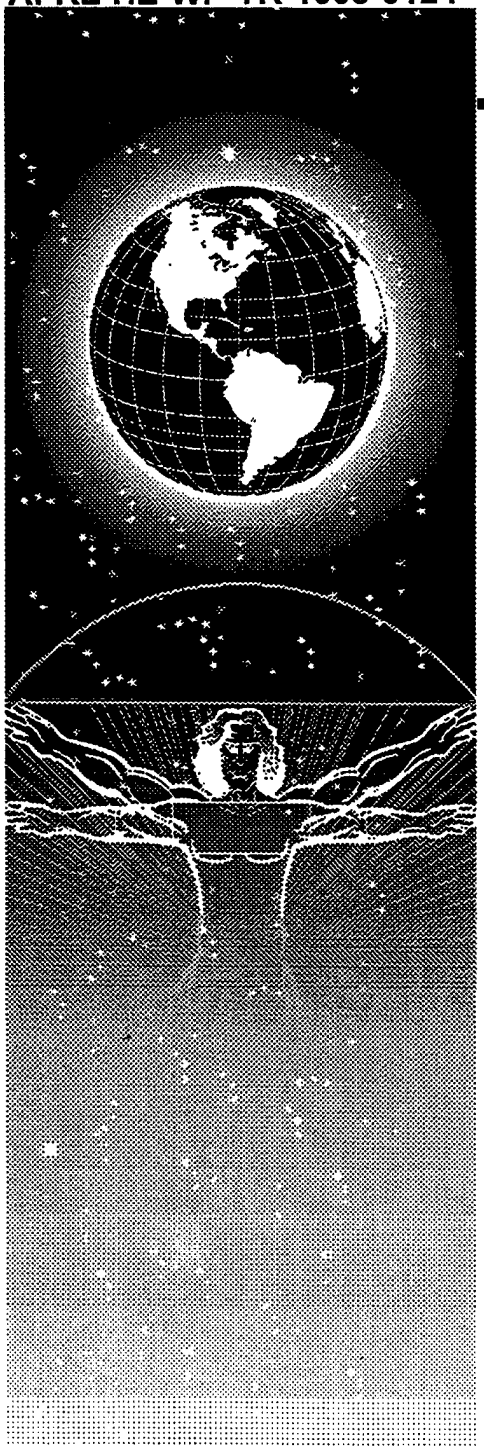
AFRL-HE-WP-TR-1998-0124



UNITED STATES AIR FORCE RESEARCH LABORATORY

HYPERBARIC OXYGEN MODULATES GENE EXPRESSION IN THE ISCHAEMIC GERBIL BRAIN

K.T. Geiss
MANTECH-GEO CENTERS JOINT VENTURE
TOXIC HAZARDS RESEARCH
PO BOX 31009
DAYTON, OH 45437-0009

K.L. Hensley
FREE RADICAL BIOLOGY AND AGING RESEARCH PROGRAM
OKLAHOMA MEDICAL RESEARCH FOUNDATION
OKLAHOMA CITY, OK 73104

R.A. Henderson
DEPARTMENT OF COMMUNITY MEDICINE
WRIGHT STATE UNIVERSITY SCHOOL OF MEDICINE
DAYTON, OH 45435

November 1998
Interim Report - June 1996 - August 1997

Human Effectiveness Directorate
Deployment and Sustainment Division
Operational Toxicology Branch
2856 G Street
Wright-Patterson AFB OH 45433-7400

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Air Force Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Service
8725 John J. Kingman Rd., Ste 0944
Ft. Belvoir, Virginia 22060-6218

DISCLAIMER

This Technical Report is published as received and has not
been edited by the Technical Editing Staff of the Air Force Research Laboratory.

