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Zoological Research

Enhancement of $GABA_A$ Receptor-Mediated Inhibitory Postsynaptic Currents Induced by "Partial Oxygen-Glucose Deprivation"

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Abstract: Oxygen/glucose deprivation (OGD) has been widely used as an *in vitro* model of focal ischemia, where the blood flow is severely reduced and neurons rapidly die. However, adjacent to the focal region is 'penumbra', where residual blood flow remains oxygen and glucose supplies are at low levels. To model this pathological genesis, we developed a partial OGD (pOGD) protocol in a rat brain slice. This model met two requirements: oxygen was partially deprived and glucose was reduced in the perfusion buffer. Therefore we investigated the effect of pOGD on gama-aminobutyric acid ($GABA_A$) receptor-mediated inhibitory postsynaptic currents (IPSCs) in CA1 neurons of a hippocampal slice through whole-cell patch-clamp technique. We found that the amplitude and decay time of IPSCs were increased immediately during pOGD treatment. And the enhancement of IPSCs amplitude resulted from an increase of the synaptic conductance without a significant change in the reversal potential of chloride. These results suggested that the nervous system could increase inhibitory neurotransmission to offset excitation by homeostasis mechanisms during the partial oxygen and glucose attack.

Key words: Partial oxygen-glucose deprivation (pOGD); $GABA_A$ receptor; IPSCs; Amplitude; Decay time

"不完全氧糖剥夺"对 A 型 γ-氨基丁酸受体介导的抑制性突触后膜电流的增强作用

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摘要："氧糖剥夺"模型作为研究脑缺血的离体模型被广泛使用，该模型模拟了局灶性脑缺血的主要病理变化。然而在缺血病灶核心区与正常脑组织之间称为缺血半暗带的区域，脑血流也有程度不一的降低。为了模拟这种病理变化，发展了一种"不完全氧糖剥夺"的离体脑片模型，该模型满足两个条件，灌流液里氧气部分剥夺而葡萄糖含量降低；"氧糖剥夺"可以导致谷氨酸介导的兴奋性毒性，从而引起神经细胞的坏死。而 A 型 γ-氨基丁酸受体（$GABA_AR$）介导的神经元抑制性活动可以对抗谷氨酸引起的兴奋性毒性，因此近年来引起广泛的研究兴趣。而谷氨酸受体和 γ-氨基丁酸受体功能在缺血半暗带是否有改变尚不得而知。因此本文采用海马脑片全细胞膜片钳的记录方法，研究"不完全氧糖剥夺"对海马 CA1 区神经元的 A 型 γ-氨基丁酸受体介导的抑制性突触后膜电流 (IPSCs)的影响。研究发现"不完全氧糖剥夺"使 $GABA_AR$ 介导的 IPSCs 的峰值增加而衰减时程延长。进一步研究发现该电流的峰值增加是由于 $GABA_AR$-氯离子通道的电导增加所致，而与氯离子的反转电位变化无关。这些发现提示在脑缺血的缺血半暗带区域 $GABA_AR$ 介导的神经元抑制性活动可能是增强的，这可能是神经元面对缺血状态产生自我保护的一种内稳态机制。

关键词：不完全氧糖剥夺；A 型 γ-氨基丁酸受体；抑制性突触后膜电流；峰值；衰减时程
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In focal strokes, the 'core' territory refers to the region with the lack of blood flow and within which brain cells rapidly die. Adjacent to the core is the 'penumbra', a peripheral zone of mild to moderate

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ischemia where residual blood flow might transiently sustain tissue viability. In this meta-stable tissue, cells die more slowly and the lesion expands over time (Lo, 2003; Ginsberg, 1999).

Oxygen/glucose deprivation (OGD) protocol in a hippocampal slice has been widely used to model the 'core' territory of focal strokes (Lipton, 1999; Lobner, 1993). In this instance the bathing solution is rapidly changed from O_2/CO_2 equilibrated to N_2/CO_2 equilibrated with glucose omitted. The accumulated data about ischemia has been obtained through the OGD protocol. For example, OGD initially causes rapid depression of glutamate-mediated excitatory synaptic transmission in the hippocampus (Martin,1994; Rosen, 1993) and suppresses GABA (gama-aminobutyric acid)-mediated synaptic transmission in the striatum (Centonze, 2001). These effects are mainly caused by the inhibitory action of adenosine released in the extracellular space, and precede the development of irreversible neuronal death and extracellular accumulation of both excitatory and inhibitory amino acids (Lipton, 1999; Martin, 1994).

