

# Effect of Naloxone and Morphine on Survival of Conscious Rats After Hemorrhage

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The endogenous opioid system has been reported to depress the cardiovascular system during shock states, since naloxone, a potent opiate antagonist, enhances recovery of hemodynamic variables in various shock states. However, the effect of naloxone on long-term survival of experimental animals exposed to hypovolemic hypotension is not clear. The present studies tested the capacity of various doses of naloxone to protect conscious rats from mortality following various bleeding paradigms. In addition, the effect of morphine on survival of rats exposed to hemorrhage was also examined. In the six different experimental protocols tested, naloxone treatments failed to improve short- or long-term survival; in fact, naloxone treatment reduced short-term survival in two of the experimental protocols. Morphine injection, however, enhanced the mortality of rats exposed to hemorrhage in a dose-dependent manner. It is concluded that while opiates administered exogenously decrease survival after acute bleeding, naloxone has no protective action in such states and, like morphine, it may decrease survival in some situations.

**Key words:** shock, opioid peptides, hypovolemic hypotension

## INTRODUCTION

Opiates have been long known to affect the cardiovascular system [1,2]. The endogenous opioid system has also been implicated in cardiovascular regulation since a) opioid peptides and their receptors are found in cardiovascular nuclei in the central nervous system [3,4]; b) opioid peptides have pronounced effects on cardiorespiratory variables when administered centrally or peripherally [5-8]; c) stress responses that are accompanied by hemodynamic changes were shown to activate the endogenous opioid system as shown by analgesia and other behavioral changes [for review, see Holaday, 5].

Hemorrhage is another cardiovascular stress situation in which the endogenous opioid system has been stated to play a detrimental role since naloxone, a potent

Submitted for publication January 7, 1986.

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opiate antagonist, was shown to enhance cardiovascular recovery [9–15]. However, although many studies have been able to demonstrate a pressor response to naloxone in hypovolemic hypotension, only a few studies examined the effect of naloxone treatment on short-term survival. An increase in survival of naloxone-treated conscious rats bled to a fixed low blood pressure [9] or fixed volume withdrawal [15] was shown in two studies. An increase in survival rate in naloxone-treated animals was also found in anesthetized dogs exposed to hypovolemic hypotension and treated with naloxone [12,13] or the partial opiate antagonist/agonist nalbuphine [16]. However, Travero et al [17] failed to demonstrate any beneficial effect on short- or long-term survival in a swine model of hemorrhagic shock in which similar treatments of naloxone were given. Also, Gurll et al [18] failed to demonstrate any significant effect of naloxone on survival rate of the anesthetized cynomolgus monkeys exposed to hemorrhage.

Since the ultimate goal of treatment is to provide a superior long-term outcome from the original insult, surviving a hemorrhagic event is the ultimate end result of treatment. Unfortunately, very few studies have examined the effect of naloxone on the survival of animals in addition to studying hemodynamic, biochemical, and neuroendocrine responses; in only two studies, increased survival of conscious animals was reported [9,15]. In one of the reports [9] only one hemorrhagic paradigm was studied while in the other [15] no beneficial effect of naloxone was found in severe hemorrhage; moreover, in this later study [15] morphine also tended to increase survival of rats exposed to hemorrhage.

In view of the overall limited information on the effect of naloxone on the survival rate of conscious animals following hypovolemic hypotension, and the present controversy on the potential therapeutic value of naloxone in this and other shock situations, we decided to study the effect of naloxone on survival of conscious rats exposed to hemorrhage. A fixed volume bleeding approach was selected for these studies since such a bleeding better represents the nature of profuse bleeding resulting from injuries to civilian or military personnel in combat situations. Although this mode of bleeding results in some variability in systemic hypotension at the end of the bleeding in some species, it is not the case in the conscious rat where mean arterial blood pressure consistently drops to 40–45 mmHg [19]. In order to produce graded survival curves, a second bleeding event was introduced to the protocol, which resulted in LD<sub>30</sub>–LD<sub>100</sub>. The doses of naloxone selected for this study as well as the treatment schedules are based on previous studies [5,9, 15, 17]. In addition, the effect of morphine on the survival of conscious rats exposed to hemorrhage was also studied since no clear data exist on the survival of conscious rats after bleeding.

