HYPOXIA-MEDIATED CHANGES IN N-METHYL-D-ASPARTATE - EVOKED CALCIUM FLUX IN THE NAKED MOLE-RAT CORTEX

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Abstract

Hypoxia induces neuronal excitotoxicity and excitotoxic cell death (**ECD**) in the brains of most adult mammals. ECD is mediated by Ca^{2+} influx through N-methyl-D-aspartate (**NMDA**) receptors and consequently, reductions in Ca^{2+} influx may be neuroprotective. Channel arrest is a reduction in membrane permeability through down-regulation of ion channel activity and has been documented in the NMDA receptors of some anoxia-tolerant vertebrates as a neuroprotective mechanism against hypoxia. Naked mole rats (**NMRs**) are among the most hypoxia-tolerant mammals and avoid neuronal ECD during hypoxia through unknown mechanisms. In this study, **we hypothesize that hypoxia-induced channel arrest occurs in NMDA receptors in the NMR cortex**. NMDA-evoked Ca^{2+} currents were measured through fluorescent calcium imaging using Fura-2AM dye in cortical neurons from NMR live brain slices during normoxia and hypoxia (approximately 1% O_2). Although we predicted a decrease in NMDA-evoked Ca^{2+} currents during hypoxia, we found no differences in the peak NMDA-evoked changes in cytosolic Ca^{2+} (**$[Ca^{2+}]_c$**) between treatments. Conversely, during normoxia, $[Ca^{2+}]_c$ recovered completely during the ten minutes following NMDA application, whereas during hypoxia, $[Ca^{2+}]_c$ remained elevated until normoxia was restored. Our findings suggest that in the hypoxic NMR cortex, either NMDA-mediated Ca^{2+} conductance remains elevated, or neuronal Ca^{2+} clearance mechanisms are inhibited. This is inconsistent with the extreme hypoxia-tolerance of the NMR brain and suggests that the NMR achieves its resistance to ECD by mechanisms other than acute modulation of NMDA receptors during hypoxia.

Introduction

1. Oxygen is necessary for mitochondrial energy production, and hypoxia results in a decrease in ATP supply.
2. In neurons from hypoxia-intolerant brains, ATP-dependent pumps that maintain ion gradients fail, leading to Ca2+-mediated ECD through the over-activation of NMDA receptors. (Choi, 1992)
3. The prevention of hypoxia-induced ECD requires a decrease in energy production to be met with a decrease in energetic demand. This can be achieved through channel arrest; a reduction in channel permeability (Hochachka, 1986). Channel arrest has been demonstrated in the brains of hypoxia tolerant vertebrates such as the painted turtle, the pond slider (Perez-Pinzon et al, 1992; Pamenter et al, 2008; Buck and Bickler 1995) and the goldfish (Wilkie et al, 2008).
4. NMRs are among the most hypoxia-tolerant mammals, although the mechanisms behind how their brains avoid hypoxia-induced ECD have yet to be elucidated.

