PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **184,** 267–277 (1987)

Naloxone Pretreatment Prevents the Bloody Diarrhea of Canine Endotoxic Shock (42478)

ERIC GANES, NELSON J. GURLL, AND DAVID G. REYNOLDS

Surgical Service, Iowa City Veterans Administration Medical Center and Department of Surgery, University of Iowa College of Medicine, Iowa City, Iowa 52242

Abstract. We examined the importance of timing with endorphin involvement in shock by giving the opiate receptor antagonist naloxone as a pretreatment in canine endotoxic shock. Dogs anesthetized with pentobarbital (30 mg/kg iv) were given *Escherichia coli* endotoxin at LD_{80} doses iv. Naloxone (2 mg/kg plus 2 mg/kg/hr iv, $N = 10$) started 15 min before endotoxin attenuated the fall in mean arterial pressure, cardiac index, and the first derivative of left ventricular pressure due to endotoxin in comparison with control animals given 0.9% NaCl ($N = 10$). Naloxone attenuated the endotoxin-induced decrease in superior mesenteric arterial blood flow and the increases in portal venous pressure and pulmonary arterial pressures. Moreover, naloxone pretreatment prevented the characteristic bloody diarrhea and reduced mortality. Our findings implicate endorphins acting on opiate receptors as important mediators of endotoxin-induced cardiovascular failure and bloody diarrhea in canine endotoxemia. These are early manifestations and dictate expeditious use of naloxone in endotoxic shock.

In 1978 and 1979 Holaday and Faden suggested that endorphins (endogenous morphinelike substances) might be involved in the pathophysiology of shock since the opiate receptor antagonist naloxone increases systemic arterial pressures (both mean and pulse) in rodent hemorrhagic and endotoxic shock (1, 2). They also intimated that opiate receptor antagonists might prove beneficial in the treatment of shock although survival was not improved in their animal model of endotoxic shock. In 1980 we reported that naloxone administration improved cardiovascular function (mean arterial pressure, cardiac output, and myocardial performance) as well as survival in a canine model of endotoxic shock (3). These initial reports have been substantiated by the work of others showing improved cardiovascular function in canine endotoxic shock following the administration of opiate receptor antagonists (4–7). Since naloxone treatment without induction of shock had minimal cardiovascular effects, endogenous substances need to be activated in shock in order for naloxone to work (1, 3); the most likely candidates for these substances are endorphins.

The endorphins, moreover, would appear to play a role early in the cardiovascular pathophysiology of endotoxic shock since mean arterial pressure responses were inversely proportional to the duration of time naloxone was given after endotoxin (8). Naloxone pretreatment studies therefore were indicated. Naloxone was given as pretreatment in some earlier studies (1, 6, 7), but these were limited in either cardiovascular information, because of the small size of the experimental animals used (1), or survival data (6, 7). We studied naloxone pretreatment in canine endotoxic shock in order to clarify its effects on survival and cardiovascular function. The finding that naloxone prevented bloody diarrhea was fortuitous.

Materials and Methods. *Animal preparation.* Ten adult mongrel dogs of either sex weighing 19.0 ± 0.7 kg (mean ± standard error of the mean (SEM)) were anesthetized with sodium pentobarbital, 30 mg/kg iv, intubated with a soft cuffed endotracheal tube, and allowed to breathe room air spontaneously. Body temperature was kept at 37 ± 2°C with a heating pad. Bilateral groin incisions were made and femoral vessels isolated. Large polyethylene catheters were placed in the right femoral vein for drug infusions and in the aorta via the right femoral artery to monitor mean arterial pressure (MAP, mm Hg). A triple lumen Swan–Ganz catheter equipped with a thermistor tip (Model 93A 131 7F, Edwards Laboratories, Santa Ana, CA) was inserted into the left femoral vein and advanced into the

