least 30 min before exposure to hypoxia and hypoglycemia. To induce hypoxia, slices were superfused with aCSF equilibrated with 95% N_2/5% CO_2. To examine the effect of glucose concentration during hypoxia, slices were superfused with aCSF in which the glucose had been replaced with an equal concentration of sucrose.

For determination of ATP concentration, slices taken from the recording chamber at the time point representing baseline, before, during, and after the TR were immediately frozen in liquid nitrogen. Later, slices were homogenized in 0.5 ml of ice-cold perchloric acid (0.3 M), neutralized with 3 M K_2CO_3, centrifuged to remove precipitates, and used for determination of ATP with a bioluminescence assay kit (Sigma–Aldrich). Protein concentration was estimated by the bicinchonic acid (BCA) reagent method (Sigma–Aldrich). All numerical data are expressed as the mean ± S.E.M. Data were tested for statistical significance with paired, two-tailed, Student's *t*-test. The level of significance was set at $P < 0.05$. The stock solutions of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, Tocris) were prepared using dimethylsulfoxide. Further dilutions were prepared using aCSF. The final concentration of dimethylsulfoxide in the perfusion medium was 0.05%. The stock solutions of omega-conotoxin-GVIA and omega-conotoxin-MVIIC (Alomone Labs) were each prepared at a concentration of 0.3 mM and 0.1 mM in a solution containing 100 mg ml^{-1} bovine serum albumin (Sigma–Aldrich). The stock solution was added directly to the re-circulating perfusion solution.

As shown in previous studies [12], application of the hypoxic hypoglycemic insult consistently induced a typical TR ($n = 15$, Fig. 1A). When reperfusion with oxygenated aCSF commenced just after the termination of aPS, another TR developed, together with rapid regain of aPS, showing a mirror image. The appearance related to aPS amplitude was not changed in this secondary TR, but was brief and low in its duration and intensity, concomitantly with a more rapid and steep raising phase of aPS. During the recovery, TR and synaptic depression occurred in the reverse

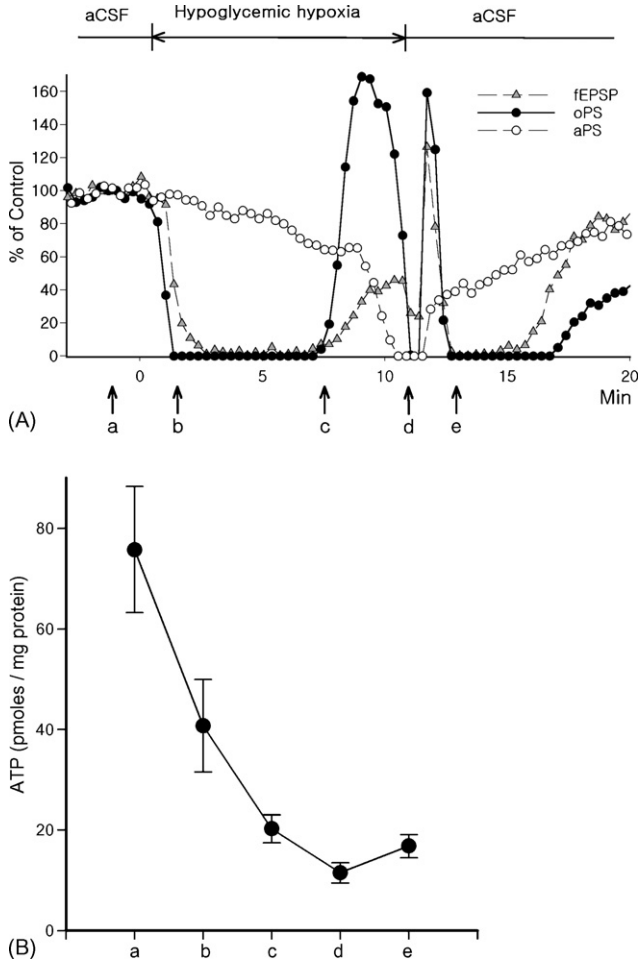

Fig. 1. Transient recovery (TR) of synaptic transmission occurs during the recovery period as well as during hypoxic hypoglycemic insult. (A) Representative time course of the effect of hypoxic hypoglycemia (applied as indicated by the arrow) and reperfusion with oxygenated artificial cerebrospinal fluid on the normalized data of evoked potentials. (B) Mean concentrations of ATP measured at each time point. The time points indicated, refer to baseline control (a), commencement of hypoxic depression (b) and transient recovery (c), disappearance of TR (d), and redisappearance of TR during the reperfusion (e) as shown in (A).