Since neurons in the 'penumbra' zone can potentially be salvaged by timely intervention (Lo, 2003), it is important to investigate the pathological changes due to oxygen and glucose decline gradually in this region. However, how this pathological condition affects inhibitory synaptic transmission is still unclear. Since the 'penumbra' zone barely receives enough blood flow to keep neurons alive (Newman,1988;Hakim,1998;Fisher, 2006), we developed a partial OGD protocol in the brain slices to mimic this pathological process. This model met two requirements: oxygen was partially deprived and glucose was reduced in the perfusion buffer.

We investigated the effect of pOGD on $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs) in CA1 neurons of the hippocampal slice through whole-cell patch-clamp technique. The results suggested that the nervous system can increase inhibitory neurotransmission to offset excitation by homeostasis mechanisms during the partial oxygen and glucose attack and targeting these mechanisms might provide important information for both researchers and clinicians.

1 Method

1.1 Slice preparation

The experimental protocols were approved by the Department of Biology of the Chinese Academy of Sciences, PR China. The slice preparation and electrophysiological protocols were similar to those described previously (Li, 2005; Zhang, 2005; Sun, 2005). 14–21 day old Wistar rats (inbred strain, Animal House Center, Kunming General Hospital, Kunming) were decapitated and the brain was quickly removed and immersed in ice-cold artificial cerebral spinal fluid (ACSF) in vibroslicer chamber. Hippocampal slices (400 μm thick) were cut coronally and then transferred into a submersion-type incubation chamber containing 300 mL ACSF heated to 35±1°C for 1 h recovery. During incubation, slices were placed on nylon mesh and both sides of the slices were perfused by oxygenated ACSF. The ACSF contained (in mol/L): NaCl 120, KCl 2.5, $NaHCO_3$ 26, NaH_2PO_4 1.25, $CaCl_2$ 2.0, $MgSO_4$ 2.0, and D-glucose 10; saturated with 95% O_2 and 5% CO_2. Then slices were gently transferred into a submerged recording chamber (4–5 mL/min, 22–25°C).

1.2 Eletrophysiology

Blind whole-cell recordings were obtained in CA1 pyramidal neurons using glass electrode (3–6 MΩ) filled with (in mmol/L): $CsCH_3SO_3$ 100, CsCl 60, HEPES 10, EGTA 0.2, Mg-ATP 2, $MgCl_2$ 1, QX-314 5, pH adjusted to 7.2 with CsOH (285 mOsm)(Alger,1996). The QX-314 was used to block $GABA_B$-mediated currents. For this internal solution, the equilibrium potential of chloride (E_{Cl})= −20 mV and $GABA_A$ receptor-mediated currents were inward at the holding potential of −65 mV and they were outward at the holding potential of 0 mV. Glutamergic synaptic transmission was inhibited by 3 mmol/L kynurenic acid and IPSCs were induced by 0.05 Hz electrical stimuli (0.1 ms in duration) to the schaffer/collateral of CA1.

1.3 Partial oxygen and glucose deprivation

The partial (i.e. not entirely oxygen-deprived) OGD-ACSF contained (in mmol/L): NaCl 120, KCl 2.5, $NaHCO_3$26, NaH_2PO_4 1.25,$CaCl_2$ 2.0, $MgSO_4$ 2.0, and D-glucose 2.0; oxygenating (95% O_2 and 5% CO_2) it for 10 min (pH=7.4). Then the gas supply was stopp-

