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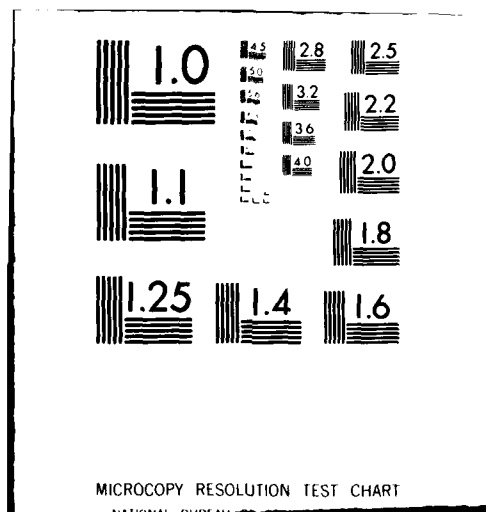
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THE ROLE OF ENDORPHINS IN THE PATHOPHYSIOLOGY OF SHOCK AND  
THE THERAPEUTIC BENEFIT OF OPIATE ANTAGONISTS (U)

JOHN W. HOLADAY, Ph.D.  
ALAN I. FADEN, MAJ, MC

JUN 1980

DEPARTMENT OF MEDICAL NEUROSCIENCES, DIVISION OF NEUROPSYCHIATRY  
WALTER REED ARMY INSTITUTE OF RESEARCH  
WASHINGTON, D.C. 20012

INTRODUCTION

The initial management of shock and trauma on the battle-field involves the rapid administration of intravenous fluids. Not only does this therapy require a skilled technician, but the problems of storage and availability of these fluids results in potentially fatal delays in treatment. In addition, the standard therapies employed in treating shock (e.g., fluids, steroids, and vasoactive agents) may not always reliably reverse the shock states which result from endotoxemia, hemorrhage, or spinal cord injury. We have sought a drug which would allow for the rapid stabilization of shock on the battlefield with minimal demands for technical skills and without the problems of storage and availability. We have shown that the opiate antagonist naloxone may be such a drug.

Within the past five years, it has been determined that opiate-like substances exist within the body and are involved in a variety of physiological and pathophysiological functions. Available evidence suggests that these endogenous opiates, collectively termed "endorphins", alter pain perception, body temperature, respiration, and may subserve other roles as well (1).

It is also known that endorphin systems are activated by stress (2,3) and that endorphins, like morphine, can produce marked hypotension following pharmacological administration (4). Since shock states are characterized by profound physiological stress, we investigated the possibility that the stress of shock would activate endorphin systems and thus contribute to the hypotension which

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characterizes the shock syndrome. More importantly, if endorphins contribute to this pathophysiological effect of shock, then blockade of endorphins by opiate antagonists should reverse the hypotension of shock.

~~In this report, we present~~ <sup>no presented</sup> evidence that the pure opiate antagonist naloxone rapidly increases blood pressure and decreases mortality associated with shock caused by endotoxemia, hemorrhage, and spinal-cord transection. Shock studies conducted in rats, cats, and dogs demonstrate these therapeutic effects of naloxone across a variety of species. Additionally, naloxone treatment significantly improves neurologic recovery (paralysis) after spinal-cord injury in the cat. Results from additional experiments provide evidence for the site and mechanisms of these therapeutic effects of naloxone.

#### MATERIALS AND METHODS†

Endotoxic and Hemorrhagic Shock in Rats. Since many anesthetic agents cause release of endorphins, we elected to study unanesthetized rats. Twenty-four hours prior to study, catheters were placed in the external jugular vein and tail artery; both cannulae were passed subcutaneously and threaded through a wire spring which was secured to the occipital area. This methodology, described in detail elsewhere (5), permitted evaluation of conscious, freely moving animals which remained in their home cages and which were not subjected to the stresses of handling immediately prior to study. Blood pressure (BP) and heart rate (HR) were continuously recorded using a microtransducer connected to a polygraph.

Escherichia coli lipopolysaccharide endotoxin was administered intravenously at a dose of 12 mg/Kg. When mean arterial pressure (MAP) had declined to a pre-established level of 65-70 mm Hg, animals received equal volumes of either saline or naloxone hydrochloride in varying doses.