## MATERIALS AND METHODS

Male Sprague Dawley rats (250–300g) were purchased from Taconic Farms (Germantown, NY) and kept at 22°C and 12 hr/12 hr light/dark cycle. Rats were anesthetized with pentobarbitone (40 mg/kg, i.p.) and polyethylene catheters (PE 50) were inserted into the femoral artery and vein through a small incision in the groin. The catheters were then threaded under the back skin and exteriorized at the back of the neck; thereafter, the catheters were secured by a spring wire and an adhesive collar loosely attached around the neck. This method is a modified technique previously described by Chiueh and Kopin [20] and slightly modified as previously

described in detail [6]. Rats were allowed to recover from surgery for 24–36 hr with food and tap water *ad libitum*.

Hemorrhage was instituted by withdrawal of blood from the arterial line through a hand-driven syringe as described below for the various protocols. The shed blood was not replaced. Control animals received injections of normal (0.9%) saline at the same time points where naloxone was injected. A separate group of rats was always included with each different naloxone experiment. Naloxone (kindly provided by DuPont Pharmaceuticals, Wilmington, DE) was dissolved in 0.9% NaCl and injected as a bolus intravenously (0.2 ml), followed by 0.2 ml flush. The following experimental protocols were used:

1. Bleeding 9.5 ml/300 g body weight over a 5-min period; 5 min later, naloxone (10 mg/kg) was injected in a single bolus dose. Survival of rats was followed hourly up to 24 hr after the bleeding. This protocol represents a situation in which profuse bleeding over a short period of time results in only short-term survival for most of the animals. This model represents the clinical scenario of rapid but not immediately fatal hemorrhage.
2. Bleeding 8.5 ml/300g over a 5-min period; naloxone (2 mg/kg) was injected 30 min after the original bleeding. Ninety minutes after the first hemorrhage, a second bleeding of 2 ml/300g was added; 60 min later, a second injection of naloxone (2 mg/kg) was given. Survival rate was followed for 24 hr after the bleeding.
3. The same bleeding protocol was used as in protocol number 2 except for a 3-ml/300-g second bleeding and a third naloxone injection 120 min after the second one.
4. The same protocol of bleeding and naloxone injections as described in protocol 3 except 5 ml/300 g for the second bleeding.
5. Bleeding 8.5 ml/300 g followed by 1.5 ml/300 g 90 min later; naloxone (5 mg/kg) was injected 30 min after the first bleeding, followed by six consecutive injections of 2.5 mg/kg naloxone. Survival was monitored for 72 hr.
6. Bleeding was conducted as described in protocol 3. Naloxone treatment was started 30 min after the first bleeding as a 2- or 5-mg/kg bolus followed by six consecutive injections of naloxone, 1 mg/kg in the group that received a bolus of 2 mg/kg, and four consecutive additional injections in the group that received a 5-mg/kg bolus. Survival of rats was followed for 24 hr.
7. Bleeding was done as described in protocol number 2; morphine sulphate (dissolved in 0.9% NaCl) was administered 30 min after the first bleeding; two different doses of morphine (0.5 and 5 mg/kg) were tested in separate groups of rats. Control rats received an injection of 0.9% NaCl at the same time points of morphine injections to the treated group.

In all the experimental protocols, control animals received the same volume of 0.9% NaCl as naloxone-treated rats. Also, injections of saline or naloxone were done by a blinded procedure and survival of rats monitored by a technician unaware of the treatments.

## STATISTICS

The Fisher exact probability test was used to compare differences in survival rate of naloxone- and saline-treated rats.

## RESULTS

The different bleeding paradigms resulted in 35–100% mortality of the control rats (Fig. 1–6). The highest volume loss ( $8.5 \pm 5$  mg/300 g hemorrhage) had a LD<sub>100</sub>, while some variability existed in the mortality after the other bleeding schedules. Therefore, it is important to note that in each study, a separate control group was always exposed to bleeding concomitantly with the corresponding drug treated group.

