PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 173, 76–81 (1983)

Beneficial Actions of Nalorphine during Hemorrhagic Shock in Cats[1] (41612)

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Abstract. Endogenous opiates have been reported to have detrimental effects on the circulatory system during hemorrhagic shock. However, the specific opiate receptor subtype which mediates these actions has not been defined. In the present study, we have utilized the mixed agonist/antagonist, nalorphine (*N*-allylnormorphine), which exhibits kappa (κ) and sigma (σ) receptor agonism as well as mu (μ) receptor antagonism, to investigate the role of the mu receptor in hemorrhagic shock. Nalorphine (2 mg/kg) produced no significant changes in any observed experimental variable in sham-shocked animals. Shocked animals treated with nalorphine (2 mg/kg) maintained significantly higher final mean arterial blood pressures (MABP) than animals which received only vehicle (102 ± 3.8 vs 61 ± 6.6 mm Hg, respectively, $p < 0.001$). In addition, nalorphine significantly reduced the rise in plasma MDF activity observed in untreated hemorrhaged animals (42 ± 3.0 vs 59 ± 4 U/ml, $p < 0.02$). Our results support a significant role for the mu receptor in the deleterious actions of endogenous opioids during hemorrhagic shock.

Both exogenously administered and endogenously released opiates have been reported to depress myocardial function as well as arterial blood pressure (1, 2). Opiate receptors have been identified in the central nervous system (CNS) since 1973 (3). However, it is now recognized that enkephalin-like and β-endorphin-like immunoreactivity as well as opiate receptors are widely distributed in the periphery as well (4, 5). These endogenous opiate systems are activated by various types of circulatory perturbations (6).

The opiate antagonist, naloxone has been reported to have protective effects and improve survival in endotoxic shock (7), hemorrhagic shock (8, 9, 10), spinal cord injury shock (11), and splanchnic artery occlusion shock (12). CNS opiate receptor sites have recently been demonstrated to be important in mediating the cardiodepressant effects of endorphins in hypovolemic shock (8). However, the inhibition of proteolysis and stabilization of lysosomal membranes reported by Curtis and Lefer (9) in naloxone-treated hemorrhagic shock cats is likely mediated by peripheral receptors, based on [^{3}H]naloxone tissue uptake studies (13).

Since the work of Martin and co-workers (14), both central and peripheral opiate receptors have been thought to be of multiple types, with the three main receptor subtypes designated as mu (μ), kappa (κ), and sigma (σ) receptors. Since their initial report, delta (δ) and epsilon (ϵ) receptors have also been postulated (15, 16). Several endogenous opioid peptides exist and may exhibit receptor specificity. Both methionine and leucine enkephalins have been reported to bind primarily to delta receptors, having a greater than 90% affinity in the mouse vas deferens preparation (17). It is possible, that since the opiate receptors appear to possess ligand specificity, they may possess functional specificity as well. If so, it would be important to know which opiate receptor subtype is responsible for a given biological effect.

Most of the previous studies reporting protective effects of opiate receptor blockade during shock states utilized the broad based opiate antagonist, naloxone which blocks at μ, κ, and δ receptors, but is devoid of any agonistic effect (15, 18). This type of antagonist gives limited information on the specific opiate receptor type involved in the protective effect, if indeed there is only one. In the present study,

[1] Supported in part by the W. W. Smith Charitable Trust.

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0037-9727/83 $1.50

we have further investigated opiate receptor involvement in hemorrhagic shock using the opiate agonist/antagonist nalorphine (*N*-allylnormorphine), which is a competitive antagonist only at the μ receptor and is a partial agonist at the κ and σ receptors (18). The effects of nalorphine on the δ receptor have not been clearly established. It was our purpose to investigate the possible protective effects of nalorphine in the pathogenesis of hemorrhagic shock, and in particular the role of the μ receptor.

Materials and Methods. Experiments were performed on adult male cats (2.5 to 4.1 kg) anesthetized intravenously with sodium pentobarbital (30 mg/kg). The forelimb withdrawal reflex was monitored throughout the experiment, and supplemental doses of anesthetic were given, if needed, to maintain a constant level of anesthesia. Food and water were given *ad libitum* prior to the experiments. The animals were divided into three major experimental groups: (i) sham-shocked cats given nalorphine (2 mg/kg), (ii) hemorrhaged cats given vehicle (0.9% NaCl), and (iii) hemorrhaged cats given nalorphine (2 mg/kg). In addition, smaller groups of hemorrhaged cats given either 1 or 4 mg/kg of nalorphine were studied. Preliminary studies indicated 2 mg/kg to be the optimal dose of nalorphine in maintaining circulatory homeostasis after reinfusion of shed blood.