For hemorrhagic shock, rats were prepared as described above. On the day following surgery, hemorrhagic shock was produced by withdrawing blood from the venous catheter. MAP was maintained at 40 mm Hg for a period of 20 minutes. This methodology resulted in the withdrawal of approximately 50% of the animals total blood volume by

†In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care', as promulgated by the Committee of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

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the time of treatment and produced a 50% mortality in untreated rats. Animals were matched in pairs according to pre-treatment MAP, with 15 animals each assigned to either a saline or naloxone treatment group. A naloxone dose of 1 mg/Kg, given as a single iv bolus, was employed; saline controls received equal-volume iv injections.

Endotoxic and Hemorrhagic Shock in Dogs. Because of surgical procedures required for monitoring more complex cardiovascular parameters in dogs, studies were conducted on pentobarbital anesthetized animals. It was shown that naloxone has no significant effect on these parameters in the pentobarbital anesthetized, unshocked dogs used as controls. MAP was monitored through a femoral artery catheter. A pigtail catheter was placed in the left ventricle (LV) and the first derivative of LV pressure with respect to time (LV dp/dt max) was used as an index of LV contractility. A triple lumen Swan-Ganz catheter was passed into the pulmonary artery to measure pulmonary arterial wedge pressure (PA<sub>w</sub>). This catheter was equipped with a thermistor tip to permit determination of cardiac output (CO) by a thermodilution method. Total peripheral vascular resistance (TPVR) and stroke volume were calculated from the CO, MAP and HR.

All animals received iv E. coli endotoxin at a dose (0.1 mg/kg) which produced a mortality of 80% at 24 hours in untreated animals. Following endotoxin administration, dogs were treated intravenously with either saline (14/group) or naloxone (2 mg/Kg; 6 dogs/group) in an equivalent volume. Survival was monitored at 24 hours.

For hemorrhagic shock studies, anesthetized dogs were prepared as above. These animals were bled down to 45 mm Hg and this MAP was maintained for 60 minutes. At this time the animals were treated with either naloxone (2 mg/Kg iv bolus, 2 mg/Kg/hr, n=5) or saline at equal volumes. One hour after treatment, shed blood was returned to surviving animals.

Spinal Shock and Spinal Trauma in Rats and Cats. Pentobarbital anesthetized rats and cats were studied. Rats were prepared with cannulae in the external jugular vein and tail artery; a guide tube was also affixed to the cranium for intraventricular (ivt) drug injections. Cats were anesthetized with pentobarbital, immobilized with gallamine triethiodide, and artificially ventilated. A femoral artery catheter was connected to a transducer to measure BP; heart rate (HR) was also monitored. A pigtail catheter was placed in the left ventricle (LV) through the right common carotid artery for measurement of cardiac contractility. Drugs were administered iv through a cannula in the femoral vein.

Both rats and cats were subjected to a dorsal laminectomy which exposed the spinal cord at the level of the seventh cervical vertebral (C7) segment. Following transection of the spinal cord, MAP fell to 20-30 mm Hg below baseline. In rats, the effects of iv as well as ivt injections of saline or naloxone were studied. Cats received only iv injections of these drugs. Doses and numbers of animals studied are depicted in figure legends.

A spinal-cord trauma technique was used to study the effects of naloxone on blood pressure and neurological outcome after spinal injury. After cannulating the femoral artery and vein, a calibrated weight (20 grams) was dropped 25 cm (a 500 gram-cm force) onto a 10 mm<sup>2</sup> plastic impact plate placed upon the spinal cord exposed at C7 as above. Forty-five minutes after injury, cats were treated intravenously with equal volume of saline or 2 mg/Kg naloxone. Cardiovascular parameters were monitored for four hours, after which catheters were removed and the laminectomy site was surgically closed. Animals were allowed to recover in home cages, and neurological examinations using a 5 point scale described elsewhere (6) were performed at 24 hours, 1, 2, and 3 weeks later.

## RESULTS

Figure 1 demonstrates the rapid improvement in blood pressure produced by 10 mg/Kg naloxone in an unanesthetized rat subjected to endotoxic shock when compared to a saline-injected control animal. Following the precipitous drop in blood pressure produced by endotoxin, saline was without effect on this parameter. By contrast, naloxone produced a rapid return in blood pressure to control levels within seconds following administration (Fig 1).