The results of the various studies with naloxone are presented in Figures 1–5. In none of the experimental protocols did naloxone improve the survival rate of rats exposed to bleeding.

In protocol 1 and 5, naloxone-treated rats showed enhanced mortality that reached statistical significance; in protocol 3, a tendency of enhanced mortality in naloxone-treated rats was also observed.

In experiments in which a single dose of morphine was administered to rats 30 min after the first bleeding, the survival of rats was substantially reduced in a dose-

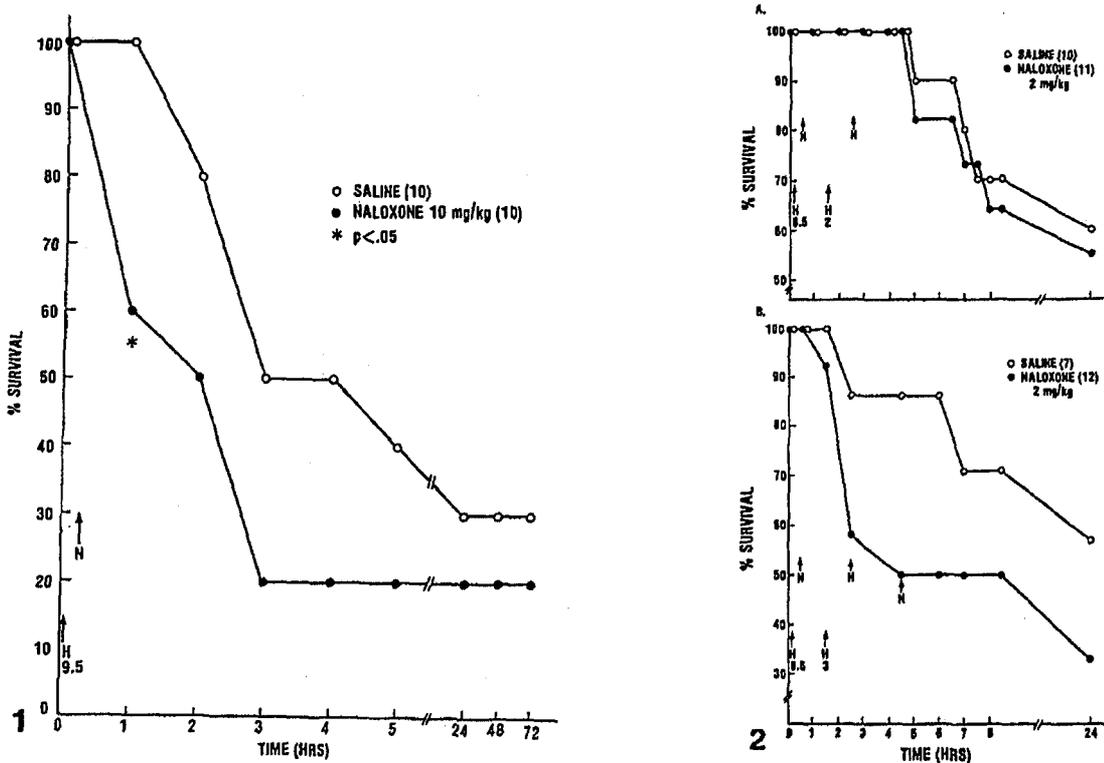


Fig. 1. Effect of naloxone on survival rate of rats exposed to hemorrhage of 9.5 ml/300 g. Percent survival is indicated in the ordinate. Time (hours) is indicated in the abscissa. ○, saline-treated hemorrhaged rats; ●, naloxone-treated rats. Time of bleeding and volume is indicated as ↑H; time of naloxone treatment is given as ↑N. Asterisks denote statistical significance by Fisher exact probability test. Number of rats in each group is given in parentheses.

Fig. 2. Effect of naloxone on survival of rats exposed to 8.5 ml/300 g hemorrhage followed by 2-ml (panel A) or 3 ml/300 g (panel B) bleeding. Percent survival is indicated in the ordinate. Time (hours) is indicated in the abscissa. ○, saline-treated hemorrhaged rats; ●, naloxone-treated rats. Time of bleeding and volume is indicated as ↑H; time of naloxone treatment is given as ↑N. Asterisks denote statistical significance by Fisher exact probability test. Number of rats in each group is given in parentheses.