Materials and methods

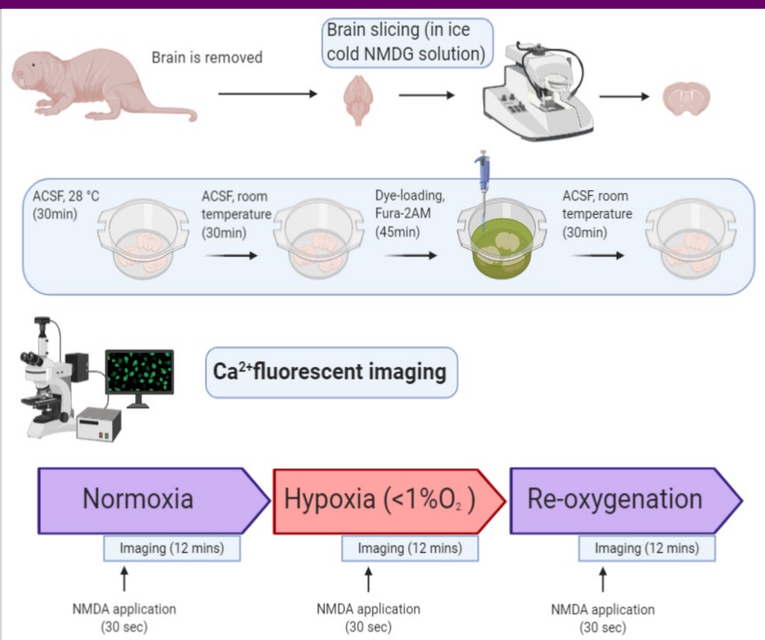
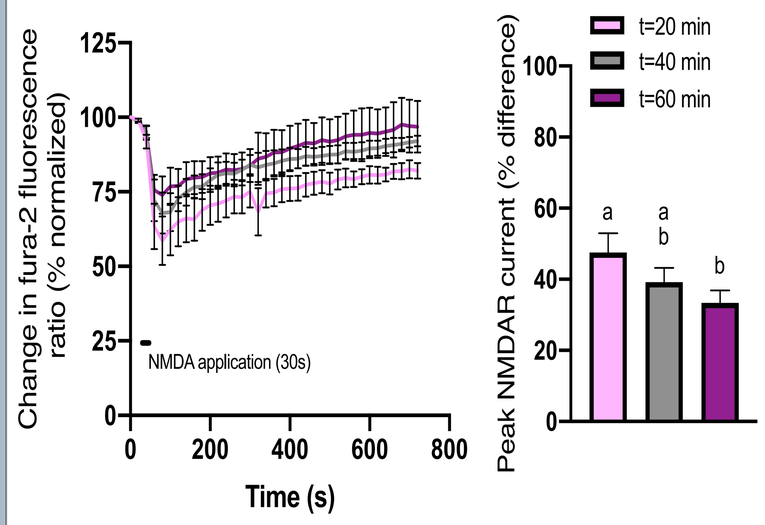
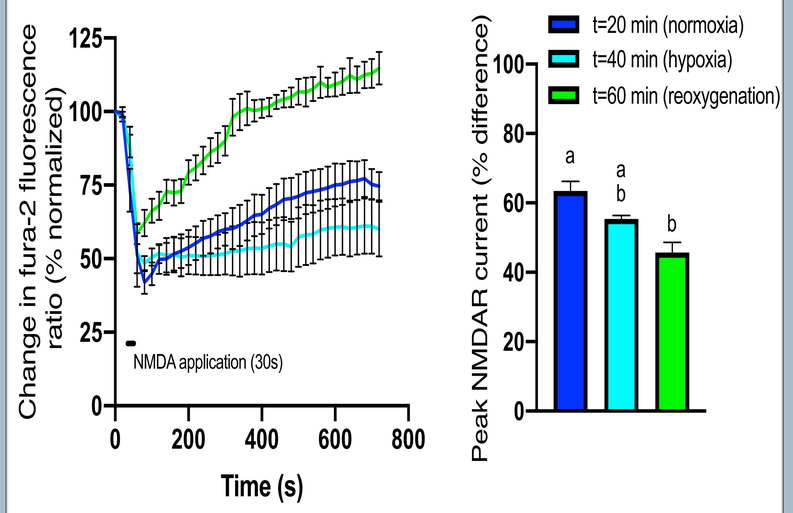


Figure 1. NMDA-evoked changes in $[Ca^{2+}]_c$ in NMR cortical neurons during normoxia



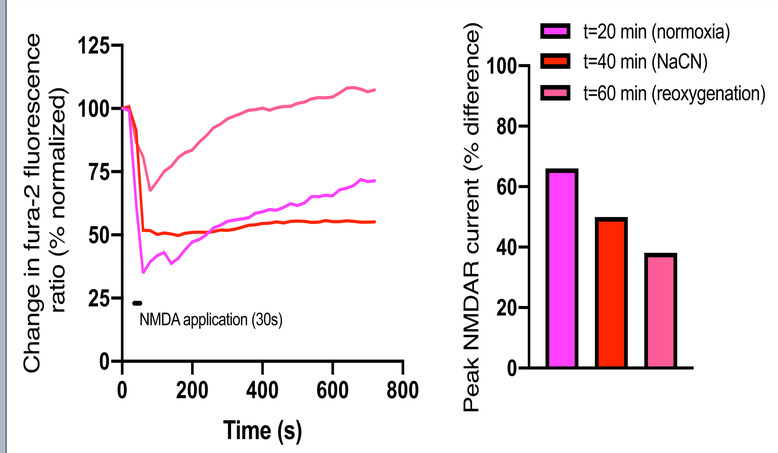
NMDA-evoked changes in $[Ca^{2+}]_c$ decline with repeated exposure during normoxia.