A comparison of MAP and pulse pressure (PP; a crude index of cardiac performance) with and without naloxone for a group of rats is seen in Fig 2. After endotoxin hypotension, naloxone treatment resulted in a significant improvement in MAP and PP, whereas saline treatment was without effect. Additionally, naloxone administered to normotensive control rats not subjected to endotoxemia had no effect on these parameters, indicating a selective action in reversing shock hypotension instead of a direct effect of the drug by itself.

In other studies (data not shown), dose response effects of naloxone were determined (7,8). A dose of naloxone as low as 0.1 mg/Kg was shown to significantly improve MAP and PP, however maximum responses were obtained with 1.0 mg/Kg and 10.0 mg/Kg naloxone.

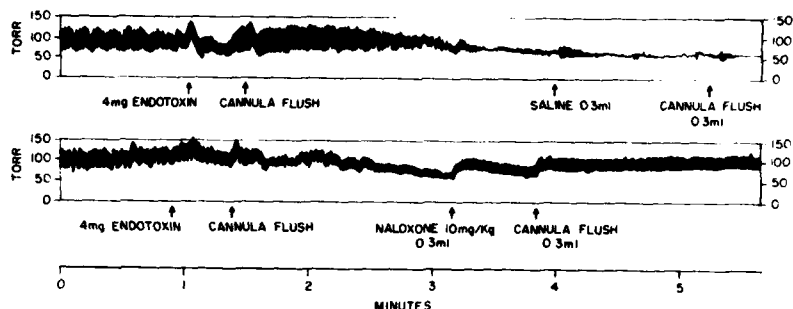


Fig. 1. Effects of intravenous saline (top) and naloxone (bottom) on the precipitous fall in blood pressure produced by 4 mg endotoxin in representative rats. Saline (0.3 ml) or 10 mg/Kg naloxone were injected after blood pressure fell to 65-70 mm Hg (torr).

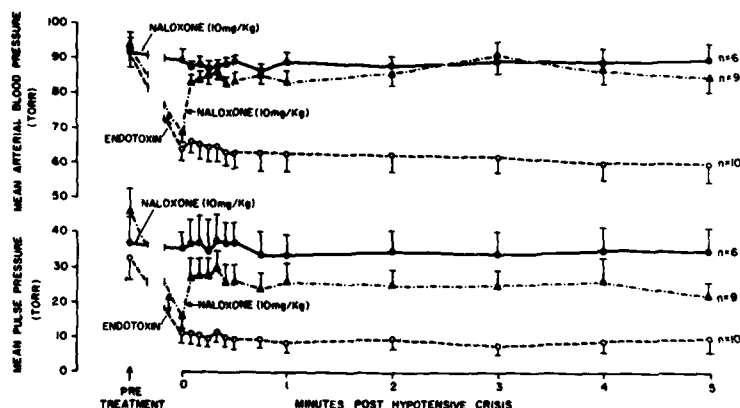


Fig. 2. The effects of naloxone or saline on mean arterial pressure (MAP, top) or pulse pressure (bottom) are compared with and without endotoxin-induced hypotension. Naloxone alone (●—●) did not affect MAP or PP. Endotoxin produced a significant drop in both MAP and PP which was reversed by naloxone (▲—▲) but unaffected by saline (○—○). Points represent averages  $\pm$  SEM.

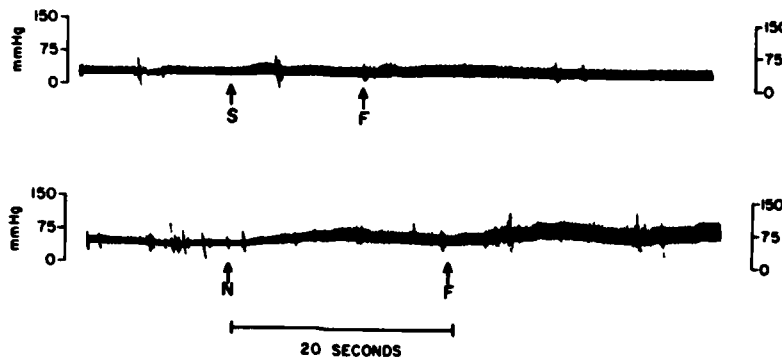


Fig. 3. Representative polygraph recordings showing the effect of saline (S, top) or 1 mg/Kg naloxone (N, bottom) on blood pressure following hemorrhagic hypotension. A 0.3 ml saline flush (F) was used to ensure drug delivery.