0037-9727/87 $1.50

pulmonary artery to record pulmonary arterial pressures, including mean (MPAP, mm Hg) and wedged (PAWP, mm Hg). Cardiac output was determined in triplicate by the thermodilution technique using a cardiac output computer (Model 9520A, Edwards Laboratories). A pigtail catheter was passed via the left femoral artery into the left ventricle to record left ventricular pressure (LVP, mm Hg). This signal was processed by a differentiating circuit incorporated in the carrier preamplifier to obtain the first derivative of the left ventricular pressure over time, the maximum value of which (LV dP/dt max, mm Hg $\cdot 10^3$/sec) is an indicator of myocardial performance. Heart rate (HR, beats/min) was recorded from lead II of the ECG signal via a cardiotachometer. All pressure catheters were connected to a physiological recorder with pressure transducers (P23 ID, Statham Gould, Hato Rey, Puerto Rico) which were calibrated manometrically each day. All cardiovascular parameters were monitored simultaneously on an eight-channel physiological recorder (Model R612, Beckman Instruments, Schiller Park, IL). Standard formulae were used to calculate cardiac index (CO, ml/min/kg), stroke volume (SV), and vascular resistances, including total peripheral (TPR), mesenteric, and pulmonary.

Another 10 dogs weighing 18.8 ± 0.6 kg were instrumented as above. In addition, portal venous pressure (PVP, mm Hg) was measured via a polyethylene catheter placed via a mesenteric branch, and superior mesenteric arterial blood flow (SMAQ, ml/min) was determined from a flowprobe on the artery and connected to an electromagnetic flowmeter (Model BL-610, Biotronex Laboratory, Kensington, MD).

Conduct of the experiment. After instrumentation, porcine sodium heparin, 250 units/kg, was given iv and baseline readings were taken. After 30 min of stabilization naloxone was given as a bolus at 2 mg/kg iv ($t = 0$) followed by a continuous iv infusion at 2 mg/kg/hr for 2 hr. Control animals received 0.9% sodium chloride iv in equivalent volumes. *Escherichia coli* endotoxin (Lot 680518, Difco, Detroit, MI), 1.5 mg/kg, was given as an iv bolus at $t = 15$ min. In the animals having measurements of PVP and SMAQ, *E. coli* endotoxin (Lot 649448), 0.1 mg/kg, was given as an iv bolus at $t = 15$ min; this is the same lot and dose used by us previously (3). These doses are lethal for 80% of the animals at 24 hr (LD_{80}) for the two batches respectively based on pilot studies. Thirty minutes after the infusion of naloxone or 0.9% NaCl was stopped the catheters were withdrawn, the vessels were ligated, the incisions were closed, and the animals were returned to their cages. Survival was noted at $t = 150$ min, 24 hr, and 72 hr. Animals surviving 72 hr were sacrificed with a lethal dose of sodium pentobarbital.

Blood samples. Blood was obtained periodically for determination of serum glucose by the glucose oxidase method and arterial pH and blood gases (Radiometer Corporation, Copenhagen, Denmark).

Statistics. The results are shown as means ± SEM. The data were evaluated for statistical significance using repeated measurements analysis of variance. In certain instances a split-plot analysis of covariance was used to adjust for possible differences before treatment. Multiple comparisons were performed using the Bonferroni method accepting as significant $P < 0.05/k$ where k is the number of tests performed (9). Data on survival and the presence of bloody diarrhea were evaluated by Fisher's exact test.

Results. *Hemodynamics, 1.5 mg/kg endotoxin.* There were no differences in any parameter between the two groups during the baseline stabilization period. MAP (Fig. 1), LV dP/dt max (Fig. 2), and CO (Fig. 3) rose slightly from $t = 0$ to $t = 15$ min in response to naloxone. The group × time interaction was highly significant only for MAP over this time period ($F(3,24) = 5.23$, $P = 0.006$). This indicates the group effect is not constant over time. Multiple comparisons demonstrated significantly higher MAP in the naloxone group at 5, 10, and 15 min.