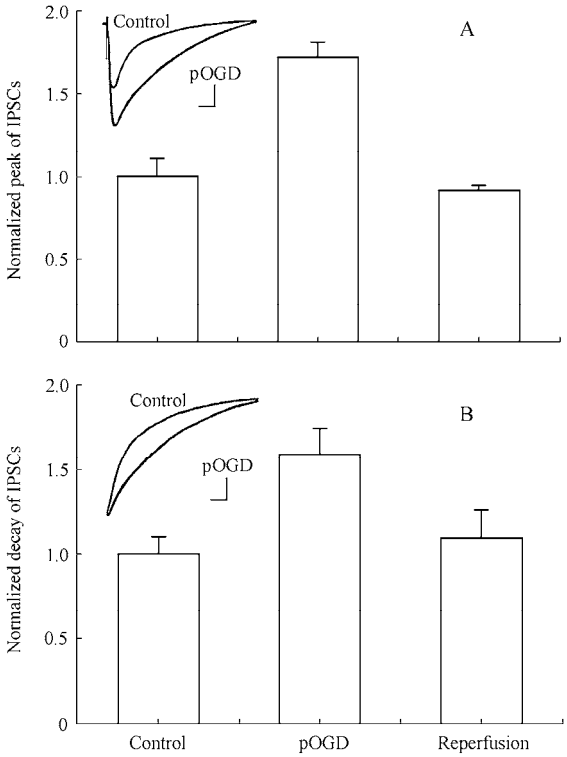

Fig. 1 Enhancement of GABAergic transmission by partial OGD (pOGD)

A: $GABA_A$ receptor-IPSCs were inward currents when cells were holding at –65 mV and pOGD caused a marked increase of the amplitude of the IPSCs. This effect was reversible when the pOGD solution was washed out (baseline= −280.1±30 pA, pOGD= −472.6± 49.9 pA, reperfusion=−257.7±10.3 pA, n=8, pOGD vs. baseline P< 0.05). The inset traces were IPSCs recorded before and during the pOGD treatment. B: pOGD significantly slowed the deactivation of IPSCs and this effect was recovered after reperfusion (baseline=45.1+ 4.8 ms, pOGD=82.2±11.1 ms, reperfusion=49.6±8.4 ms, n=8, pOGD vs. baseline P<0.05). The inset traces showed current deactivation before and during the pOGD treatment. For the purpose of this comparison, the level of the current at the peak was normalized. The scale bar was 20 ms, 50 pA.

ed for at least 30 min to let the O_2 partially diffuse out before the solution entered into the recording chamber. After 5 min of exposure to the pOGD solution, the slices were perfused with an oxygenated ACSF (reperfusion).

1.4 Data analysis and Statistics

Signals were filtered at 5 kHz and digitized at 20 kHz and stored on the computer. All values were reported as mean±*se*, *n* being the number of slice. Student's *t*-test was used for statistical comparison. Significance level was set at P<0.05.

2 Results

2.1 Effect of partial oxygen and glucose deprivation (OGD) on hippocampal GABAergic IPSCs

We recorded $GABA_A$ receptor-IPSCs from pyramidal neurons in area CA1, induced by stimulation of the schaffer collateral-commissural pathway. IPSCs were inward currents when cells were holding at –65 mV. After obtaining stable IPSC amplitude, partial oxygen and glucose deprivation (pOGD) was performed. The pOGD caused a marked increase in the amplitude of IPSCs. The average of IPSCs amplit-

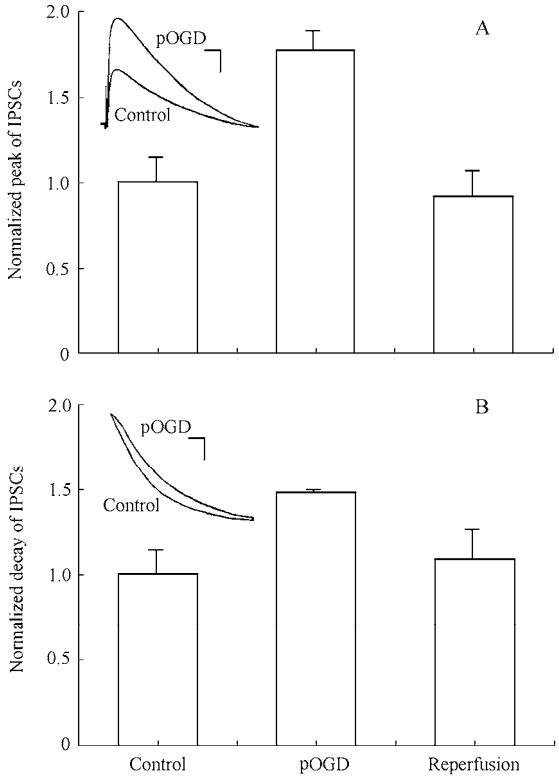

Fig. 2 Enhancement of GABAergic transmission by partial OGD (pOGD)