Figure 3 shows the effects of naloxone on blood pressure following hemorrhagic shock in representative rats. Naloxone, at a dose of 1 mg/Kg, significantly improved MAP and PP when compared to saline-injected, control rats (Fig. 4). More importantly, in this model of hemorrhagic shock, naloxone significantly improved survival with 13 of 15 naloxone-treated and 8 of 15 saline-treated rats surviving 24 hours (Fishers exact probability test,  $p < .05$ ).

As seen in rat studies, the hypotension resulting from both endotoxic and hemorrhagic shock in dogs was rapidly reversed by naloxone at a dose of 2 mg/Kg iv (data not shown). Cardiac output (CO) was shown to be significantly improved (Figure 5), probably as a consequence of the increased cardiac contractility (CC) produced by naloxone in both shock models (data not shown). The fact that CO, and MAP all increased following naloxone injection, and venous return as well as total peripheral resistance were unchanged, suggests that naloxone was exerting its therapeutic effects directly or indirectly by improving cardiac contractility.

More critically, naloxone significantly improved survival following endotoxic or hemorrhagic shock in dogs. Twenty four hours following endotoxin treatment, 3 of 14 saline treated dogs (21%) were alive, whereas 5 of 6 naloxone treated animals (83%) survived. With hemorrhagic shock, results were even more striking. All of the naloxone-treated dogs survived 24 hours following hemorrhage and none of saline-treated animals remained alive.



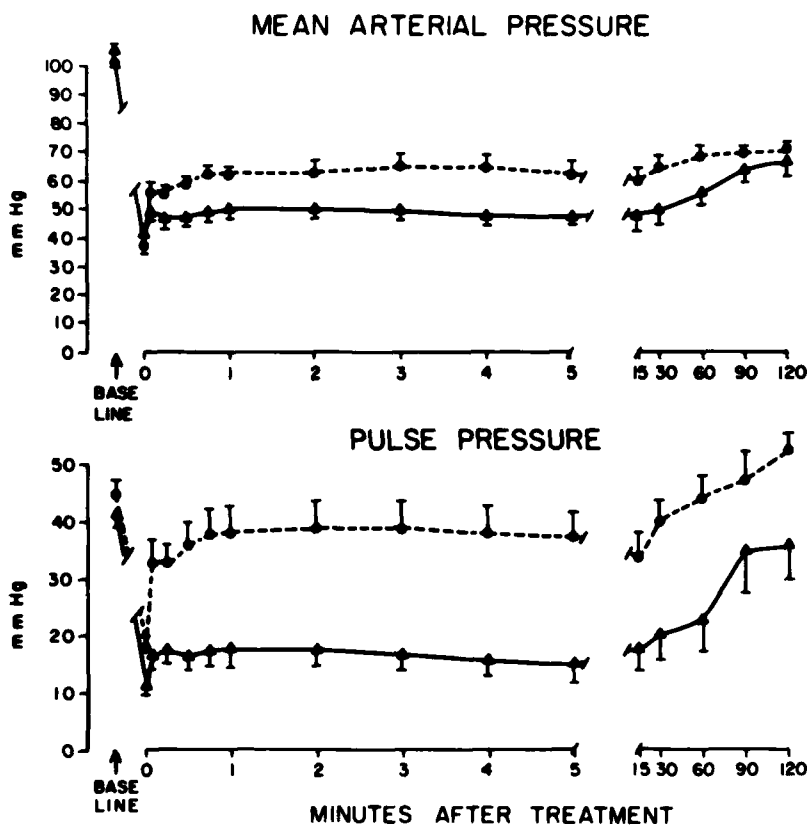


Fig. 4. The effects of 1.0 mg/Kg naloxone (●—●) and saline (▲—▲) treatment on MAP (top) and PP (bottom) following hemorrhagic shock. Naloxone treatment significantly improved these cardiovascular parameters, whereas saline was without a significant effect. Fifteen rats were studied in each group; vertical bars are  $\pm$  SEM.