Saline-treated animals experienced a fall in MAP, CO, and LV dP/dt max after endotoxin. There was a shorter fall in MAP due to endotoxin in the naloxone-pretreated group (152 ± 8 to 99 ± 11 mm Hg or 35%, Fig. 1) compared to the saline-pretreated controls (140 ± 4 to 51 ± 5 mm Hg or 64%). There was a significant group × time interaction for MAP over $t = 20$ to 150 min ($F(10,73) = 3.29$, $P = 0.002$) which was predominantly due to changes before 45 min since the group × time interaction was not significant at $t = 45$ to 150

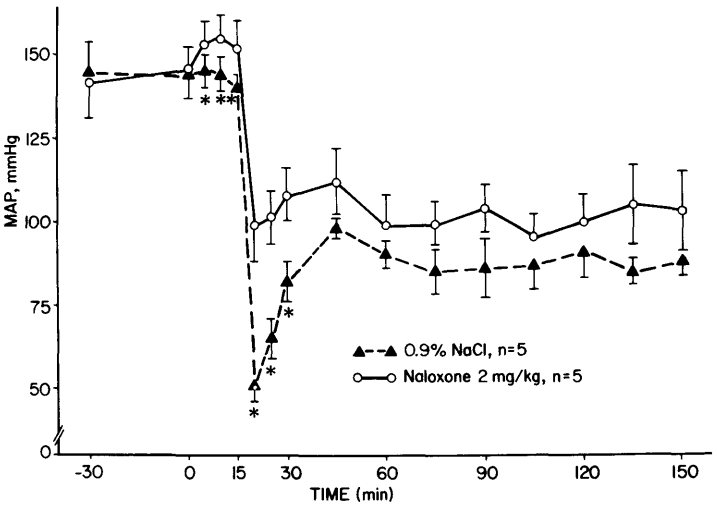

FIG. 1. Naloxone pretreatment at $t = 0$ raises mean arterial pressure (MAP, mm Hg) and attenuates its fall after *E. coli* endotoxin, 1.5 mg/kg iv injection (at $t = 15$ min), in dogs. Values are shown as means ± SEM. $^*P < 0.05$, NaCl cf. naloxone.

min. The MAP response to naloxone is significantly higher than that due to saline at $t = 20$, 25, and 30 min, after which time the curves are no longer different. Over $t = 20$ to 150 there was a significant group main effect of naloxone on MAP ($F(1,8) = 5.8$, $P = 0.043$); however, when adjusted for differences at $t = 15$, the naloxone effect was no longer significant ($F(1,7) = 2.07$, $P = 0.194$). Thus the group effect difference was due to changes before $t = 15$.

The LV dP/dt max response to naloxone was similar to the MAP response in that naloxone attenuated its fall (from 2.08 ± 0.19 to 0.75 ± 0.06 mm Hg · 10^3/sec or 64% in controls compared with a 45% fall from 2.53 ± 0.35 to 1.38 ± 0.18 with naloxone, Fig. 2). The groups were also different over $t = 20$ to

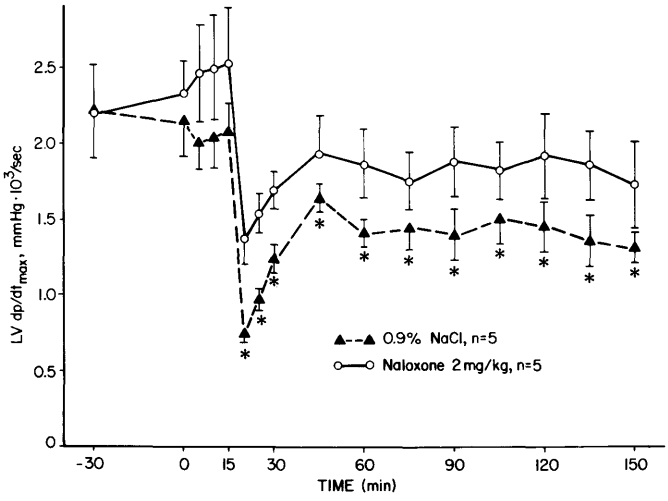

FIG. 2. Naloxone pretreatment blunts the fall in left ventricular performance (LV dP/dt max, mm Hg · 10^3/sec) due to endotoxin, 1.5 mg/kg iv. $^*P < 0.05$, NaCl cf. naloxone.