TECHNICAL REVIEW AND APPROVAL

AFRL-HE-WP-TR-1998-0124

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

STEPHEN R. CHANNEL, Maj, USAF, BSC
Branch Chief, Operational Toxicology Branch
Air Force Research Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 1998	Interim Report - June 1996 - August 1997

4. TITLE AND SUBTITLE
Hyperbaric Oxygen Modulates Gene Expression in the Ischaemic Gerbil Brain

5. FUNDING NUMBERS
Contract F41624-96-C-9010
PE 61102F
PR 2312
TA 2312A2
WU 2312A202

6. AUTHOR(S)
Geiss, K., Hensley, K., and Henderson, R.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ManTech Geo-Centers Joint Venture
PO Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Human Effectiveness Directorate
Air Force Research Laboratory
Wright-Patterson AFB, OH 45433-7400

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
AFRL-HE-WP-TR-1998-0124

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Interruption of blood supply (ischaemia) results in tissue injury. However, most damage occurs as blood rapidly returns (reperfusion), generating reactive oxygen species (e.g. superoxide) and causing damage to DNA and other cellular components. Along with superoxide, increased nitric oxide (NO) levels ensue. NO, normally present in low concentrations, at higher concentrations may cause cellular damage. Hyperbaric oxygen (HBO) treatment suppresses NO production *in vivo*. Mongolian gerbils underwent transient global ischaemia via bilateral carotid ligation with, or without, HBO (2 atm) and allowed to recover for 2 or 24 hrs. Brain tissue was analyzed for heat shock protein 70 (HSP70), ornithine decarboxylase (ODC), and inducible NO synthase (iNOS) mRNAs. ODC mRNA levels decreased 50% when HBO was given before ischaemia, compared to ischaemia alone. iNOS mRNA was lower when HBO was provided before, versus after, ischaemic insult. HSP70 mRNA was increased for all treatments, compared to the sham control. HBO appears to impact the mRNA levels of ischaemia-related genes and likely the production of NO and radical species. These results are relevant to cases of ischaemic injury, as well as to operational exposures of personnel to radical producing chemicals in non-isobaric conditions.

14. SUBJECT TERMS
Ischaemia, Reperfusion, Brain, RNA, HBO, Hyperbaric oxygen

15. NUMBER OF PAGES
18

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

THIS PAGE INTENTIONALLY LEFT BLANK.

PREFACE

This one of a series of technical reports describing the results of experimental laboratory programs conducted at AFRL/HEST. This document serves as an interim report concerning predictive toxicology methods. Interruption of blood supply (ischaemia) by wounds, surgery, or blunt trauma results in tissue injury. However, most damage occurs as blood rapidly returns (reperfusion) to the tissues. This research was conducted to better understand the mechanisms of ischaemia/reperfusion toxicity and the potential effects of hyperbaric oxygen treatment, in order to better establish the potential hazards for humans. Previous studies have demonstrated that hyperbaric oxygen treatment may suppress some of the detrimental effects of the ischaemia/reperfusion injury. The results of this study provide some of this necessary insight by describing the response of molecular biomarkers of effect, caused by exposures to hyperbaric oxygen and ischaemia/reperfusion conditions. The research described in this report began June 1996 and was completed in August 1997 under U.S. Air Force Contract No. F41624-96-C-9010 (ManTech/Geo-Centers Joint Venture). LtCol. Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. Participation of Kenneth L. Hensley, PhD., and Jennifer Raker, graduate student, was supported in 1996 by the Air Force Office of Scientific Research Summer Research Program.

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

TABLE OF CONTENTS

SECTION	PAGE
PREFACE	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	v
LIST OF FIGURES	vi
ABBREVIATIONS	vii
INTRODUCTION	1
MATERIALS AND METHODS	3
Animal Exposures and Sampling	3
RNA Analysis	3
Statistical Analysis	4
RESULTS AND DISCUSSION	5
CONCLUSIONS	9
REFERENCES	10

LIST OF TABLES

TABLE		PAGE
1	Experimental Design	4
2	iNOS mRNA Multiple Comparisons	7
3	ODC mRNA Multiple Comparisons	8

LIST OF FIGURES

FIGURE		PAGE
1	Effects of HBO and/or ischaemia on iNOS mRNA Expression	7
2	Effects of HBO and/or ischaemia on ODC mRNA Expression	8
3	Effects of HBO and/or ischaemia on HSP70 mRNA Expression	9