In all animals, the right carotid artery and the left external jugular vein were catheterized to record mean arterial blood pressure (MABP) and central venous pressure (CVP), respectively, using Statham P23-AC pressure transducers and a Grass Model 7 oscillographic recorder. The trachea was cannulated in order to maintain a patent airway and to facilitate positive-pressure ventilation in the event of severe respiratory depression during hemorrhage. Polyethylene catheters were inserted in the right femoral artery for blood removal and the right femoral vein for drug infusion. The left femoral vein was catheterized and used for blood sampling for biochemical analysis. After completion of all surgery the animals were observed for 20 min prior to further study.

At the end of the stabilization period, control readings were taken for MABP, heart rate (HR), and CVP. The animals were heparinized (500 units/kg), and a blood sample was withdrawn and iced for later biochemical analysis. Hemorrhagic shock was produced by lowering the MABP to 40–45 mm Hg over a 20-min period via blood removal from the femoral artery. The shed blood was held in an aerated, heparinized (2000 Units heparin sodium), silicon-coated, Plexiglass reservoir during oligemia. MABP was maintained at 40–45 mm Hg during the 150-min oligemic phase by means of a servopump. At the end of this period, the blood remaining in the reservoir was reinfused into the cat over 15–20 min, at a rate which was slow enough to prevent large increases in CVP. Experiments in which the animals did not attain a post-reinfusion pressure of at least 100 mm Hg were discarded. Hemodynamic variables were monitored for 120 min after reinfusion.

Drug-treated animals were given a loading dose of nalorphine (2 mg/kg) 40 min after the beginning of the hemorrhage. Nalorphine was infused at a rate of 2 mg/kg/hr into the femoral venous cannula starting 40 min later and continuing throughout the experiment. Animals which were not given nalorphine, received the 0.9% NaCl vehicle. During the 290-min observation period, 4 ml blood samples were taken at −20, 60, 150, 170, 230, and 270 min. The volume removed was replaced with an equal volume of heparinized saline. At the end of the experiment, a 30- to 40-ml blood sample was taken for myocardial depressant factor (MDF) analysis. Cats given 1 or 4 mg/kg nalorphine were given the same dose as an hourly infusion rate. Thus, cats given 1 mg/kg were then infused with nalorphine at a rate of 1 mg/kg/hr.

Plasma analysis. All plasma samples were stored on ice until they were centrifuged at 2500*g* for 20 min at 0°C. The plasma was collected and analyzed for protein concentration by the biuret method of Gornall *et al.* (19). The activity of the lysosomal hydrolase, cathepsin D, was determined by the method of Anson (20), using hemoglobin as substrate. The cathepsin D activity was indexed to the plasma protein concentration. MDF activity in the plasma was determined using paper chromatography by the method of Barenholz *et al.* (21). The chromatographic spots corresponding to MDF were developed with ninhydrin and eluted in $NaHCO_3$ according to

the method of Yamada and Pettit (22). The absorbance of each eluted spot G was measured and the value related to the absorbance of an eluted serine standard. One MDF chromatographic unit is equal to the absorbance value of 1.3 nmole of serine. A conversion factor of 6 was used to divide all chromatographically determined MDF values to normalize them to MDF values previously published using the cat papillary muscle bioassay method (23).

In vitro tissue preparations. Right ventricular papillary muscles were prepared according to previously described methods (24) and contractile force was monitored using Grass FT-03 force transducers and a Grass Model 7 oscillographic recorder. Concentrations in the range of 1 to 300 μg/ml of bathing solution were tested in order to assess possible inotropic actions of injected and infused nalorphine. The direct lysosomal stabilizing properties of nalorphine were determined using isolated cat large granule fractions prepared from liver homogenates according to the method of Bridenbaugh *et al.* (25).

Statistics. Student's t test was utilized for data analysis; $P < 0.05$ was considered statistically significant. Mean values are expressed with the standard error of the mean as the index of variation (mean ± SEM).