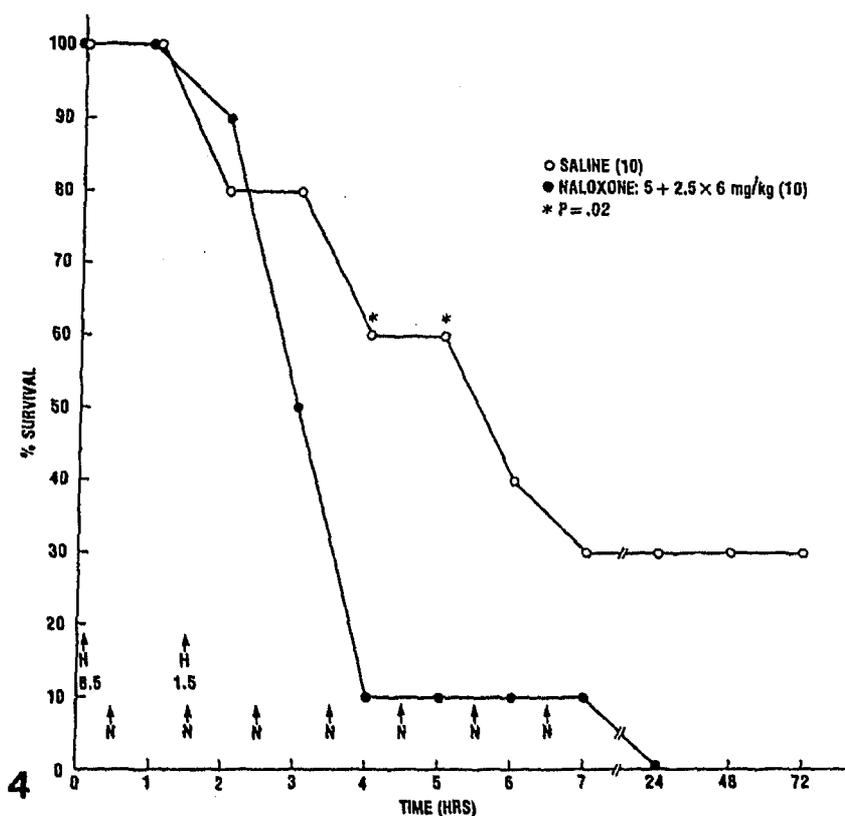
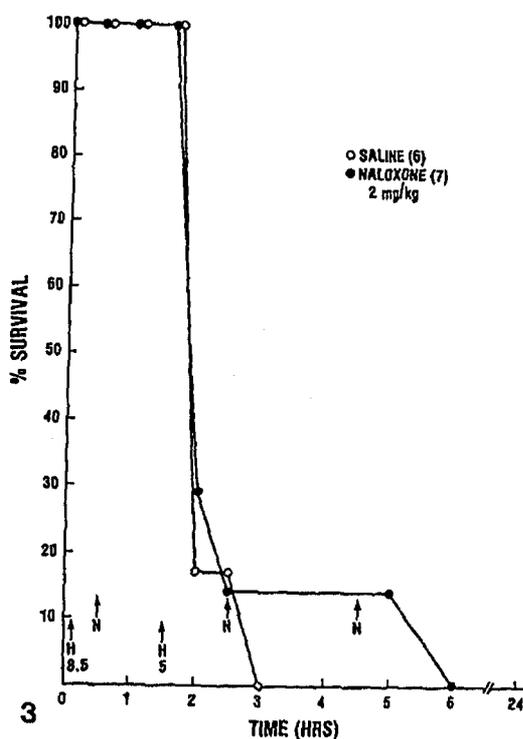


Fig. 3. Effect of naloxone on survival of rats exposed to 8.5 ml/300 g hemorrhage followed by 5 ml/300 g bleeding. Percent survival is indicated in the ordinate. Time (hours) is indicated in the abscissa. ○, saline-treated hemorrhaged rats; ●, naloxone-treated rats. Time of bleeding and volume is indicated as ↑H; time of naloxone treatment is given as ↑N. Asterisks denote statistical significance by Fisher exact probability test. Number of rats in each group is given in parentheses.