LIST OF ABBREVIATIONS

HBO,h	hyperbaric oxygen
INOS	inducible nitric oxide synthase
ODC	ornithine decarboxylase
HSP	heat shock protein
I/R	ischaemia/reperfusion
DNA	deoxyribonucleic acid
ROS	reactive oxygen species
NO	nitric oxide
O_2•	superoxide radical
RNA	ribonucleic acid
RT-PCR	reverse transcriptase-polymearse chain reaction
PCR	polymerase chain reaction
cDNA	copy DNA
OD	optical density
SEM	standard error of the mean
S	sham
I	ischaemia

I. INTRODUCTION

The efficacy of hyperbaric oxygen (HBO) as a therapeutic agent, especially for the treatment of stroke or other ischaemia/reperfusion (I/R) injuries, has been researched and reviewed (1, 2, 3). However, there have been no clear conclusions on this matter. At the heart of the problem is that the exact molecular mechanisms of effect of combined HBO and I/R treatments is not fully understood. In this presentation, we consider the experimantal model for HBO and I/R effects on the brain.

Whereas extended periods of ischaemia alone generally lead to tissue necrosis, brain damage resulting from ischaemia/reperfusion can occur at a number of stages. Early phase damage is thought to be largely mediated by calcium influx into cells. Along with this, oxidative damage arises as a primary culprit for the ensuing degeneration of neuronal tissue after reperfusion (4, 5, 6). Endpoints for DNA damage and fragmentation (markers for apoptosis) are evident after I/R events (7, 8). In later stages of the response, neutrophils and inflammatory cytokines can participate in the subsequent cascade of events (5, 6). These cytokines can mediate the attraction of additional macrophages, activate cells, and stimulate gene expression. Nitric oxide (NO) and reactive oxygen species (ROS) are particularly cogent to macrophage- and microglia-mediated post-ischaemic events (4). Levels of both of these molecules are increased as a result of I/R events. Whereas NO may be protective or beneficial to neurons, in the presence of superoxide radical ($O_2^{-}\bullet$) NO readily reacts, forming peroxynitrite species (9). Peroxynitrite is consistently found to cause cell damage and death.

The expression of certain genes is modulated in response to I/R events. In particular, the inducible form of nitric oxide synthase (iNOS) present in macrophages, glia, and vascular cells, is induced by I/R and accounts for the related increase in NO production (10). The significance of this increased NO concentration in later stage tissue damage (11, 12) is discussed above. Polyamines are another group of chemicals that play a potential role in I/R injury. These molecules can disrupt neuronal integrity and facilitate cerebral edema formation (13, 14, 15, 16). Ornithine decarboxylase (ODC), the rate-limiting enzyme for polyamine biosynthesis (from ornithine), is regulated at the transcriptional level by I/R events and the increased enzyme activity closely correlates with increased mRNA levels (17). The increased production of polyamines has been hypothesized to be in competition for arginine with NO

synthase enzymes (18). Among other genes modulated in response to I/R are the heat shock proteins (HSPs). Specifically, HSPs 27, 60, and 70/72 have exhibited increased expression due to I/R exposure. HSP70 response is elicited by many different stressors, but in particular, it is related to the development of a limited tolerance to ischaemia (19). Although this tolerance is not evidenced specifically by changes at the transcriptional level, increased amount of HSP70 mRNA remains an indicator of I/R injury (9).

The effects of hyperbaric oxygenation on gene expression varies somewhat in comparison to those of I/R injury. The ability of HBO to prevent delayed neuronal death, is a central reason to consider HBO in the context of I/R effects on gene expression (20). This preventive effect sustains, while HBO still induces mild oxidative stress (21), but without associated early brain damage (22). HBO has been shown specifically to decrease the expression of iNOS, potentially resulting in decreased amount of NO (21). The response of the ODC gene to HBO has not been previously characterized. The consideration of potential HSP70 response to HBO is confounded by previous studies, such as those reporting minimal response (20) or a response that increased with multiple HBO exposures (23).