Spinal shock, which occurs following transection of the spinal cord, is also known to significantly reduce blood pressure. In rats, 48  $\mu$ g of naloxone's active (-) or "levo-" isomer injected into the ventricles of the brain (ivt) produced an equivalent improvement in blood pressure as seen with 10 mg/Kg (-) naloxone injected parenterally. The (+) isomer of naloxone, which is chemically identical to (-) naloxone but biologically inactive at opiate receptors, had no effect on BP following ivt injection (Figure 6). These findings indicate that the effects of naloxone in this model are mediated by opiate

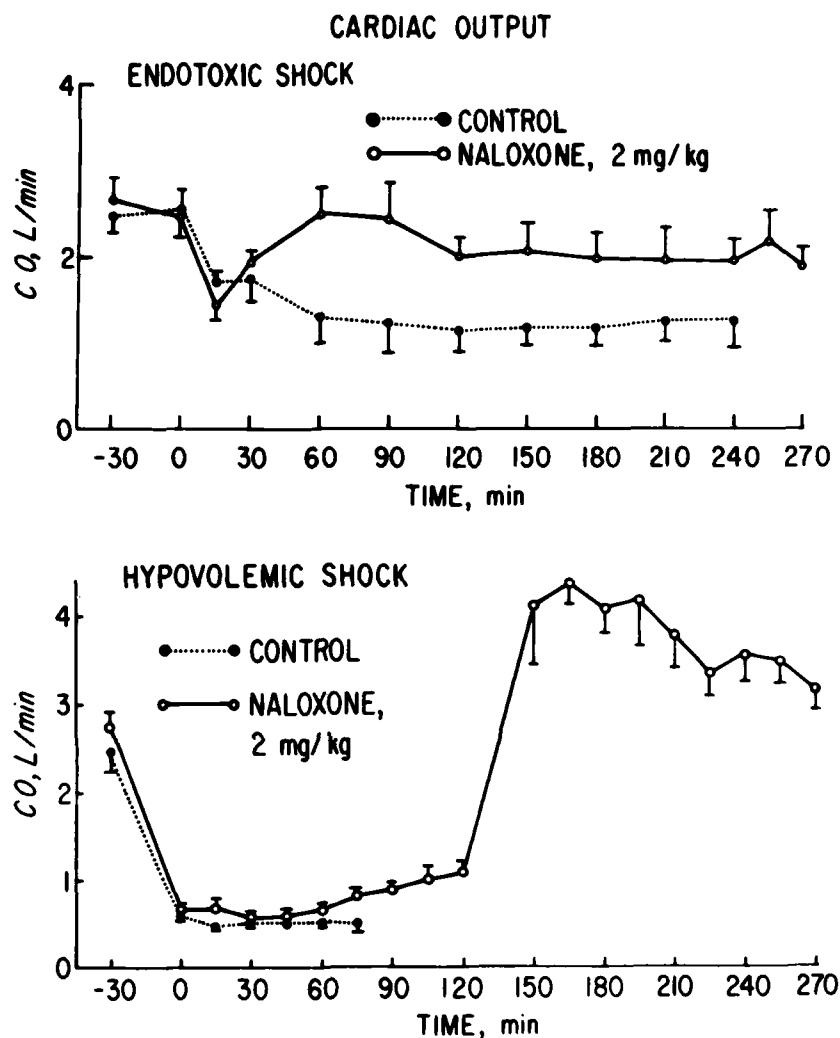


Fig. 5. Cardiac output (CO) was measured before and after treatment with naloxone or saline in canine models of shock. The upper figure demonstrates the decrease in CO produced by endotoxic shock; the lower figure represents the effects of hemorrhagic shock on this measure. Naloxone-treated dogs (○—○) experienced a sustained increase in CO, whereas CO in saline treated animals (●—●) continued to decline over time (injections at arrow). Data points for saline-treated dogs subjected to hemorrhagic shock end abruptly (bottom figure) since all 6 dogs died before shed blood could be reinfused. Vertical bars are  $\pm$  SEM.

receptors within the central nervous system (9,10). Injection of this very small ivt dose of (-) naloxone parenterally produced no effect. The fall in body temperature as well as respiratory depression following spinal shock were also reversed by naloxone (data not shown).