Fig. 4. Effect of naloxone on survival of rats exposed to 8.5 ml/300 g hemorrhage followed by 1.5 ml/300 g bleeding. Percent survival is indicated in the ordinate. Time (hours) is indicated in the abscissa. ○, saline-treated hemorrhaged rats; ●, naloxone-treated rats. Time of bleeding and volume is indicated as ↑H; time of naloxone treatment is given as ↑N. Asterisks denote statistical significance by Fisher exact probability test. Number of rats in each group is given in parentheses.

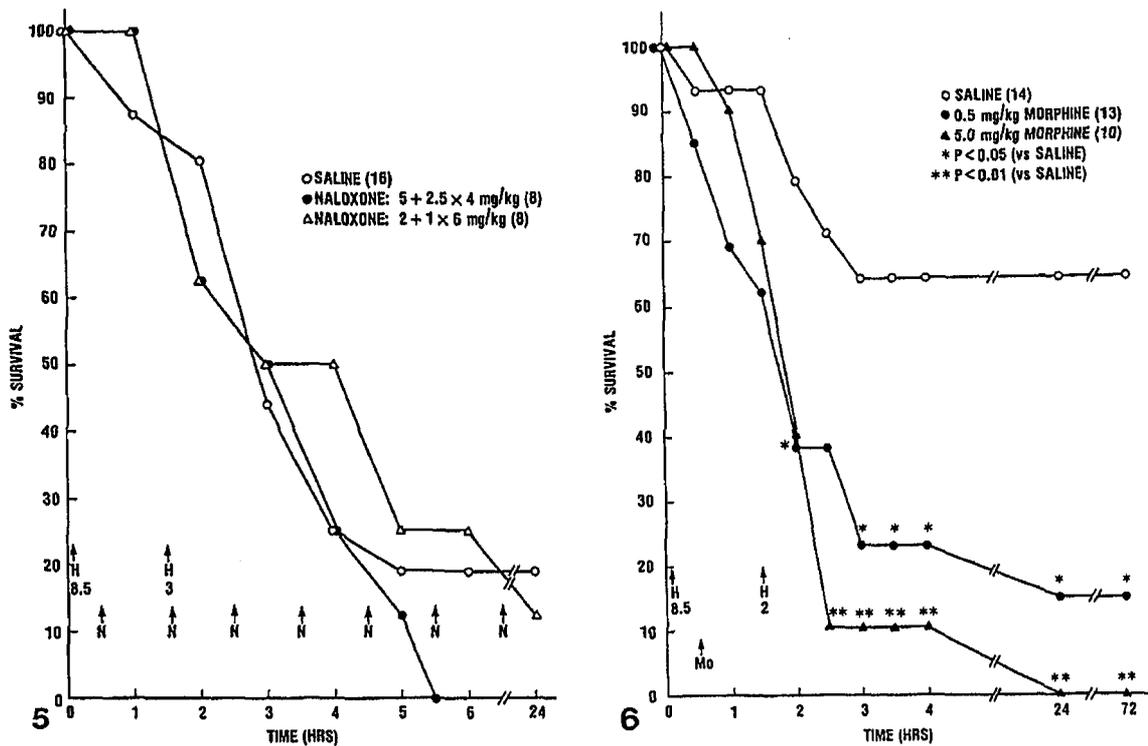


Fig. 5. Effect of naloxone on survival of rats exposed to 8.5 ml hemorrhage followed by 3 ml/300 g bleeding. Percent survival is indicated in the ordinate. Time (hours) is indicated in the abscissa.  $\circ$ , saline-treated hemorrhaged rats;  $\bullet$ , naloxone-treated rats. Time of bleeding and volume is indicated as  $\uparrow$ H; time of naloxone treatment is given as  $\uparrow$ N. Asterisks denote statistical significance by Fisher exact probability test. Number of rats in each group is given in parentheses.