The purpose of this study was to better define the molecular events associated with ischaemia/reperfusion injury and hyperbaric oxygen exposure by analyzing the expression of specific mRNAs of genes that responded to these treatments (iNOS, ODC, and HSP70). We intend to define whether there is regulation of ODC at the transcriptional level for HBO exposed animals and how it compares with ODC expression for animals exposed to ischaemia. We have exposed animals to combinations of ischaemia and HBO, and assessed the ability of HBO to ameliorate or enhance the response of certain genes (ODC, HSP70, and iNOS) to the ischaemic insult.

II. MATERIALS AND METHODS

Animal Exposures and Sampling

Male Mongolian gerbils were anesthetized with pentobarbital (40 mg/kg, i.p.) and prepared for transient global ischaemia via carotid artery occlusion as previously described by Chandler and others (See 24, 35, 26). Following surgery, animals were individually housed and allowed to recover for 2 days. Animals (5 animals per treatment group) were subjected to a single treatment scenario as described in the table below (Table 1). Ischaemia was accomplished by bilateral carotid occlusion for a period of 10 min. Animals exposed to hyperbaric oxygen (HBO, 2 atm) were placed in a pressure- and gas- regulated steel chamber for a single 1 hour exposure. The sham and HBO groups underwent the same surgical procedures as those recieving ischaemia, but without carotid ligature. Animals were sacrificed by decapitation at specific times following reperfusion (2 hrs and 24 hrs) and the hippocampus was frozen in liquid nitrogen for RNA analysis, while other portions of the brain were also frozen in liquid nitrogen for subsequent enzyme and protein analysis. Samples were then transferred to a -140°C freezer to be kept for analysis.

RNA Analysis

Total RNA was extracted from brain tissue using a commercial reagent (RNA Stat 60) and dissolved in specially prepared formamide (Formazol), both from Tel-Test "B", Inc. (Friendswood, TX). Extracted RNA was stored at -140°C until Northern analysis. RNA samples were electrophoresed (40μg/lane) on 1% formaldehyde gels and transferred to positively charged nylon membranes (MAGNA-NT, Micron Separations, Inc., Westboro, MA). Membranes were then UV-crosslinked and baked at 80°C for 1hr.

The ODC cDNA probe was made from a 720bp PstI restriction fragment isolated from a plasmid clone of mouse ODC (pMK934) obtained from Dr. Rosemary Elliott (Roswell Park Memorial Institute, Buffalo, NY). The HSP70 cDNA probe (651bp) was made from a HSP70 cDNA fragment we amplified by RT-PCR from gerbil mRNA. The iNOS cDNA probe (616bp) was prepared from a PCR fragment provided by Dr. Ken Hensley (OMRF, OK). The cDNAs were labeled with [32]P-dCTP by random primer method. Hybridizations of cDNAs were performed at 42°C. After overnight hybridization, membranes were exposed to X-ray film at -70°C for 1 to 5 days. Band intensities on developed films were quantified by laser scanning

densitometry (Bio-Rad Instruments Model 670 Densitometer with Molecular Analyst Software, Version 1.3, Bio-Rad, Laboratories, Hercules, CA). Equivalent loading among gel lanes of RNA was determined by hybridization with a rat 18S rRNA probe (AMBION, Austin, TX).

TABLE 1. Experimental design

Time Course of Experiment ⟶	Group	
60 min HBO — 2 hrs 40 min — Sacrifice	H	2 hrs
10 min ISCH — 2 hrs — Sacrifice	I	
60 min HBO — 30 min — 10 min ISCH — 2 hrs — Sacrifice	H+I	
10 min ISCH — 60 min HBO — 1 hr — Sacrifice	I+H	
60 min HBO — 24 hrs 40 min — Sacrifice	H	24 hrs
10 min ISCH — 24 hrs — Sacrifice	I	
60 min HBO — 30 min — 10 min ISCH — 24 hrs — Sacrifice	H+I	
10 min ISCH — 60 min HBO — 23 hrs — Sacrifice	I+H	
24 hrs — Sacrifice	S	

Statistical Analysis

RNA levels measured by densitometry and reported in optical density (OD, mean ± SEM) units were analyzed by one-way ANOVA with BMDP (BMDP Statistical Software, Inc., Los Angeles, CA), with multiple comparisons between groups by *t* test. Differences were identified as statistically significant at $p \leq 0.05$. Unexpected mortality accounts for group sizes less than n=5.

