THE ROLE OF ENDORPHINS IN THE PATHOPHYSIOLOGY OF
HEMORRHAGIC AND ENDOTOXIC SHOCK IN THE PRIMATE(U) IOWA
UNIV IOWA CITY COLL OF MEDICINE* N J GURLL ET AL.
REPORT NUMBER 1

THE ROLE OF ENDOPHINS IN THE PATHOPHYSIOLOGY OF
HEMORRHAGIC AND ENDOTOXIC SHOCK IN THE PRIMATE

ANNUAL REPORT 1980-1981

NELSON J. GURILL, M.D., THOMAS VARGISH, M.D.,
AND DAVID G. REYNOLDS, Ph.D.

JULY 1981

SUPPORTED BY
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK, FREDERICK, MARYLAND 21701

CONTRACT NO. DAMD-17-80-C-0094

UNIVERSITY OF IOWA COLLEGE OF MEDICINE
IOWA CITY, IOWA 52242

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authority documents.
THE ROLE OF ENDOPHINS IN THE PATHOPHYSIOLOGY OF
HEMORRHAGIC AND ENDOTXIC SHOCK IN THE PRIMATE

Nelson J. Guril, M.D.
Thomas Vargish, M.D.
David G. Reynolds, Ph.D.

University of Iowa College of Medicine
Iowa City, Iowa 52242

U.S. Army Research and Development Command
Fort Detrick
Frederick, MD 21701

Approved for public release - Distribution unlimited.

shock, endorphin, naloxone, hemorrhage, endotoxemia, primate shock,
optiate receptors, pathophysiology of shock

In order to investigate the possible role and involvement of endogenous morphine-like substance (endorphins) in shock we studied cynomolgus monkeys subjected to hemorrhagic and endotoxemic shock. Blockade of opiate receptors with naloxone improved cardiovascular performance and survival in endotoxemic shock but produced only mild improvement in cardiovascular function and no increased survival in hemorrhagic shock. We conclude that endorphin systems are activated in and contribute to the cardiovascular depression in primate shock. Further work in this area is justified including development of a better primate hemorrhagic shock model.
REPORT NUMBER 1

THE ROLE OF ENDOPHINS IN THE PATHOPHYSIOLOGY OF
HEMORRHAGIC AND ENDOTOXIC SHOCK IN THE PRIMATE

ANNUAL REPORT 1980-1981

NELSON J. GURLL, M.D., THOMAS VARGISH, M.D.,
AND DAVID G. REYNOLDS, Ph.D.

JULY 1981

SUPPORTED BY

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK, FREDERICK, MARYLAND 21701

contract no. DAMD-17-80-C-0094

university of iowa college of medicine
iowa city, iowa 52242

approved for public release; distribution unlimited.

the findings in this report are not to be construed as an
official department of the army position unless so designated
by other authority documents.
SUMMARY