A: $GABA_A$ receptor-IPSCs were outward currents when cells were holding at 0 mV and pOGD caused a marked increase of the amplitude of the IPSCs. This effect was reversible when the pOGD solution was washed out (baseline=177.1±36.5 pA, pOGD=343.6± 98.3 pA, reperfusion =161.2±24.2 pA, n=5, pOGD vs. baseline P< 0.05). The inset traces were IPSCs recorded before and during the pOGD treatment. B: pOGD significantly prolonged the deactivation of IPSCs and this effect was recovered after reperfusion (baseline =78.2±19.0 ms, pOGD=114.2±26.3 ms, reperfusion =84.5±15.2 ms, n=5, pOGD vs. baseline P<0.05). The inset traces showed current deactivation before and during the pOGD treatment. For the purpose of comparison, the level of the current at the peak was normalized. The scale bar was 20 ms, 50 pA.

ude was –280.1±30 pA (*n*=8) and decay time was 45.1±4.8 ms (*n*=8) when cells were holding at –65 mV. During the onset of pOGD treatment the amplitude of IPSC was increased to –472.6±49.9 pA (*n*=8, *P*<0.05 compared with baseline) and the decay time was increased to 82.2±11.1 ms (*n*=8, *P*<0.05 compared with baseline). After reperfusion, the amplitude of IPSC was recovered to –257.7±10.3 pA (*n*=8, *P*>0.05 compared with baseline) and the decay time was reversed to 49.6±8.4 ms (*n*=8, *P*>0.05 compared with baseline).

The IPSCs were outward currents when cells were holding at 0 mV. After obtaining stable IPSC amplitude, pOGD was performed. The average of IPSCs amplitude was 177.1±36.5 pA (*n*=5) and decay time was 78.2±19.0 ms (*n*=5). During pOGD treatment the amplitude of IPSCs was increased to 343.6±98.3 pA (*n*=5,*P*<0.05 compared with baseline) and decay time was increased to 114.2±26.3 ms (*n*=5, *P*<0.05 compared with baseline). After reperfusion, the amplitude of IPSC was recovered to 161.2±24.2 pA (*n*=5, *P*>0.05 compared with baseline) and the decay time was reversed to 84.5±15.2 ms (*n*=5, *P*> 0.05 compared with baseline). Taken together, these results revealed that pOGD significantly increased the amplitude and prolonged the deactivation of IPSCs either at –65 mV or at 0 mV.

2.2 Effect of pOGD on GABA-gated Cl¯ channel conductance

The IPSCs reversal potential (Er) was –22.7±3.3 mV (*n*=7) at normal ACSF and was –21.2±3.2 mV (*n* = 7) during pOGD treatment (*P* > 0.05). The current-voltage (Ⅰ–Ⅴ) relation of the IPSCs revealed that the enhancement of IPSCs by pOGD was due to a 36.8% increase of $GABA_A$-R/ chloride channel conduction from 7.2±0.7 nS in control to 11.4±1.4 nS during pOGD (*n* = 7, *P* < 0.05) (Fig. 3).

2.3 Effect of pOGD on the GABAergic synaptic plasticity

After a stable baseline recording, high-frequency stimulation (HFS; two trains of 100 pulses at 100 Hz, separated by 20 s) induced long-term depression of the IPSCs amplitude to 69.4±5.0% of baseline (*n*=7, Fig.4) under control conditions. After pOGD treatment, slices were resuperfused for 30 min and the HFS induced LTD of IPSCs amplitude to 74.3±5.6% of baseline (*n*=7, Fig.4). There was no significant difference between the two groups (*P*>0.05).