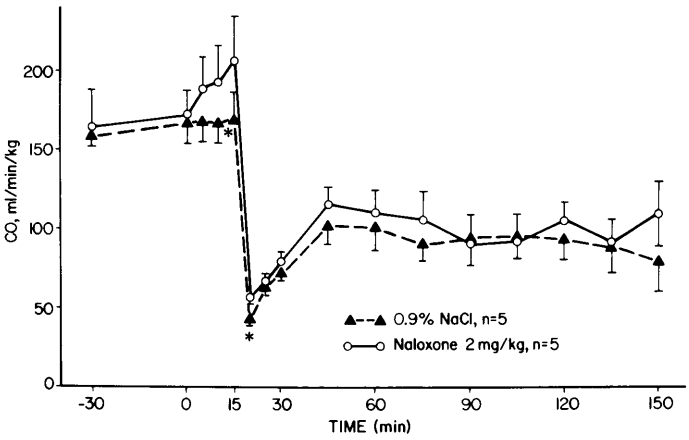

FIG. 3. Naloxone pretreatment has no significant effect on cardiac index (CO, ml/min/kg) responses to endotoxin, 1.5 mg/kg iv. $*P < 0.05$, NaCl cf. naloxone.

150 min in this parameter ($F(1,8) = 5.13$, $P = 0.053$). This borderline difference in group main effect is lost when adjusted for differences at 15 min. The CO response differs from that of MAP and LV dP/dt max. In both saline- and naloxone-pretreated groups CO fell similarly (169 ± 20 to 43 ± 2 ml/min/kg or 75% in controls vs 206 ± 28 to 57 ± 4 or 72% with naloxone, Fig. 3). A significant difference between the groups in CO was evident only at $t = 15$ and $t = 20$ min, after which time there were no longer any significant differences.

HR was depressed with endotoxin by a similar magnitude in both groups of animals. TPR increased slightly after endotoxin administration which was accentuated by naloxone pretreatment to levels significantly above controls at $t = 20$, 25, and 30 min. SV changed in a way similar to CO. Pulmonary arterial pressure responses to endotoxin were somewhat unusual in that MPAP and PAWP did not increase. There was a slight fall in MPAP and PAWP over time after endotoxin but there were no significant differences between controls and naloxone-pretreated animals.

Hemodynamics, 0.1 mg/kg endotoxin. Naloxone increased MAP, CO, and LV dP/dt max slightly as seen before (Figs. 1–3). Decreases in these parameters after endotoxin were not quite as dramatic as after 1.5 mg/kg endotoxin but were similar quantitatively to what we have reported previously with 0.1 mg/kg of this batch of endotoxin (3). Naloxone attenuated the fall in MAP (5% versus 16% in controls), CO (21% cf. 41%, Fig. 4), and LV dP/dt max (6% increase versus a 16% fall). MAP was higher in those animals pretreated with naloxone compared to those pretreated with saline over $t = 20$ to 75 min ($F(1,8) = 5.14$, $P = 0.0531$). CO was also higher with naloxone over this same time period, but this difference was not statistically significant ($F(1,8) = 3.04$, $P = 0.1194$). Naloxone pretreatment resulted in significantly higher LV dP/dt than saline over $t = 20$ to 75 min ($F(1,8) = 8.13$, $P = 0.0214$) and $t = 90$ to 150 min ($F(1,8) = 7.32$, $P = 0.0269$). MAP and LV dP/dt max did not recover over 45 min as they had after 1.5 mg/kg endotoxin (Figs. 1 and 2). Consequently, these were higher in the naloxone group than controls after $t = 60$. CO was better maintained with naloxone than with saline (Fig. 4).

There were other differences in the cardiovascular responses to naloxone and endotoxin, depending on the dose and lot of endotoxin used. HR did not fall with 0.1 mg/kg as it had after 1.5 mg/kg; as with the other dose, there were no differences between the naloxone and saline groups. The increase in TPR with endotoxin was accentuated by saline and not by naloxone, just the reverse of what we had observed with the 1.5 mg/kg dose of endotoxin.

