In-vitro Ischemia Alters the Pharmacological Profile of GABA<sub>A</sub> Receptors

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INTRODUCTION

Global cerebral ischemia is a significant cause of morbidity and mortality in the United States and around the world. This results in significant functional neurological losses that can range from mild memory impairment to a persistent vegetative state. Despite considerable interest in techniques that might reduce neurological injury after ischemia, only induced hypothermia has shown efficacy in clinical trials for treatment of patients after sudden cardiac arrest. Numerous pharmacological neuroprotection strategies have been investigated, thus far without consistent results. One such strategy is to alter the balance of excitatory and inhibitory neurotransmission in the brain, reducing the damage caused by excessive excitatory signaling (excitotoxicity) which occurs after restoration of cerebral blood flow (reperfusion). Attempts to increase inhibitory neurotransmission have focused on the GABA<sub>A</sub> receptor. GABA<sub>A</sub> receptors mediate the majority of fast inhibitory neurotransmission in the brain. The receptor is a hetero-oligomeric transmembrane protein with a central chloride ion-selective pore. The subunits of the receptor generally have a stoichiometry of 2:2:1 (α, β, and γ subunits most commonly). Cerebellar Purkinje cells have GABA<sub>A</sub> receptors comprised of α<sub>1</sub>, β<sub>2</sub>, and γ<sub>2</sub>. These cells have a robust excitatory and inhibitory drive and are particularly vulnerable to ischemic damage. Using these cells in culture, we have observed changes in GABA<sub>A</sub> pharmacology after OGD. The diagram to the left shows a simplified model of the structure of the GABA<sub>A</sub> receptor, as used in this study.

HYPOTHESIS

Ischemia causes an alteration in the pharmacology of the GABA<sub>A</sub> receptor.

METHODS

Electrophysiology: Whole cell voltage clamp experiments were made from the somas of Purkinje cells (selected from their large soma and extensive dendritic arbor) in culture, using an Axopatch 200B amplifier interfaced to a Dell computer. Data was collected and analyzed using pCLAMP software. A gravity-fed microperfusion system was used to measure the response of Purkinje cells to GABA. The amplitude of GABA activated currents was measured using Clampfit analysis software and normalized to each cell’s capacitance. A pre-stored 8-value microperfusion system (ALA Scientific Instruments) for rapid solution exchange with a gravity fed bath flow rate of 2.5ml/min was used to measure the response of PCs to varying concentrations (see figure legends) of GABA, etomidate, propofol, zolpidem, and diazepam. The composition of the bathing Saline solution was (in mM): 140 NaCl, 5 KCl, 0.8 MgCl<sub>2</sub>, 1 CaCl<sub>2</sub>, 10 HEPES, 1 MgATP, pH 7.3 with NaOH. Internal pipette solution was (in mM): 140 CsCl, 1 EGTA, 10 HEPES, 5 MgATP, pH 7.3 with CsOH. Propofol, Diazepam and GABA were obtained from Sigma-Aldrich. Etomidate and zolpidem are obtained from Tocris Bioscience.

RESULTS

Figure 1. Oxygen and glucose deprivation causes decreased GABA mediated current. Following 2 hours OGD, cerebellar Purkinje cells display significantly reduced GABA<sub>R</sub> current within the 1st hour of reperfusion.

Figure 2. Oxygen and glucose deprivation does not change GABA<sub>R</sub> affinity for GABA. GABA<sub>R</sub> current response to increasing concentrations of GABA (0.1, 3, 30, 300, 1000, 5000 µM) was measured before and after OGD. As expected from previous experiments, exposure to OGD causes decreased GABA<sub>R</sub> current, OGD cells had smaller current amplitudes at higher doses. However, there was no significant change in affinity for GABA (Kd=3.8 and 7.4, Control and OGD).

Figure 3. Oxygen and glucose deprivation does not alter GABA<sub>A</sub> sensitivity to Propofol. Response to 2µM GABA plus increasing concentrations of propofol (1, 3, 5, 10, 15 µM) was tested before and after OGD. Last response is GABA alone (1 µM). Neither affinity nor efficacy for propofol decreased (Control Kd=5.6 ± 0.5 µM, n=9, OGD Kd=3.8 ± 0.5 µM, n=12).

Figure 4. Etomidate has decreased affinity and efficacy following oxygen and glucose deprivation. Response to 5µM GABA plus increasing concentrations of etomidate (0.1, 1, 3, 5, 10 µM) was tested before and after OGD. Last response is GABA alone (1 µM). Affinity for etomidate decreased (Control Kd=2.9 ± 0.3 µM, n=8, OGD Kd=3.8 ± 0.2 µM, n=6). Also, etomidate efficacy was significantly reduced after OGD.

Figure 5. Oxygen and glucose deprivation does not alter GABA<sub>A</sub> sensitivity to zolpidem and diazepam. Response to 5µM GABA plus increasing concentrations of zolpidem (0.1, 1, 3, 10 µM) was tested before and after OGD. Efficacy of zolpidem did not change (Control Kd=46.5 ± 75.9 µM, n=10, OGD Kd=42.6 ± 62.4 µM, n=8). Response to 5µM GABA plus increasing concentrations of diazepam (0.1, 0.3, 1, 3, 10 µM) was tested before and after OGD. Efficacy and affinity for diazepam did not change (Control Kd=2.27 ± 0.4 µM, n=7, OGD Kd= 2.81 ± 0.4 µM, n=6).

Figure 6. Model: Oxygen and glucose deprivation may modify PC GABA<sub>R</sub> pharmacology by altering phosphorylation or receptor subunit stoichiometry.

SUMMARY

1.) OGD results in decreased efficacy of GABA at its receptor, but does not alter affinity.

2.) GABA<sub>A</sub> receptor affinity and efficacy for propofol, zolpidem, and diazepam does not change following OGD. However, etomidate affinity & efficacy are significantly reduced.

3.) These findings suggest that the GABA<sub>A</sub> receptor may be subject to disease induced plasticity, which may influence treatment for cerebral ischemia.

SELECTED REFERENCES


Kelley, M. H., et al. Ischemic insult to cerebellar Purkinje cells causes diminished GABA<sub>A</sub> receptor function and allospregnanolone neuroprotection is associated with GABA<sub>A</sub> receptor stabilization. J Neurochem.; 2008, Nov; 107(5): 668-78


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Figure 2. Oxygen and glucose deprivation causes decreased GABA$_A$R α1 subunit protein. Protein from cerebellar cultures subjected to 2 hours OGD demonstrates there is a significant decrease in GABA$_A$R α1 subunit protein after 1 hour of reperfusion.