Results. *Hemodynamics.* The initial mean arterial blood pressures for the three groups of cats were not significantly different from each other (Fig. 1). The sham-shocked group maintained a relatively stable MABP during the 270-min experimental period. Nalorphine (2 mg/kg) had no significant effect on MABP over time or when compared to that of nonhemorrhaged cats receiving only 0.9% NaCl (not shown). Controlled hemorrhage reduced

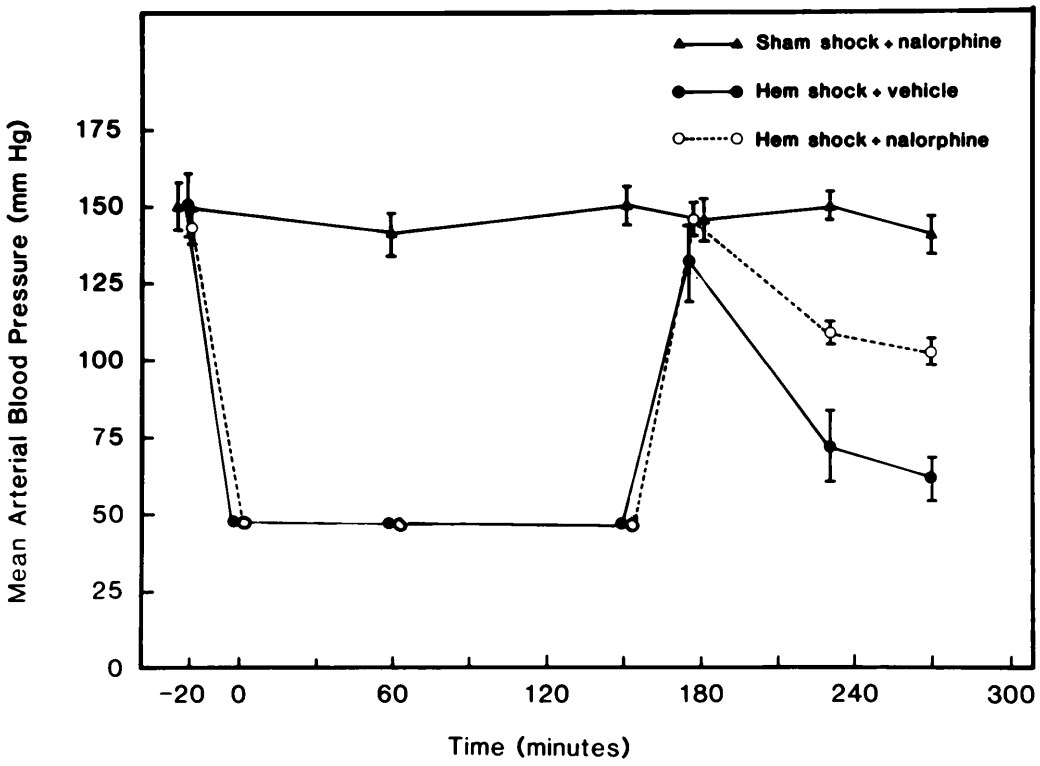

FIG. 1. Mean arterial blood pressure (MABP) for hemorrhaged and sham-shocked animals. In all groups nalorphine (2 mg/kg) was injected 20 min after time zero and was infused at 2 mg/kg/hr beginning 60 min after time zero for the duration of the experiment. The final postreinfusion MABP ($P < 0.001$) of both hemorrhagic shock groups was significantly lower than that of the five sham-shocked animals. The seven hemorrhaged cats treated with nalorphine maintained significantly higher ($P < 0.001$) postreinfusion MABP than those six cats receiving only the vehicle.

TABLE I. PLASMA CATHEPSIN D ACTIVITIES IN SHAM SHOCK AND HEMORRHAGIC SHOCK CATS

Plasma cathepsin D activity (U/mg protein)	Sham shock + nalorphine (5)	Hemorrhagic shock + vehicle (6)	Hemorrhagic shock + nalorphine (7)
Initial (−20 min)	2.9 ± 1.6	0.98 ± 1.0	0.7 ± 0.4
Postoligemic (150 min)	1.1 ± 0.9	12.1 ± 3.3*	9.0 ± 2.5*
Final (270 min)	2.0 ± 0.7	19.7 ± 5.5*	10.2 ± 2.7*

Note. All values are means ± SEM, numbers in parentheses are numbers of cats in each group. *$P < 0.01$ compared to 0 min values.

