

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 15-08-2004		2. REPORT TYPE Final Technical Report		3. DATES COVERED (From - To) 24-10-2000 to 31-01-2004	
4. TITLE AND SUBTITLE Nitric Oxide and CNS O2 Toxicity Biochemical Modeling and Risk Prediction.				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER N0014-01-1-0240	
				5c. PROGRAM ELEMENT NUMBER	
				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
6. AUTHOR(S) C. A. Piantadosi, B. W. Allen, I. T. Demchenko				8. PERFORMING ORGANIZATION REPORT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Duke Center for Hyperbaric Medicine and Environmental Physiology Box 3315 Durham, NC 27710				10. SPONSOR/MONITOR'S ACRONYM(S) ONR	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Department of the Navy Office of Naval Research 800 North Quincy Street Arlington, VA 22217-5660				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Unlimited

DISTRIBUTION STATEMENT A
Approved for Public Release
Distribution Unlimited

20040820 031

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of this project was to elucidate the biological mechanisms and specific pathways that implicate the gaseous signaling molecule nitric oxide (NO) as a critical factor in producing the convulsions of central nervous system (CNS) oxygen (O2) toxicity and to obtain data that could be the basis for mathematical risk predictions of O2 convulsions. For the past 3 years, data obtained in this project support the hypothesis that NO indeed contributes to CNS O2 toxicity by several mechanisms: a) by increasing the availability of NO in the brain which in turn eliminates cerebral vasoconstriction, leading to hyperemia and the delivery of a toxic dose of oxygen; b) by stimulating NO production and O2- generation, both of which are implicated in the formation of ONOO-, a potent neurotoxic agent; c) by altering the excitatory/inhibitory balance in vulnerable brain regions during the early stage of extreme hyperoxia, prior to the appearance of O2 seizures. In rats protected with the inhibitor of NO production L-NAME no significant changes were observed in the excitotoxic index.

15. SUBJECT TERMS

High Pressure Oxygen Toxicity, Nitric Oxide, Seizures, CNS Oxygen Toxicity

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unlimited	18. NUMBER OF PAGES 4	19a. NAME OF RESPONSIBLE PERSON Claude A. Piantadosi, MD
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code) (919) 684-6143

FINAL TECHNICAL REPORT

GRANT #: N00014-01-1-0240

PRINCIPAL INVESTIGATOR: Claude A. Piantadosi, MD

INSTITUTION: Duke Center for Hyperbaric
Medicine and
Environmental Physiology,

GRANT TITLE: Nitric Oxide and CNS O₂ Toxicity
Biochemical Modeling and Risk
Prediction.

AWARD PERIOD: 24 November 2000 - 31 January 2004

OBJECTIVE: To elucidate the biological mechanisms and specific pathways that implicate the gaseous signaling molecule nitric oxide (NO) as a critical factor in producing the convulsions of central nervous system (CNS) oxygen (O₂) toxicity. Although the precise mechanism by which CNS O₂ toxicity leads to convulsions is unknown, the fact that NO plays a critical role indicates that changes in its bioactivity (i.e. that fraction of its concentration that is available to exert a biological effect) lead to predictable responses *in vivo* that can be the basis for mathematical risk predictions of O₂ convulsions.

APPROACH: We measured *in vivo*, in anesthetized rodents, levels of reactive oxygen and nitrogen species (including NO), catecholamines, glutamate, and GABA as functions of PO₂ and time in the brain. We have correlated these with escape from cerebral vasoconstriction and with brain electrical activity. We postulated a biochemical mechanism of O₂ toxicity in order to derive a mathematical model to predict probability and time of onset of O₂ seizures. We also measured regional cerebral blood flow (rCBF) using hydrogen clearance, interstitial PO₂ and NO with microelectrodes, as well as significant neurotransmitters and products of brain metabolism, using microdialysis.

ACCOMPLISHMENTS: For the past 3 years, data obtained in our laboratory support our hypothesis that HBO decreases rCBF by increasing superoxide (O₂⁻) production, which inactivates NO and produces vasoconstriction. This protects the brain against the damaging molecular effects of extreme hyperoxia. However, prolonged exposure to HBO in the 3 to 6 ATA range restores NO production and leads to generation of reactive nitrogen species (RNS) such as peroxyxynitrite (ONOO⁻), which is responsible for nitration of vascular and brain proteins, especially tyrosine and cysteine amino acid residues. Some (but not all) of these events interfere with molecular function. We also found critical roles for the depletion of the inhibitory amino acid GABA (gamma-aminobutyric acid) and the production of hydrogen peroxide and

ammonia by monoamine metabolism, which has allowed us to develop a biochemical model in which accelerated NO production leads to escape from vasoconstriction through the production of carbamyl phosphate.

NO-induced escape from autoregulation is followed by neuronal excitability, stimulation of metabolic activities that decrease seizure threshold and, ultimately, cause convulsions. Thus, we have been able to gather quantitative evidence in support of our hypothesis that changes in NO activity govern the escape of CBF from constrictor control that precedes neuronal excitotoxicity and predicts electrical hyperactivity during HBO exposure

CONCLUSIONS: NO indeed contributes to CNS O₂ toxicity by several mechanisms:

a) by increasing the availability of NO in the brain which in turn eliminates cerebral vasoconstriction, leading to hyperemia and the delivery of a toxic dose of oxygen;

b) by stimulating NO production and O₂⁻ generation, both of which are implicated in the formation of ONOO⁻, a potent neurotoxic agent. Rats pretreated with the systemic blocker of NO production L-NAME maintained a low CBF and did not show increases in interstitial NO and ONOO⁻ or EEG signs of oxygen toxicity;

c) by altering the excitatory/inhibitory balance in vulnerable brain regions during the early stage of extreme hyperoxia, prior to the appearance of O₂ seizures. In rats protected with L-NAME no significant changes were observed in the excitotoxic index.