3 Discussion

The concept of the ischemic penumbra is an important one for both basic investigators of cerebral ischemia and for clinicians who treat stroke patients, since this portion of the ischemic territory is still potentially salvageable if an appropriate treatment is given (Heiss, 1994; Hakim, 1998; Fisher, 2006). The penumbra is an elegant concept, however, in practice it has been a difficult one to exploit/explore. For one thing, ischemic penumbra is unstable in both time and space. Depending on the severity and the duration of

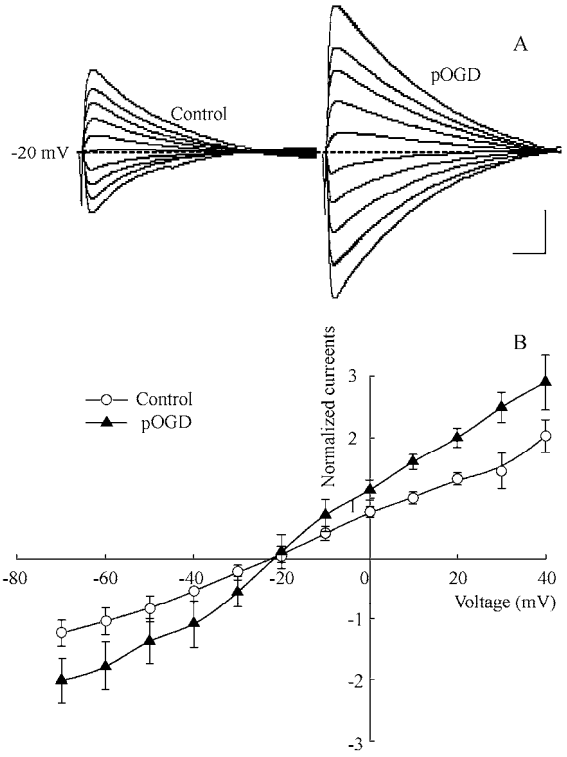

Fig. 3 Enhancemnt of $GABA_A$-gated Cl– channel conductance by partial OGD (pOGD)

A: Exemplar depolarized pulses before and during pOGD stepped by 10 mV. The traces showed an enhancement in IPSCs during pOGD at all the explored membrane potentials. B: Current-voltage (Ⅰ–Ⅴ) relation of IPSCs before and during the pOGD. The polarity was reversed at approximately –20 mV in both experimental conditions ($Er_{(baseline)}$ =–22.7±3.3 mV, $Er_{(pOGD)}$=–21.2+3.2 mV, *n*=7, *P*>0.05). For the purpose of comparison, the peak of the current at all the explored membrane potentials was normalized to the peak of +20 mV (Conduction $_{(control)}$ =7.2± 0.7 nS, $Conduction_{(pOGD)}$ =11.4±1.4 nS, *n*=7, *P*<0.05). The scale bar was 20 ms, 150 pA.

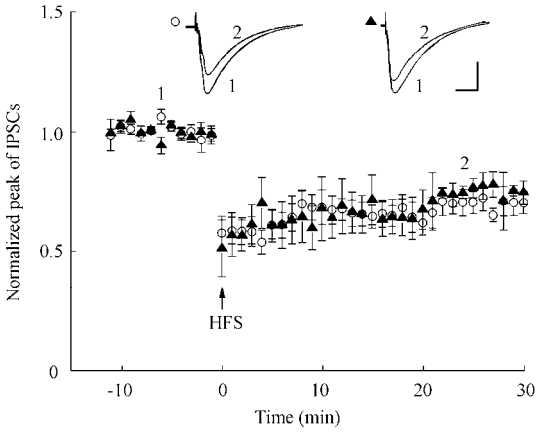

Fig. 4 Partial OGD treatment did not impair the GABAergic synaptic plasticity

High-frequency stimulation (HFS) induced long-term depression of the IPSCs amplitude to 69.4±5.0% of baseline under control condition (n=7). After pOGD treatment, slices were reperfused for 30 min and the HFS induced LTD of IPSCs amplitude to 74.3±5.6% of baseline (n=7, P>0.05). HFS was given at the time indicated by the arrow and the time of HFS for each experiment was aligned and zeroed. Sample traces (indicated by numbers) taken before and 20—25 min after HFS were shown superimposed on top. The scale bar was 20 ms, 100 pA.