MABP to a stable value of 45 mm Hg by time zero. This pressure was maintained for 150 min using a servopump mechanism. The severity of this hypotensive, oligemic period was similar in the shocked groups since the maximum bleedout volumes were not significantly different, 38.8 ± 1.4 vs 41.6 ± 2.4 ml/kg for the untreated and nalorphine-treated shock groups, respectively. Reinfusion of the shed blood was initiated after 150 min of hypotension, and maximal postreinfusion pressures were recorded at 180 min. As illustrated in Fig. 1, these postreinfusion values for the two shocked groups were not significantly different from each other nor were they significantly different from MABP obtained at the same time in the sham-shock cats. Following reinfusion, the MABP at 230 min for the hemorrhaged cats given the vehicle was significantly lower than for the animals receiving nalorphine, (71 ± 8.5 vs 109 ± 3.2 mm Hg, $P < 0.001$). At 270 min, the MABP of the hemorrhaged animals receiving vehicle and nalorphine was 61 ± 6.6 and 102 ± 3.8 mm Hg ($P < 0.001$), respectively. The MABP for the two hemorrhaged groups were significantly less than for the sham-shock animals at both 230 and 270 min. While postreinfusion MABP values did not return completely to control levels, these results suggest that nalorphine (2 mg/kg) acts to maintain postoligemic blood pressure at normal values.

Plasma analysis. Table I summarizes the initial and final plasma cathepsin D activities in the three groups of cats under study. No significant difference was observed among any group initially. After 150 min of hemorrhage, both hemorrhaged groups exhibited a significant increase in circulating cathepsin D activity of about 8- to 10-fold. However, 2 hr after reinfusion of shed blood, the cathepsin D activity in the untreated hemorrhaged animals continued to increase markedly, whereas that for the nalorphine cats, did not change appreciably. Although the final cathepsin D activities were not significantly different between the two hemorrhaged groups, nalorphine appeared to modestly attenuate the lysosomal disruption of hemorrhagic shock. The *in vivo* data tends to be supported by the *in vitro* liver large granule suspensions where nalorphine (20 μg/ml) produced a modest but significant decrease in cathepsin D activity of 11.9 ± 3.7% ($P < 0.02$) from that of the vehicle treated homogenates.

Figure 2 illustrates plasma MDF activities determined at the end of the 270-min experimental period. The sham-shock cats given nalorphine exhibited activities of 25 ± 3 U/

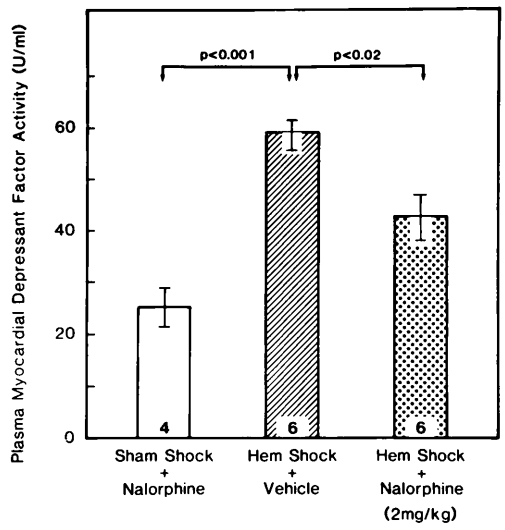

FIG. 2. Plasma myocardial depressant factor (MDF) activity in hemorrhaged and sham-shocked cats. Bar height and brackets indicate means and standard errors; numbers in bars indicate number of samples assayed. Hemorrhage causes a significant increase in MDF compared to sham-shocked animals ($P < 0.001$). This increase in plasma MDF is significantly ($P < 0.02$) blunted in hemorrhaged animals receiving 2 mg/kg of nalorphine.

ml while the untreated hemorrhaged group was 59 ± 4 U/ml ($P < 0.001$). Hemorrhaged animals treated with nalorphine exhibited an MDF activity of 42 ± 3 U/ml, a value significantly ($P < 0.02$) lower than that observed in the untreated hemorrhaged cats. These data indicate that nalorphine significantly reduces the formation of MDF in hemorrhagic shock.