In order to investigate the possible role and involvement of endogenous morphine-like substance (endorphins) in shock we studied cynomolgus monkeys subjected to hemorrhagic and endotoxemic shock. Blockade of opiate receptors with naloxone improved cardiovascular performance and survival in endotoxemic shock but produced only mild improvement in cardiovascular function and no increased survival in hemorrhagic shock. We conclude that endorphin systems are activated in and contribute to the cardiovascular depression in primate shock. Further work in this area is justified including development of a better primate hemorrhagic shock model.
FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Title Page</td>
<td>1</td>
</tr>
<tr>
<td>2.</td>
<td>Summary</td>
<td>2</td>
</tr>
<tr>
<td>3.</td>
<td>Foreword</td>
<td>3</td>
</tr>
<tr>
<td>4.</td>
<td>Table of Contents</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Body of Report</td>
<td>5</td>
</tr>
<tr>
<td>a)</td>
<td>problem</td>
<td>5</td>
</tr>
<tr>
<td>b)</td>
<td>background</td>
<td>5</td>
</tr>
<tr>
<td>c)</td>
<td>approach</td>
<td>5</td>
</tr>
<tr>
<td>d)</td>
<td>results: hemorrhagic shock</td>
<td>6</td>
</tr>
<tr>
<td>e)</td>
<td>results: endotoxemic shock</td>
<td>7</td>
</tr>
<tr>
<td>f)</td>
<td>results: hormones &amp; neurohumors</td>
<td>8</td>
</tr>
<tr>
<td>g)</td>
<td>discussion</td>
<td>8</td>
</tr>
<tr>
<td>h)</td>
<td>conclusions</td>
<td>8</td>
</tr>
<tr>
<td>i)</td>
<td>recommendations</td>
<td>9</td>
</tr>
<tr>
<td>6.</td>
<td>Literature Cited</td>
<td>10</td>
</tr>
<tr>
<td>7.</td>
<td>Appendices</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Figure 1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Figure 2</td>
<td>13</td>
</tr>
<tr>
<td>8.</td>
<td>Distribution</td>
<td>14</td>
</tr>
</tbody>
</table>
a) problem. Shock due to hemorrhage and trauma is the most serious and lethal threat to the soldier in war. Even during peacetime septic and hemorrhagic shock are frequent and important conditions in military as well as civilian medical practice. These shock states do not always respond to appropriate therapies suggesting the possibility of other pathophysiological mechanisms and treatment modalities. Furthermore, the exigencies of the battlefield and rapid evacuation techniques favors the administration of specific anti-shock agents that don't require extensive preparation.

b) background. Endogenous opioids are elevated in the plasma during shock and, like exogenous opiate compounds, depress cardiac function. The possible involvement of endorphin systems in shock was suggested by Holaday and Faden and substantiated in rodent and canine models of hemorrhagic and endotoxemic shock. Blockade of opiate receptors with naloxone improves cardiovascular performance and survival in these models. Preliminary clinical studies of naloxone in human shock have been encouraging but poorly controlled and generally unscientific. In contrast to most species where adrenocorticotropic hormone (ACTH) and beta-endorphin rise and fall concomitantly, steroids fail to suppress beta-endorphin in humans and monkeys at doses that attenuate ACTH. This suggests that endorphin control mechanisms are different in primates than other animals. For these reasons basic studies in primates are urgently needed.

c) approach. Cynomolgus monkeys were lightly anesthetized and instrumented to monitor mean arterial pressure (MAP), heart rate (HR), pulmonary arterial pressures, cardiac output (CO by thermal dilution) and left ventricular contractility (LV dp/dt max by differential amplification). Endotoxemic and
hemorrhagic shock were induced. Treatment with either naloxone 2 mg/kg i.v.
bolus plus 2 mg/kg/hr i.v. infusion for 4 hours with 0.9% NaCl in equivalent
volumes was begun when MAP reached 75 mm Hg after E. coli endotoxin 5 mg/kg
i.v. In the hemorrhagic shock model the monkeys were bled to a MAP of
25-45 mm Hg which was maintained for 1-3 hours with an open reservoir system.
The reservoir was then clamped and treatment begun with either naloxone
2 mg/kg/hr i.v. bolus plus 2 mg/kg/hr i.v. infusion for 3 hours or equivalent
volumes of 0.9% NaCl. Shed blood was reinfused 1 hour later. The depth and
duration of shock were modified (vide infra) because of problems with the
original model.

d) results: hemorrhagic shock. In the first 14 monkeys we could find no
difference in survival between naloxone (n=4) and saline treatment (n=10).
These animals were unexpectedly non-acidotic despite MAP as low as 25 mm Hg.
We discovered this was due to a factitiously low MAP because of large arterial
pressure catheters relative to small femoral vessels. A smaller arterial
pressure catheter inserted via the axillary artery gave accurate readings of
MAP and were used subsequently.