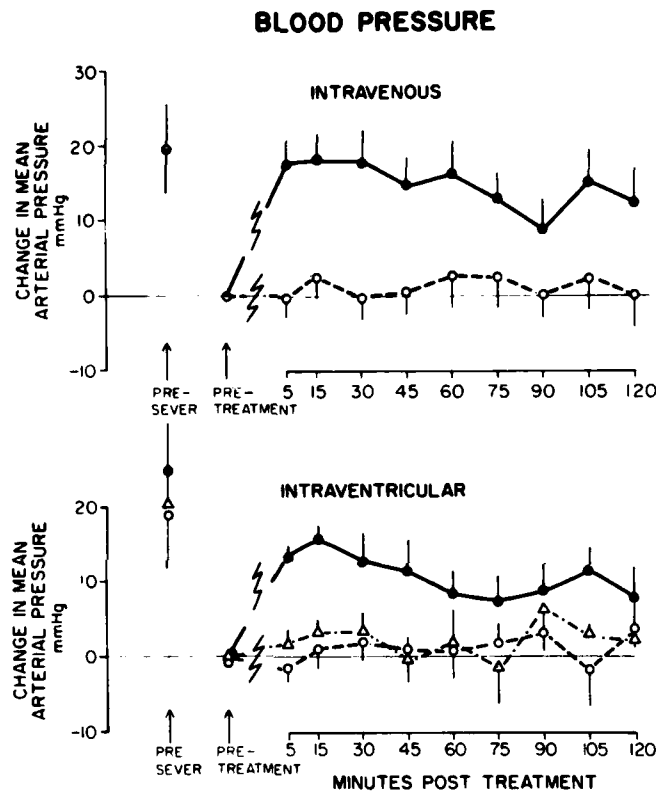


Fig. 6. Following rapid transection of the spinal cord in rats, MAP fell about 20 mm Hg. Rats receiving intravenous naloxone (top graph, 10 mg/Kg, 11 rats/group) had a sustained increase in MAP. In the bottom graph, the effects of 48  $\mu$ g (-) naloxone ( $\bullet$ - $\bullet$ ) were compared to the effects of 48  $\mu$ g (+) naloxone ( $\Delta$ - $\Delta$ ) or drug vehicle ( $\circ$ - $\circ$ ) following intraventricular injection in spinally transected, hypotense rats (6-7 rats/group). Vertical bars are  $\pm$  SEM.

Spinal shock in cats also produced a significant fall in MAP and cardiac contractility (11,12). In these studies, four treatment groups were used. Cats were treated with naloxone, naloxone following vagotomy, naloxone following atropine, or saline. Since transection of the spinal cord leaves intact only parasympathetic innervation from the brain to the heart, the effect of naloxone on cardiovascular parameters would appear to be mediated by the vagus nerves. As in the rat, naloxone treatment significantly improved cardiovascular parameters. These effects of naloxone were completely blocked by vagotomy or atropine (Fig. 7). Thus, at least in spinal shock, naloxone acts upon parasympathetic centers in the CNS to improve cardiac performance via the vagus nerves which provide cholinergic innervation to the heart.

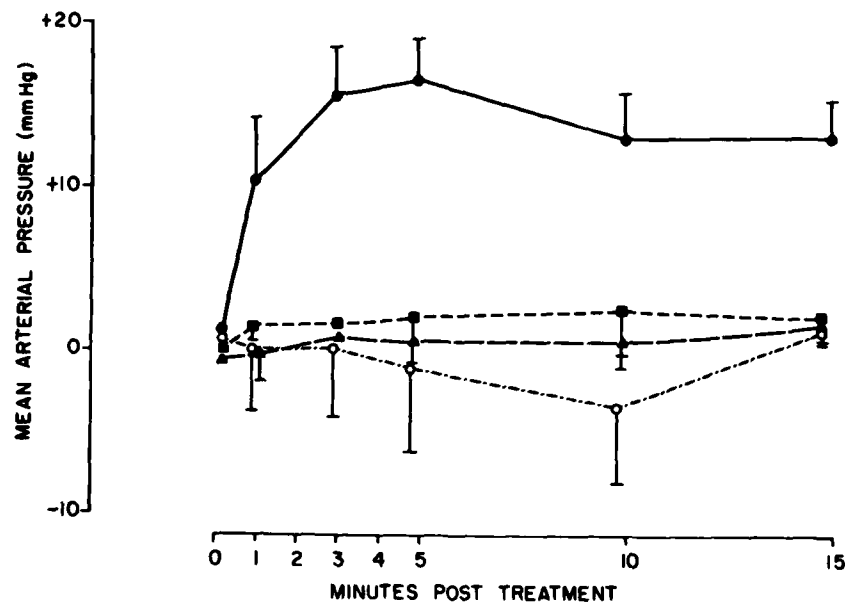


Fig. 7. Following hypotension caused by spinal cord transection in cats, animals received either naloxone (1 mg/Kg, ●—●) or saline (▲—▲). Additionally, naloxone was injected into vagotomized (□---□) or atropinized (○---○) cats following the hypotension produced by spinal cord transection. Naloxone only improved MAP in cats without vagotomy or atropine; saline was without effect as well.