III. RESULTS AND DISCUSSION

Expression of iNOS mRNA was detected in all samples (Fig. 1). The levels of response ranged from 140-550% that of the sham control group. Table 2 shows the statistical significance of comparisons made between groups ($p \leq 0.05$). HBO-only and ischaemia-only groups did not have iNOS mRNA levels different from the sham. The largest elevations (almost 2 times that of the next highest level) of iNOS were seen in the groups that received post-ischaemic HBO (540-550% over sham control). Responses in most other groups were significantly lower than in those two groups. The effect of HBO before ischaemia did show an increase over the sham for both the 2hr and 24hr time points (230% and 300%, respectively). These results are not consistent with HBO decreasing available NO, in vivo, based in a decrease in iNOS expression. To the contrary, in the case of HBO exposure after an ischaemic insult, iNOS mRNA levels are markedly increased. Thus, any potential beneficial effect of HBO exposure is indicated by this specific response.

ODC mRNA levels were detected in all treatment groups (Fig. 2). Table 2 shows the statistical significance of comparisons made between groups ($p \leq 0.05$). Levels of response as compared to that of the sham control group ranged from a decrease of 50% (HBO-only group) to an increase of 150% (2hr ischaemia-only group). The highest levels were evidenced in the ischaemia-only groups (150% at 2hr and 130% at 24hr). The effect of HBO treatment before an ischaemic exposure resulted in a significantly lower level of ODC mRNA expression at the 24hr time point (50% of sham) than the ischaemia-only group at the 24hr time point (130% of sham). When HBO was given after ischaemic insult, there was an equally elevated amount of ODC mRNA compared to the ischaemia-only group at 2hr. However, by the 24hr point, while the ischaemia-only group has an ODC mRNA level at 130% of sham, the ischaemia+HBO group has a level only 50% of the ODC expression for the sham control. These data show that ODC mRNA expression does not appear to be induced by HBO, to an extent greater than seen in the sham groups. In addition, HBO appears to suppress ischaemias induction of ODC mRNA expression. At 24hr, this is the case whether HBO precedes or follows ischaemic insult. The mechanism by which HBO elicits this response has not been strictly defined. However, the decreased levels of ODC correlate well with decreased ODC enzyme activity and polyamine production as identified elsewhere (17). Our identification of the ODC mRNA expression response to combined exposure to HBO and ischaemia has not been previously characterized.

HSP70 mRNA was detectable for all of the treatment groups (Fig. 3). The mean responses for HSP70 mRNA expression to most combinations of HBO and ischaemia range from 150-350% of the level for the sham group. However, HSP70 mRNA in only two of treatment groups (24hr ischaemia and 24hr HBO) are significantly elevated (>250%) versus the sham group. There are no apparent differences among the other treatment scenarios. HSP70 responds to a number of different chemical and physiological stressors. Previous studies of HSP70 mRNA response to HBO or I/R have not shown consistent results. Here, there is a purturbation in all of the groups, but the responses are highly variable.