In the next 19 monkeys the MAP was held at 30 mm Hg during shock with
resultant acidosis and mortality in almost all. We were able to create a
lethal shock model but were unable to demonstrate a beneficial effect of
naloxone on survival. There was no statistically significant difference
in survival rate or length of survival between naloxone (n=7) and saline
treatment (n=12). This may be related to the acidosis which developed since
acidosis decreases the binding of antagonists to opiate receptors. In the
next 4 monkeys the depth of shock and the dose of naloxone were modified so
that survival would be improved. We finally were able to achieve a shock
model with partial survival using a MAP of 45 mm Hg and a naloxone dose of 2 mg/kg plus 2 mg/kg/hr.

In the subsequent 10 animals studied under these conditions there again was no difference in survival between naloxone (n=5) and saline (n=5). Taken as a whole, there were no differences between treatment groups in survival, but naloxone did produce some improvement in MAP, CO, and LV dp/dt max in some of the 21 animals treated. The response to naloxone was "good" in 2, "moderate" in 3, "slight" in 3, transient in 5, and absent in 8 animals. Four of the 8 without responses to naloxone had catheter injuries to the heart, a problem found in 6 of the 49 monkeys studied in hemorrhagic shock. Two monkeys died of this catheter-induced injury before being randomized to any treatment group.

e) results: endotoxemic shock. Twelve monkeys were studied. Survival rate was significantly better in the naloxone treated group (6/6) than in the saline treated group (1/6, p<.01 by Fisher's exact test). Naloxone significantly improved MAP by 25-30 mm Hg over saline treated controls (p<.02 by ANOVA, Figure 1). LV dp/dt max was higher in naloxone treated monkeys (3600 mm Hg/sec) than in controls (2400 mm Hg/sec), a difference that was highly significant statistically (p<.01 by ANOVA). There were no differences between naloxone and 0.9% NaCl with respect to CO, stroke volume, HR, peripheral vascular resistance, body temperature, and metabolic measurements. Naloxone improved LV dp/dt max by 800 mm Hg/sec compared to no change with saline (p<.02 by ANOVA, Figure 2).
f) Results: hormones and neurohumors. There was no significant elevation of plasma histamine in response to the shock states. Plasma assays of β-endorphin, cortisol, and ACTH will be done presently. No results, even preliminary, are available.

g) Discussion. The results in the endotoxemia study suggest that endorphin systems are activated in and contribute to the cardiovascular pathophysiology of endotoxemic shock in primates. Furthermore, our results suggest the feasibility of using naloxone in shock states due to sepsis and endotoxemia. The dose of 2 mg/kg plus 2 mg/kg/hr is effective in converting an 80% lethal model to 100% survival. The possibility of using lower doses might be considered.

The results in hemorrhagic shock are not nearly so encouraging but may reflect deficiencies in the model and not in the concept. There are some cardiovascular responses to naloxone which suggest that some of the cardiovascular depression is due to endorphins acting on opiate receptors (since naloxone has no effect in non-shock states).\(^4\) Deficiencies in the model and in the dose of naloxone used may be clarified by the plasma β-endorphin results especially comparing the different shock models.

Failure to demonstrate increased plasma histamine in shock suggests that this compound does not contribute to the cardiovascular abnormalities found. Because of its rapid metabolism it still might be released by endogenous opioid substances.

h) Conclusions. Endorphin systems are activated in shock, especially endotoxic shock. Beneficial cardiovascular effects accrue from blockade
of opiate receptors with naloxone leading to prolonged survival at least in the endotoxemia model.

i) **recommendations.** Hemorrhagic shock is obviously of prime importance. Further work on the primate shock model is needed, the direction of which may be a function of the plasma β-endorphin levels. If β-endorphin levels are elevated in the endotoxemic but not the hemorrhage model then a more severe hemorrhage model is needed. If β-endorphin is elevated in both models then one should address the problem of naloxone dosage in hemorrhage. If plasma β-endorphin is not elevated in either model or normal in endotoxemia while elevated in hemorrhage then more consideration ought to be given to central nervous system endorphin systems (met-enkephalin, leu-enkephalin, hypothalamic β-endorphin) than pituitary endorphins in shock.