Following spinal cord trauma in cats, BP was again seen to drop approximately 15-20 mm Hg. Naloxone injections produced a significant increase in MAP, and saline was without effect on this measure. Neurologic examinations demonstrated that the naloxone-treated cats had significantly less paralysis than saline-treated animals at 24 hrs, 1, 2, and 3 weeks following spinal trauma (data not shown).

#### DISCUSSION

The therapeutic efficacy of naloxone has been demonstrated following endotoxic shock in rats (1,7,8) and dogs (13), in hemorrhagic shock in rats (14,15,16) and dogs (17), and following spinal shock in rats (9,10) and cats (11,12). Since naloxone has been described as a pure opiate antagonist, its effects in altering physiological and behavioral parameters have been used to infer a blockade of endorphin-mediated events (1). From this perspective, our findings summarized above provide experimental evidence that endorphin systems significantly contribute to the pathophysiology of shock produced by a variety of means in different species. More importantly, the therapeutic efficacy of naloxone in all of these shock models points to its potential clinical utility in improving survival and recovery in humans.

Since we have shown that doses as low as 0.1 mg/Kg naloxone have therapeutic benefit in animals requiring greater than 100.0 mg/Kg for toxic effects, the large therapeutic ratio for this narcotic antagonist indicates a wide safety margin for its clinical use in treating shock.

Studies on the site and mechanism by which endorphins contribute to shock pathophysiology have recently indicated that pituitary endorphins gain access to the CNS where they ultimately produce the cardiovascular manifestations which characterize this syndrome (15,16). In those experiments, it was demonstrated that animals without pituitary glands, (hypophysectomized) do not respond to naloxone following hemorrhagic shock. By contrast, in control animals with intact pituitary glands, 10 µg of naloxone ivt effectively restored blood pressure, thus indicating a CNS site of action for these pituitary endorphins (16).

Once in the CNS, endorphins appear to act on specific opiate receptors since their effects are stereospecifically reversed by minute amounts of naloxone. Data obtained from spinally transected rats and cats indicate that parasympathetic centers, possibly in the brainstem, are involved. The action of endorphins in this region appears to indirectly depress cardiovascular function via the vagal-cholinergic input to the heart (12). Lastly, the end result of endorphin effects in

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shock are to depress cardiac contractility and thereby decrease cardiac output and MAP concomitantly (12,13,17).

Recent data indicate that the improvement in blood pressure following injury to the spinal cord is associated with an improvement in neurological recovery (6). Collectively, these findings predict that the therapeutic effects of narcotic antagonists such as naloxone may significantly improve survival and functional recovery following battlefield injuries.

#### SUMMARY

We have shown that the specific-opiate antagonist naloxone rapidly improved blood pressure and significantly decreased mortality associated with endotoxic, hemorrhagic, and spinal shock. These therapeutic effects of naloxone in the treatment of shock were demonstrated in rats, cats, and dogs. In addition, naloxone was shown to significantly decrease the paralysis resulting from spinal-cord injury in the cat. Available evidence indicates that naloxone produces those effects by antagonizing endogenous opiates (endorphins) secreted from the pituitary gland in response to the stresses of shock or spinal trauma. Moreover, these endorphins appeared to depress cardiovascular function by acting at opiate receptors in areas of the brain which regulate cardiac contractility. Our findings predict that the use of naloxone in the care and management of battlefield injuries may significantly improve survival and functional recovery.

#### ACKNOWLEDGEMENTS

The authors are indebted to Thomas P. Jacobs and Clifton E. Johnson for their expert technical assistance as well as to Pat Connors for her preparation of this manuscript. We thank Endo Laboratories, Garden City, NY, for their generous gift of naloxone.

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FOOTNOTE

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