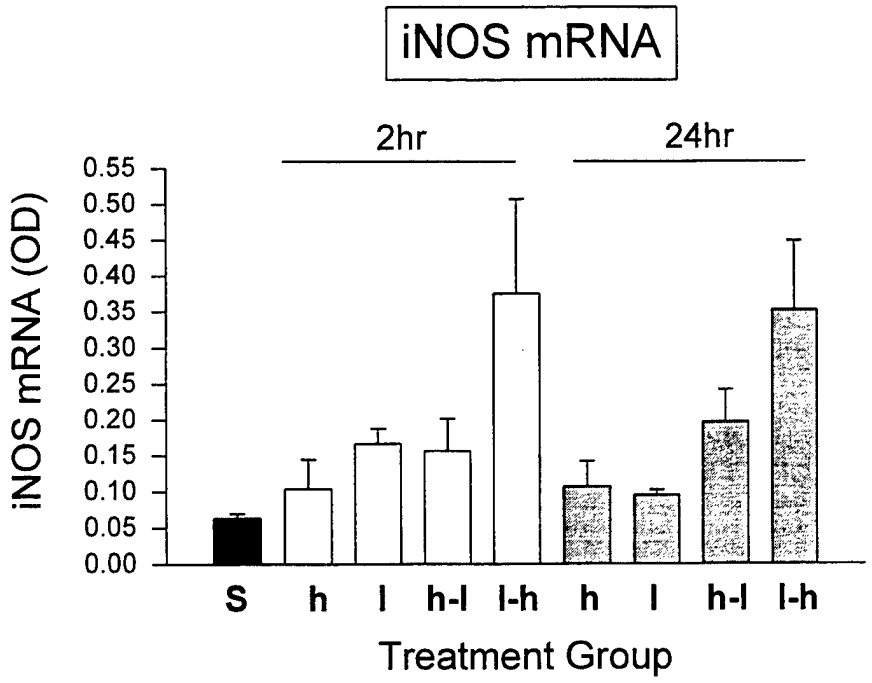


FIGURE 1.
Effects of HBO and/or ischaemia-reperfusion on iNOS mRNA Expression
Amounts of iNOS mRNA expressed as mean optical density (OD) units±SEM as determined by densitometry. The four treatments are defined on the x-axis [HBO-only (h), ischaemia-only (I), HBO before ischaemia (h+I), and ischaemia before HBO (I+h)] and are grouped by the time of sacrifice (2 or 24 hours) separately from the sham (S).

	2 hour				24 hour			
	h	l	h-l	l-h	h	l	h-l	l-h
S				*			*	*
h				*		n/a	n/a	n/a
l				*	n/a		n/a	n/a
h-l				*	n/a	n/a		n/a
l-h					n/a	n/a	n/a	
h								*
l								*
h-l								*
l-h								
n=	(3)	(3)	(3)	(3)	(4)	(3)	(2)	(3)	(4)

TABLE 2. Statistical Analysis Summary Table for iNOS mRNA
Sample size (n) is listed below each group; asterisk (*) denotes $p \leq 0.05$; n/a is used where contrasts were not made.