Figure 2. NMDA-evoked changes in $[Ca^{2+}]_c$ in NMR cortical neurons during hypoxia treatment



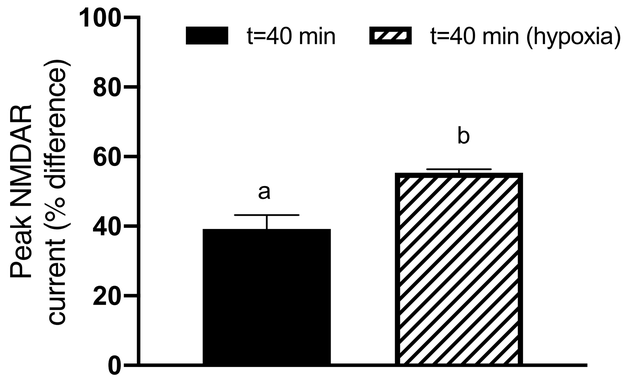
NMDA-evoked peak changes in $[Ca^{2+}]_c$ decline with repeated exposure, but are not significantly effected by hypoxia.

Figure 3. NMDA-evoked changes in $[Ca^{2+}]_c$ during cyanide (NaCN) treatment



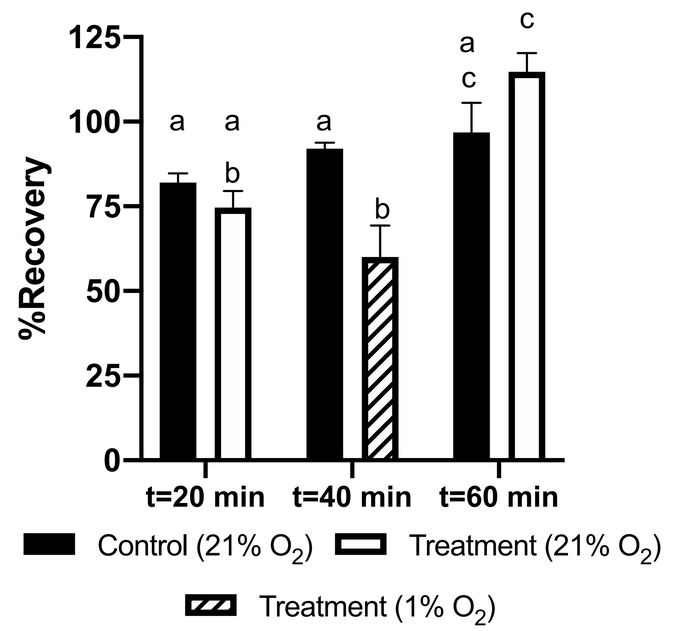
NaCN treatment (chemical hypoxia) acts as a positive control and supports results from hypoxia treatment, demonstrating no effect on NMDA-evoked peak changes in $[Ca^{2+}]_c$ as well as a reduced recovery rate.

Figure 4. Comparison of peak NMDA-mediated changes in $[Ca^{2+}]_c$ between normoxia and hypoxia



The peak NMDA-evoked increase in $[Ca^{2+}]_c$ during hypoxia (t = 40 min) is higher than the normoxic control at the same time point.

Figure 5. $[Ca^{2+}]_c$ recovery after NMDA-evoked Ca^{2+} influx



Hypoxia reduces percent recovery toward baseline $[Ca^{2+}]_c$ at the end of each imaging session.

Conclusions

1. There is no evidence of acute regulation of ion channel conductance during hypoxia in NMR cortex.
2. NMDA receptor channel arrest might occur through an alternative mechanism.
3. Hypoxia reduces the rate at which $[Ca^{2+}]_c$ levels are restored to baseline following NMDA application in NMR cortical neurons.

References

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