TITLE: EXCITOTOXINS, HYPOXIA, HYPOGLYCEMIA, CALCIUM, MAGNESIUM AND NEURONAL DAMAGE

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The excitotoxic concept postulates the excitatory neurotransmitters glutamate (Glu) and Aspartate (Asp) to play a major role in the mechanism of hypoxic-ischemic neuronal damage. Of the three types of Glu receptors known, the N-methyl-D-aspartate (NMDA) type is the one implicated to be directly involved in that mechanism (1). An activated NMDA receptor is believed to act as a Ca channel to increase intracellular Ca. Mg ions may act to attenuate such increase (1). The neuron's only defense against excitotoxicity is an energy-dependent re-uptake system for Glu and Asp. Here, the role of oxygen, glucose, Ca and Mg in NMDA-type agonists neurotoxicity was studied in vitro.

Rat hippocampal slices were prepared and maintained as described elsewhere (2). Population spikes evoked in the CA1 cell body layer by stimulating the Schaffer collaterals were used as a measurement of neuronal function. Hypoxia was produced by replacing all the oxygen in the gas atmosphere with nitrogen. "Hypoglycemia" was produced by lowering the glucose concentration in the perfusion medium (ACSF). In each experiment slices were exposed to 15 min of standard, baseline conditions followed by 30-75 min of treatment and 30 min of recovery, washout period.

Under normoxic conditions Glu or Asp were harmless. However, after hypoxia and reoxygenation the rate of recovery of neuronal function decreased with elevated levels of Glu, Asp or NMDA. The latter exerted 300 fold greater damage than Glu. Reducing the level of Ca in the ACSF during hypoxia decreased Glu, Asp and NMDA neurotoxicity. In contrast, elevated Mg level in the ACSF attenuated the neurotoxicity of NMDA. Severe "hypoglycemia" increased NMDA neurotoxicity.

We concluded that interupting energy metabolism may sensitize neurons to, and increase their damage by excitotoxins due to disturbance of ion homeostasis; low Mg-ACSF enhances NMDA-induced neuronal damage, while a low-Ca medium attenuates excitotoxicity. The premise of this study is that Ca influx and its cytosolic accumulation are the principal events leading to neuronal damage upon cerebral ischemia.

References.
1. Neuron 1:623-634, 1988
2. J Neurosci Meth 28:7-13, 1989

Proportion of hippocampal slices exhibiting neuronal function after different treatments (hypoxia, 10 min; low-glucose ACSF, 75 min; NMDA-type agonist exposure, 30-45 min; no Ca-, low Mg- or high Mg-ACSF, 45 min, including 10 min hypoxia). $X^2$-test for statistics.

| Treatment | No. of slices (functional/total) +Ca | (%) | -Ca | (%) |
|---|---|---|---|---|
| 1. Hypoxia | 35/41 | (85) | 54/54 | (100) |
| 2. #1 + 1mM Glu | 11/30[a] | (37) | 29/29 | (100) |
| 3. #1 + 1mM Asp | 12/49[a] | (24) | 25/25 | (100) |
| 4. #1 + 10uM NMDA | 12/74[a] | (16) | 41/43 | (95) |
| 5. #1 + low Mg | 5/12[a] | (42) | ----- | ----- |
| 6. #1 + high Mg | 13/13 | (100) | ----- | ----- |
| 7. #5 + 10uM NMDA | 0/12[b] | (0) | ----- | ----- |
| 8. #6 + 10uM NMDA | 9/14[c] | (64) | ----- | ----- |
| 9. Low-glucose | 20/26 | (77) | ----- | ----- |
| 10. #9 + 10uM NMDA | 2/25[d] | (8) | ----- | ----- |

[a]Significantly different from treatment #1 (P>.0005).
[b]Significantly different from treatment #4 (P>.05).
[c]Significantly different from treatment #4 (P>.0005).
[d]Significantly different from treatment #9 (P>.0005).



TITLE: **ISCHEMIA-MEDIATED METABOLIC ALTERATIONS PRODUCE ACTIVATION OF CORNEAL A-DELTA AND C FIBERS.**

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**Objectives:** Tissue injury, muscle fatigue and tourniquet application lead to local hypoxia and metabolic perturbations, resulting in pain. The mechanism of pain following ischemia remains unknown. This study was conducted to look at the effect of hypoxia and glucose depletion upon the activity of A-delta and C fibers. Advantages of the *in vitro* corneal nerve preparation for the study of ischemia-mediated nerve stimulation are: 1) the cornea is innervated by only A-delta and C fibers; 2) the electrophysiology of normal and injured corneal nerves is well characterized; 3) the corneal environment is stable and monitored with regard to temperature, pH, $pCO_2$, and tissue pressure; and 4) single metabolic perturbations can be imposed on corneal free nerve endings.

**Methods:** Corneas from New Zealand white rabbits were maintained *in vitro*, in a specially designed perfusion chamber. The endothelial side was perfused with oxygenated (95% $0_2$/5%$CO_2$) artificial aqueous humor solution (AQH) and maintained at normal intraocular pressure (18 mmHg) and temperature (35°C). A warmed and humidified $0_2/CO_2$ environment surrounded the epithelial side of the cornea. Nociceptor fibers were isolated by micro-dissection near the limbus and glass suction electrodes were used to record action potentials. Signals were amplified (x 10,000), filtered (100 Hz to 10KHz) and digitally stored for computer analysis. Control recordings were obtained and the stability of the preparation established prior to imposing metabolic alterations upon the cornea. Hypoxia was produced by substituting humidified $O_2/CO_2$ (95/5 %) with humidified $N_2/CO_2$ (95/5 %) while the gas concentration was continuously monitored using a POET 2 gas analyzer. L-glucose in physiologic concentrations was used to substitute for D-glucose.

**Results:** Corneal nerve action potential discharge was increased by both lowering oxygen and substituting L-glucose for D-glucose. Normal background discharge frequencies of 3 to 7 spikes/second were more than doubled (213 +/- 3.4% mean +/- SD; p<0.001 ANOVA) in 12 preparations following 15 to 20 minutes of exposure to hypoxia. Discharge frequency gradually decreased to below control levels (5.13 +/- 0.4 spikes/sec) after 2 hours in the $N_2/CO_2$ atmosphere, and plataued at a rate of 1.7 +/- 0.6 spikes/sec. A transient increase in discharge frequency to 8.9 +/- 0.5 spikes/sec was observed during the first 5 minutes of recovery in $O_2/CO_2$.

Substitution of L-glucose for D-glucose produced a significantly greater increase in discharge frequency (653+/- 28%; n=8, p<0.001 ANOVA compared to control). This elevated frequency lasted for 30 to 45 minutes and was followed by a reduction in activity to below control levels. Recovery required 30 minutes, proceeded by a brief period (2-3 min) of increased activity. Combining $N_2$ and L-glucose substitution did not produced a significant increase in action potential frequency (671 +/- 14%, n=6) compared to L-glucose substitution alone (p>0.05). Neither treatment altered conduction velocity or action potential amplitude evoked by electrical stimulation.

**Conclusion:** Both hypoxia and L-glucose substitution result in activation of cornea A-delta and C fibers. The time course of activation and recovery are comparable to the onset and recovery of pain produced by tourniquet application in humans. This preparation will allow further study of the mechanisms involved in the generation of ischemic pain.

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