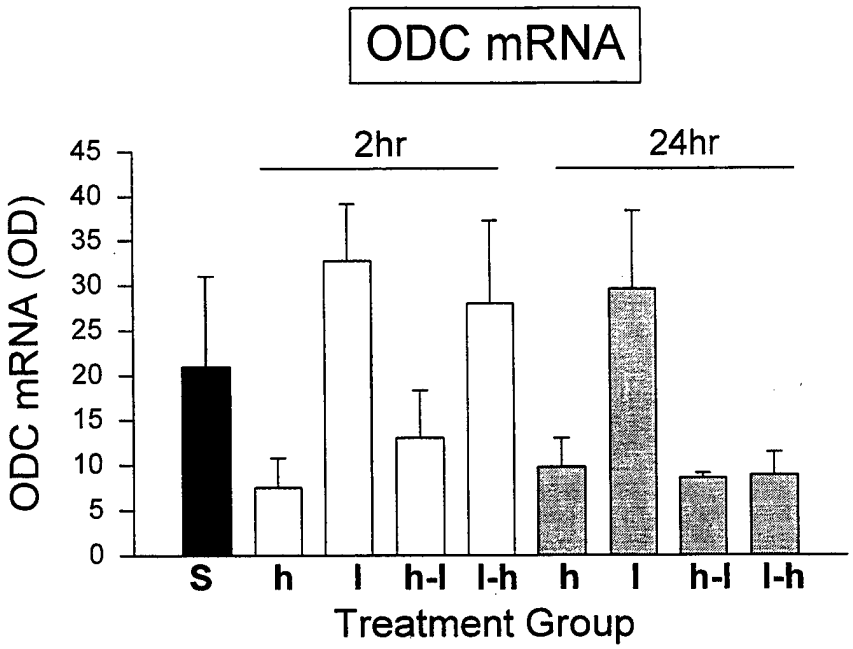


FIGURE 2.
Effects of HBO and/or ischaemia-reperfusion on ODC mRNA Expression
Amounts of ODC mRNA expressed as mean optical density (OD) units✂SEM as determined by densitometry. The four treatments are defined on the x-axis [HBO-only (h), ischaemia-only (I), HBO before ischaemia (h+I), and ischaemia before HBO (I+h)] and are grouped by the time of sacrifice (2 or 24 hours) separately from the sham (S).

		2 hour				24 hour			
		h	**l**	**h-l**	**l-h**	**h**	**l**	**h-l**	**l-h**
	S	*	*			*	*	*	*
		h	*		*		n/a	n/a	n/a
			l	*		n/a		n/a	n/a
h				**h-l**	*	n/a	n/a		n/a
					l-h	n/a	n/a	n/a	*
						h	*		
2							**l**	*	*
h								**h-l**	
									l-h
n=		(4)	(3)	(4)	(3)	(4)	(5)	(5)	(3)	(5)

TABLE 3. Statistical Analysis Summary Table for ODC mRNA
Sample size (n) is listed below each group; asterisk (*) denotes p✂0.05; n/a is used where contrasts were not made.

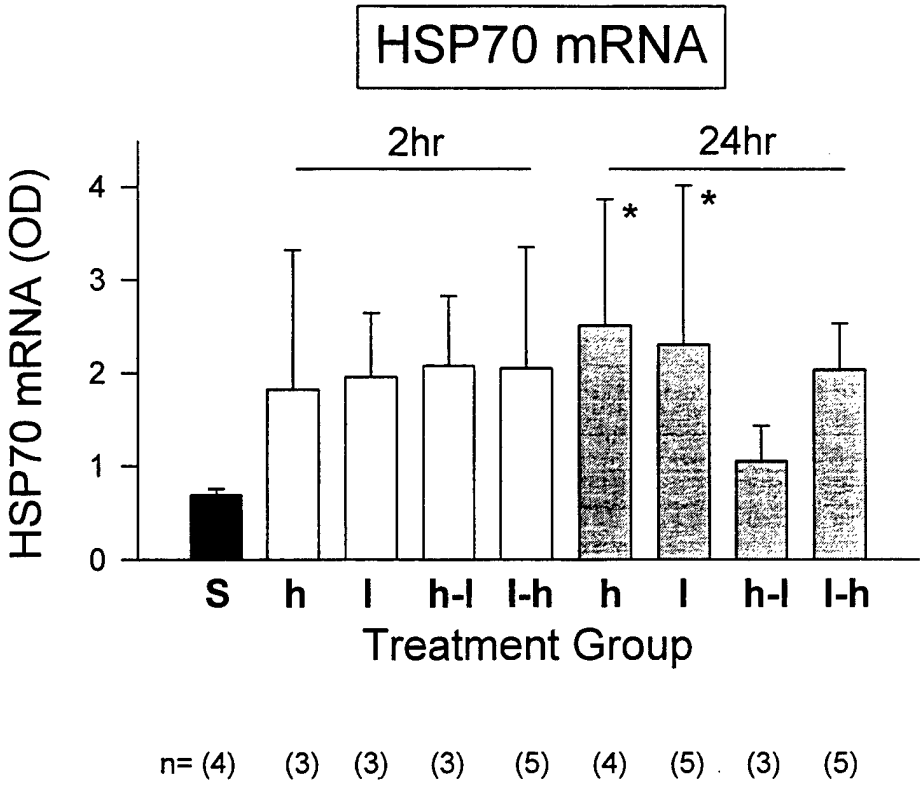


FIGURE 3.
Effects of HBO and/or ischaemia-reperfusion on HSP70 mRNA Expression
Amounts of HSP70 mRNA expressed as mean optical density (OD) units±SEM as determined by densitometry. The four treatments are defined on the x-axis [HBO-only (h), ischaemia-only (I), HBO before ischaemia (h+I), and ischaemia before HBO (I+h)] and are grouped by the time of sacrifice (2 or 24 hours) separately from the sham (S). Only the groups that are significantly different ($p<0.05$) from the sham are denoted by an asterisk (*). Group sample sizes (n) are listed below each group.