These preliminary results are exciting and warrant further work:
1) developing the hemorrhagic shock model, 2) looking at mechanisms involved and 3) exploring the use of non-opioid opiate antagonists like thyrotropin releasing hormone. The use of primates is mandatory.
LITERATURE CITED


APPENDICES

Figure 1. The response of mean arterial pressure (MAP, mm Hg) to naloxone 2 mg/kg plus 2 mg/kg/hr i.v. versus 0.9% NaCl in cynomolgus monkeys given E. coli endotoxin 5 mg/kg. Treatment begun when MAP reached 75 mm Hg. (time t=0). Results are shown as mean ± SEM.

Figure 2. The response in left ventricular contractility (LV dp/dt max, mm Hg x 10^-3/sec) to naloxone versus 0.9% NaCl in monkey endotoxemic shock. See legend of Figure 1 for details.
PRIMATE ENDOTOXIN SHOCK

MAP, mm Hg

TREATED 2 mg/kg naloxone
CONTROL 0.9 % NaCl

TIME (min)
PRIMATE ENDOTOXIN SHOCK

Δ LV dp/dt max (mm Hg · 10^3/sec)

-0.4  -0.2  0  0.2  0.4  0.6  0.8  1.0

TIME (min)

- 30  0  +30  +60  +90  +120  +150  +180  +210

- TREATED 2 mg/ml naloxone
- **CONTROL 0.9 % NaCl**

---

The graph illustrates the changes in Δ LV dp/dt max (left ventricular peak pressure) over a period of time in primate endotoxin shock. The treated group (2 mg/ml naloxone) shows a significant increase in Δ LV dp/dt max followed by a gradual decrease, whereas the control group (0.9 % NaCl) exhibits a different pattern with a more pronounced fluctuation. The error bars indicate the variability in the data points.
<table>
<thead>
<tr>
<th>DISTRIBUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 copies Commander</td>
</tr>
<tr>
<td>U.S. Army Medical Research and Development Command</td>
</tr>
<tr>
<td>Attn: SGRD-RMS/J. Idoine</td>
</tr>
<tr>
<td>Fort Detrick</td>
</tr>
<tr>
<td>Frederick, MD 21701</td>
</tr>
<tr>
<td>14 copies Commander</td>
</tr>
<tr>
<td>Letterman Army Institute of Research</td>
</tr>
<tr>
<td>Attn: SGRD-ULZ-RCM/Dr. Neville</td>
</tr>
<tr>
<td>Presideo of San Francisco, CA 94129</td>
</tr>
<tr>
<td>4 copies Commander</td>
</tr>
<tr>
<td>U.S. Army Medical Research and Development Command</td>
</tr>
<tr>
<td>Attn: SGRD-RMS</td>
</tr>
<tr>
<td>Ft. Detrick</td>
</tr>
<tr>
<td>Frederick, MD 21701</td>
</tr>
<tr>
<td>12 copies Administrator</td>
</tr>
<tr>
<td>Defense Technical Information Center</td>
</tr>
<tr>
<td>Attn: DTIC-DDA</td>
</tr>
<tr>
<td>Cameron Station</td>
</tr>
<tr>
<td>Alexandria, VA 22314</td>
</tr>
<tr>
<td>1 copy Commandant</td>
</tr>
<tr>
<td>Academy of Health Sciences, U.S. Army</td>
</tr>
<tr>
<td>Attn: AHS-CIM</td>
</tr>
<tr>
<td>Fort Sam Houston, TX 78234</td>
</tr>
<tr>
<td>1 copy Dean, School of Medicine</td>
</tr>
<tr>
<td>Uniformed Services University of the Health Sciences</td>
</tr>
<tr>
<td>4301 Jones Bridge Road</td>
</tr>
<tr>
<td>Bethesda, MD 20014</td>
</tr>
</tbody>
</table>