SIGNIFICANCE: These data provide the first direct correlation between increased NO production and the onset of hyperoxic vasodilation in prolonged HBO₂ exposure.

Our biological data provide essential parameters and mechanistic interrelationships needed to construct a basic biochemical model to describe, predict, and ultimately, perhaps, to delay the early events of O₂ toxicity.

PATENT INFORMATION: N/A

AWARD INFORMATION: N/A

PUBLICATIONS AND ABSTRACTS:

Papers

1. Demchenko, I.T., A.E. Bosso, A.R. Whorton, C.A. Piantadosi. Nitric oxide production is enhanced in rat brain before oxygen-induced convulsions. Brain Research. 917 (2001) 253-261.

2. Allen, B.W., L.A. Coury, C.A. Piantadosi. Electrochemical detection of physiological nitric oxide: materials and methods. In: Meth Enzymol (Vol. 359) Nitric Oxide, Part D: Nitric Oxide Detection, Mitochondria and Cell Functions, and Peroxynitrite Reactions. Cadenas, E, L Packer (Eds.) San Diego: Academic Press, pp. 125-134, 2002.
3. Demchenko, I.T., T.D. Oury, J.D. Crapo and C.A. Piantadosi. Regulation of the brain's vascular responses to oxygen. Circulation Research, 91:1031-1037, 2002
4. Ross, A.D., H. Sheng, D.S. Warner, C.A. Piantadosi, I.B. Haberle, B.J. Day and J.D. Crapo. Hemodynamic effects of metalloporphyrin catalytic antioxidants: structure-activity relationships and species specificity, Free Radical Biol. Med., 33: 1657-1660, 2002.
5. Atochin, D.N., I.T., Demchenko, J. Astern, A.E. Boso, C.A. Piantadosi, and P.L. Huang. Contributions of endothelial and neuronal nitric oxide synthases to cerebrovascular responses to hyperoxia. J Cerebral Blood Flow & Metabolism, 23(10):1219-1226, 2003.
6. Allen, B.W., L.A. Coury and C.A. Piantadosi. Electrochemical activation of electrodes for amperometric detection of nitric oxide. Nitric Oxide Biology and Chemistry 8 (2003)243-252.
7. Demchenko, I.T., D.N. Atochin, A.E. Boso, P.L. Huang and C.A. Piantadosi. Oxygen seizure latency in mice lacking neuronal or endothelial nitric oxide synthases, Neuroscience Letters 344(1):53-56, 2003.
8. Demchenko, I.T., Yu.I. Luchakov, A.N. Moskvina, D.R. Gutsaeva, B. W. Allen, E D. Thalmann, and C. A. Piantadosi. Cerebral Blood Flow and Brain Oxygenation in Rats Breathing Oxygen Under Pressure. (Submitted 14 July 2004: Journal of Cerebral Blood Flow and Metabolism.)
9. Wang, C., S. Huang, C.A. Piantadosi, B.W. Allen, J. Liu. Novel electrochemical nitric oxide sensor using Ru-modified carbon nanotubes. (In preparation.)

Abstracts

1. Atochin, D.N., I.T. Demchenko, J. Astern, A.E. Boso, P.L. Huang, C.A. Piantadosi. Endothelial Nitric Oxide is Involved in Hyperbaric Oxygen Induced Cerebral Vasoconstriction. Undersea and Hyperbaric Med. Soc. Annual Meeting, 14-16 June, 2001. San Antonio, TX. Undersea & Hyperbaric Medicine 28: 2001 Supplement, p. 84-A

2. Piantadosi, C.A., B.J. Day, J.D. Crapo, I.T. Demchenko. A Novel Catalytic Antioxidant Protects against Hyperbaric Oxygen Induced Convulsions. Undersea and Hyperbaric Med. Soc. Annual Meeting, 14-16 June, 2001. San Antonio, TX Undersea & Hyperbaric Medicine 28: 2001 Supplement, p. 84-A.
3. Demchenko, I.T., T.D. Oury, , J.D. Crapo, C.A. Piantadosi. Transgenic Mice Over expressing or Lacking Superoxide Dismutase Exhibit Different Tolerances to Extreme Hyperoxia. Undersea and Hyperbaric Med. Soc. Annual Meeting, 14-16 June, 2001. San Antonio, TX. Undersea & Hyperbaric Medicine 28: 2001 Supplement, p. 84-B
4. Allen, B.W. and C.A. Piantadosi. Activation of NO electrodes. 8th Annual Meeting of the Oxygen Society, November 15-19, 2001, Research Triangle Park, North Carolina.
5. Demchenko, I.T., T.Oury, J.Crapo, C.Piantadosi. Extracellular superoxide dismutase modulates balance between nitric oxide and superoxide during cerebrovascular responses to hyperoxia. 8th Annual Meeting of the Oxygen Society, November 15-19, 2001, Research Triangle Park, North Carolina.