the focal ischemia, it may be anywhere in the ischemic brain (Newman, 1988; Heiss, 1994; Hakim, 1998; Fisher, 2004). In this study, we developed a pOGD in a brain slice *in vitro* to mimic this pathological process. This model met two requirements: oxygen was partially deprived and glucose was reduced in the perfusion buffer. Both of these conditions are similar to the circumstances that occur in the penumbra when it receives inadequate blood flow to keep neurons alive.

Here we found that the amplitude and decay time of IPSCs were increased immediately after pOGD attack. Moreover, the slope of current-voltage (Ⅰ－Ⅴ) relationship for the IPSCs was increased, which suggested an increase of the inhibitory synaptic conductance.

Therapeutic evidences for ischemia based on modulating the actions of GABA on $GABA_A$ receptor-rs suggested neuronal-protection may be achieved by increasing inhibitory neurotransmission (Galeffi, 2004; Schwartz, 1995; Green, 2000; Shuaib, 1997; Schwartz-Bloom, 2001). However, there is a critical time-window after ischemia when enhancement of GABA neurotransmission is beneficial to neurons (Schwartz-Bloom, 1998). Combined with the current observation that GABAergic transmission was enhanced during pOGD attack, it is highly possible that an increase in inhibitory synaptic transmission may have a protective role against neuronal death in the penumbra.

Changes in the amplitude of synaptic currents are usually attributed to changes in the total synaptic conductance, resulting from alterations in either transmitter release or the postsynaptic responses to the transmitter. However, other mechanisms may also contribute. For example, changes in the intracellular chloride concentration ($[Cl^-]i$) could alter the reversal potential for IPSCs (E_{IPSC}), thus changing their amplitude (Ling, 1995; Thompson, 1989). Although an enhancement in $GABA_A$-mediated currents was found during the pOGD attack at all the explored membrane potentials, the polarity was reversed at approximately −20 mV in both experimental conditions. This finding indicated that the observed IPSCs enhancement was not due to a change of intracellular chloride concentration, but due to the conduction-enhancement of $GABA_A$-R/ chloride channel.

It is well known that GABA levels increase rapidly with glutamate following ischemic attack (Schwartz-Bloom, 2001; Inglefield, 1998). The consequences of this short-lived and large accumulation of extracellular GABA may offset the glutamate-induced excitotoxicity. It is highly possible that GABA levels also increase during pOGD, which may modify $GABA_A$ receptors and result in the enhancement of IPSCs. Another possibility is an increased postsynaptic sensitivity to GABA, which could also be responsible for the pOGD-induced enhancement of hippocampal IPSCs.

The basic inhibitory synaptic transmission was fully recovered from the pOGD attack after reperfusion, which suggests that pOGD is one of sub-lethal stress for the neurons in the 'penumbra' zone. Since neuronal excitability in the brain is strictly controlled by the inhibition tone set by GABAergic interneurons, the strength of inhibitory synaptic transmission should have important consequences on neuronal excitability. Our results showed that GABAergic LTD was not affected after reperfusion, which further indicated that pOGD is one form of sub-lethal stress. Selye's theory indicates that

organisms can upregulate powerful endogenous pathways following sub-lethal stress, such that resistance to subsequent injury is increased (Selye, 1956; Kitagawa, 1990; Nandagopal, 2001; Lo, 2003). According to this theory, the pOGD may make neurons more resistant to subsequent ischemic attack. The GABAergic enhancement during the pOGD may decrease neuronal excitability, thereby reducing the energy demands of the neuron (Moncayo, 2000). Once these compensating mechanisms have been exhausted (such as the condition in OGD), excitotoxicity induces ischemic neuronal death (Lipton, 1999). Therefore targeting the mechanisms of GABAergic enhancement in the penumbra may provide promising therapeutic approaches for ischemic attack.

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