Fig. 6. Effect of morphine on survival of rats exposed to 8.5 ml/300 g hemorrhage followed by 2 ml/300 g bleeding. Percent survival is indicated in the ordinate. Time (hours) is indicated in the abscissa. Symbols of the different groups are depicted in the figure. Time of bleeding and volume is indicated as  $\uparrow$ H. Asterisks denote statistical significance by Fisher exact probability test. Number of rats in each group is given in parentheses. Injection of a single dose of morphine, 0.5 or 5mg/kg, is denoted by  $\uparrow$ Mo.

related manner (Fig. 6.). The low dose of morphine reduced survival from approximately 60 to 15% while no survivors were found in the group of rats treated with the high dose of morphine (5 mg/kg).

It is also pertinent to note that in all the experiments, rats surviving 24 hr completely recovered.

## DISCUSSION

The results of the present studies clearly indicate that naloxone has no protective effect in rats exposed to fixed volume bleeding without reinfusion of the shed blood. In fact, enhanced mortality of naloxone-treated rats was found in two experiments (Figs. 1 and 4) while a tendency to reduce survival was seen in a third experiment (Fig. 2B). A decrease in average survival time after naloxone treatment was also reported in fixed volume bleeding in the swine [17]. The range of naloxone doses used in this study is in the same range used in several previous studies in which naloxone's effect on survival was studied [9,13,14,15]. The major difference of the present study from that by Faden and Holaday [9] is the mode of bleeding; in the

present study, the hemorrhage was done by volume while in the latter study, bleeding was aimed at producing a constant low blood pressure over a given period of time. However, the amount of shed blood reported in the latter study [9] was in the same range of the bleeding produced in the present study. Nevertheless, our data also vary from those by Isoyama et al [15], who used a volume-hemorrhage similar to that in the present study. However, even in the latter study [15] naloxone failed to improve survival rate in rats exposed to bleeding of an LD<sub>80</sub> and had a protective effect only in rats in which bleeding resulted in LD<sub>40</sub>. Although in the fixed-volume hemorrhage, the state of shock is less apparent when monitored by systemic hemodynamic variables, it has the advantage of being more relevant to human situations, eg, combat injuries [21] and civilian accidents [22] in which death results within a few hours after the bleeding.

Our study also shows that morphine administration to rats exposed to oligemia is definitely a detrimental pharmacological intervention. These results are different from a previous report [15] in which the same dose of morphine (0.5 mg/kg) was administered to rats exposed to hemorrhage. Bleeding procedures and anesthesia are possible factors for the discrepancy between the two studies, which were conducted in the same species using similar doses of morphine. In the previous study [15], bleeding was done while the animals were still anesthetized with pentobarbitone; moreover, the bleeding was done over 60 seconds in which almost 40% of the blood was shed. In our study, the bleeding was done over 5 min, which allowed a larger volume of bleeding both during the first and second hemorrhage to achieve comparable mortality rates. In addition, the study by Isoyama et al [15] differs from that by Faden and Holaday [9] by the lack of a pressor response to naloxone in either the moderate (LD<sub>40</sub>) or severe (LD<sub>80</sub>) hemorrhage experiments.

Although the mechanisms involved in the morphine enhancement of mortality rate were not studied, it is reasonable to propose that respiratory depression leading to hypoxemia and acidosis might have played a major role. This suggestion is supported by the known effects of opiates (eg, morphine) and opioid peptides that are  $\mu$ -agonists [23,24]. Other mechanisms, such as reduced blood flow to essential organs, were also suggested [25]. In conclusion, the studies reported in this paper failed to support previous suggestions that naloxone exerts a protective effect in severe oligemia. However, our studies suggest that exogenous opiates like morphine have a detrimental effect on survival in hemorrhagic shock.

## ACKNOWLEDGMENTS

This work was supported in part by USUHS Protocol #R09211. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the view of the Department of Defense or the Uniformed Services University of the Health Sciences. The authors are thankful to Rhoda Press and Elizabeth Powell for their excellent technical assistance and Wanda Patterson and Heidi Hatton for preparing the manuscript.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. NIH 78-23, 1978).