In vitro studies. The isolated papillary muscle preparations revealed no significant increase in contractile force at nalorphine concentrations of 1 to 300 μg/ml. These findings as well as the absence of an increase in MABP in response to nalorphine in sham-shock control cats suggest that the increase in postreinfusion MABP observed in the drug treated, hemorrhaged animals was not due to any direct inotropic actions of nalorphine.

Discussion. Since the original work of Holaday and Faden on endotoxic shock (11), endogenous β-endorphins have been purported to be involved in the pathophysiology of a variety of types of circulatory shock (8–12, 27). Naloxone has been the most widely used opiate antagonist in these studies and has been reported to reverse endotoxin-induced hypotension (7), maintain significantly increased postoligemic blood pressure (9), increase left ventricular dP/dt (10), inhibit proteolysis (9), stabilize lysosomes (9) and reduce plasma MDF activity (9). The question of which opiate receptor subtype mediates these protective actions has recently been investigated by Curtis and Lefer (26). The κ receptor does not appear to be involved in the pathophysiology of hemorrhagic shock. In this study, we have utilized the κ and σ partial agonist/μ antagonist, nalorphine, in order to further elucidate the role of the μ receptor in hemorrhagic shock.

Nalorphine (2 mg/kg) produced no significant changes in any experimental variables including blood pressure in sham-shocked animals. Doses of 1 and 4 mg/kg were also without any hemodynamic effect. However, hemorrhaged animals given nalorphine maintained their control MABP at 2 hr after reinfusion to a much higher extent than the hemorrhaged animals given vehicle (i.e., 71 vs 40% of initial values).

The nalorphine-treated animals developed significantly lower MDF activities after hemorrhage and had a tendency for lower cathepsin D activities in the plasma compared to cats receiving only the vehicle. These biochemical and hemodynamic alterations induced by nalorphine would tend to improve survival during hypovolemic shock. Since decreased cathepsin D activity as well as decreased MDF activities are associated with increased splanchinc perfusion, nalorphine may act to reduce splanchnic vasoconstriction by antagonism of regional opiate receptors. The gastrointestinal tract and splanchnic areas are known to possess rich stores of opioid peptides whose function is largely unknown (4). Nalorphine was also found to be a potent respiratory stimulant during hemorrhagic shock. All hemorrhaged cats receiving the vehicle required mechanical respiration for a short period at some point during the experimental period in contrast to the hemorrhaged animals receiving nalorphine which did not. This positive-pressure respiration did not affect the course of hemorrhagic shock when compared with earlier shocked cats not on a respirator (9).

Nalorphine is reported to act as a competitive antagonist at the μ receptor and a partial agonist at both the κ and σ receptors. Moreover, based on studies utilizing the mouse vas deferens preparation, nalorphine may be a partial antagonist at other receptors (28). The antagonism of this other receptor, later identified as the δ receptor, is unlikely to be of great significance in hemorrhagic shock since we found nalorphine to have only modest effects in the mouse vas deferens preparation. Based on the report of Curtis and Lefer (26), it is unlikely that any of the protective actions which we report here for nalorphine are mediated by κ receptors. They reported that a κ antagonist (i.e., MR-2266) afforded none of the protective effects characteristically reported with the multiple-receptor antagonist naloxone or with two newer μ antagonists (i.e., C-7000 and J-7747) all three of which possess significant δ antagonistic activity. Our results, using nalorphine, a μ antagonist having only modest δ antagonistic activity, suggest a more important role for the μ receptor compared to the δ receptor in the deleterious actions of endogenous opioids during hemorrhagic shock. However, we cannot exclude either the δ or the ε receptor mechanisms at this time.

In summary, we have shown nalorphine to have significant protective actions in hemorrhagic shock including maintenance of

postreinfusion MABP, slightly decreased plasma cathepsin D activity, and significantly decreased plasma MDF activities. There are however undesirable psychomimetic side effects previously reported (29) for nalorphine when given at analgesic dosages in man. While nalorphine itself may not prove to be the drug of choice in shock therapy, it appears that synthetic μ antagonists would be beneficial in the treatment of shock.

We gratefully acknowledge the technical assistance of Jean Edmonds during the course of these investigations. We also thank Dr. Franklin M. Robinson of Merck Sharp & Dohme, West Point, Pennsylvania for the generous supply of nalorphine.

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Received September 7, 1982. P.S.E.B.M. 1983, Vol. 173.