IV. CONCLUSIONS

Given that our analysis did not include time points between 2 and 24hrs, we are unable to make any conclusions relating to fluctuation in iNOS expression within that period. The absence of HBO's ability to have decreased the levels of iNOS expression at the 24hr time period does not help us discern whether HBO has the potential to ameliorate the potential damage by NO or subsequent reactive species induced by ischaemia-reperfusion injury. Further analysis of reactive oxygen or radical species in this experimental scenario is important to clear up this dilemma. The information concerning HSP70 mRNA expression in these experiments is equivocal. The ODC mRNA response is consistent with previously reported

data. The decrease in ODC due to HBO with or without ischaemia has not been previously reported. From this data, one promising role for HBO in countering the destructive effects of ischaemia-reperfusion lies not in the decreasing of iNOS expression, but rather in the apparent suppression of ODC expression. Whereby, the production of polyamines is impeded, decreasing their overall contribution to inflammation and tissue edema.

V. REFERENCES

1. Nighoghossian, N., and Trouillas, P. (1997) J Neurol. Sci. 150:27-31.
2. Siddiqui, A. Davidson, J.D., and Mustoe, T.A. (1997) Plast. Reconstr. Surg. 99:148-155.
3. Baiborodov, B.D., et al. (1996) Anesteziol. Reanimatol. 6:56- 58.
4. Olanow, C.W. (1993) TINS. 16:439.
5. Kogure, K., Arai, H., et. al. (1985) Prog. Brain Res. 63:237-259.
6. Arndt, H., Kubes, P., and Granger, D.N. (1991) 69:1056-1060.
7. Iwai, T., Hara, M., et. al. (1995) Brain Res. 671:305-308.
8. Nitatori, T., Sato, N., et. al. (1995) J Neurosci. 15:1001-11.
9. Lipton., et. al. (1994) Ann. N Y Acad. Sci. 738:382-387.
10. Nathan, C. (1992) FASEB J. 3:31.
11. Iadecola, C., Xu, X., et. al. (1995) J Cerebral Blood Flow Metab. 15:52-59.
12. Iadecola, C., Zhang, F., et. al. (1995) J Cerebral Blood Flow Metab. 15:378-384.
13. Paschen, W., Csiba, L., et. al. (1991) Brain Res. 566: 354-357.
14. Dempsey, R.J., Carney, J.M., and Kindy, M.S. (1990) Acta. Neurochir. Suppl. 51:189-191.
15. Roa, A.M., Baskaya, M.K., et. al. (1995) J Neurochem. 65:2639-2643.
16. Dempsey, R., Carney, J., and Kindy, M. (1991) J Cerebral Blood Flow and Metab. 11:979-985.
17. Kindy, M., Hu, Y., and Dempsey, R. (1994) J Cerebral Blood Flow and Metab. 14:1040-1045.
18. Zablocka, B., Lukasiuk, K., et. al. (1995) J Neurosci. Res. 40:233-240.
19. Kanemitsu, H., Kirino, T., et. al. (1994) Neurolog. Res. 16:209-212.
20. Konda, A., Baba, S., Iwaki, T., et al. (1996) Neuropathol. Appl. Neurobiol. 22:350-360.
21. Kurata, S., Yamashita, U., and Nakajima, H. (1995) Biochim Biophys Acta 1263:35-38.
22. Mink, R.B., and Dutka, A.J. (1995) Crit. Care Med. 23:1398-1404.
23. Wada, K., Ito, M., et. al. (1996) Brain Res. 740:15-20.
24. Chandler, M., et al. (1985) J. Pharmacol. Meth. 14:137-146.
25. Oliver, et al. (1990) PNAS-USA 87:5144-5147.
26. Carney, et al. (1991) J. Mol. Neurosci. 3:47-57.