Naloxone blocked the rise in PAWP and attenuated the early rise and prolonged depression in MPAP after endotoxin (Fig. 5). Although the rise in pulmonary vascular resistance due to endotoxin was less with nal-

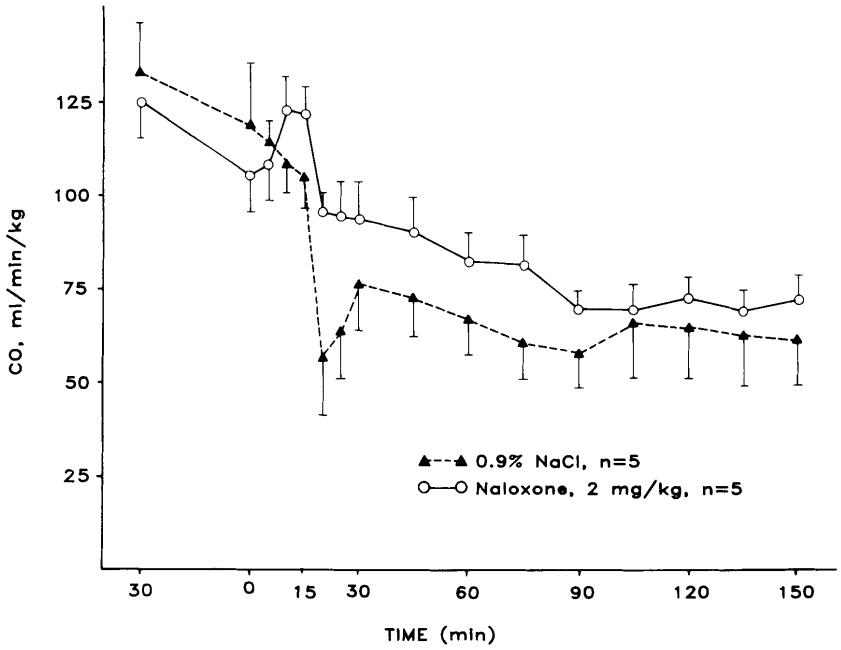

FIG. 4. Naloxone pretreatment blocks the fall in cardiac index due to *E. coli* endotoxin, 0.1 mg/kg iv.

oxone than with saline pretreatment, these differences were not significant due to large variances. SMAQ was better maintained with naloxone than with saline after a transient decrease (Fig. 6). Naloxone also blunted the increase in portal venous pressure after endotoxin so that the peak pressure was less (17 ± 1 versus 21 ± 1 mm Hg, Fig. 7). Mesenteric

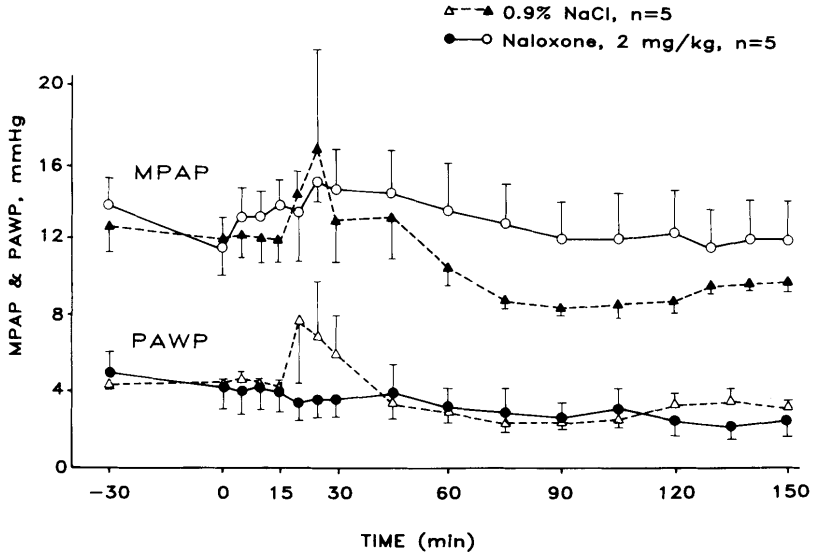

FIG. 5. Naloxone pretreatment blunts the increase in pulmonary arterial wedge pressure (PAWP, mm Hg) and the early rise and late fall in mean pulmonary arterial pressure (MPAP, mm Hg) due to endotoxin, 0.1 mg/kg iv.

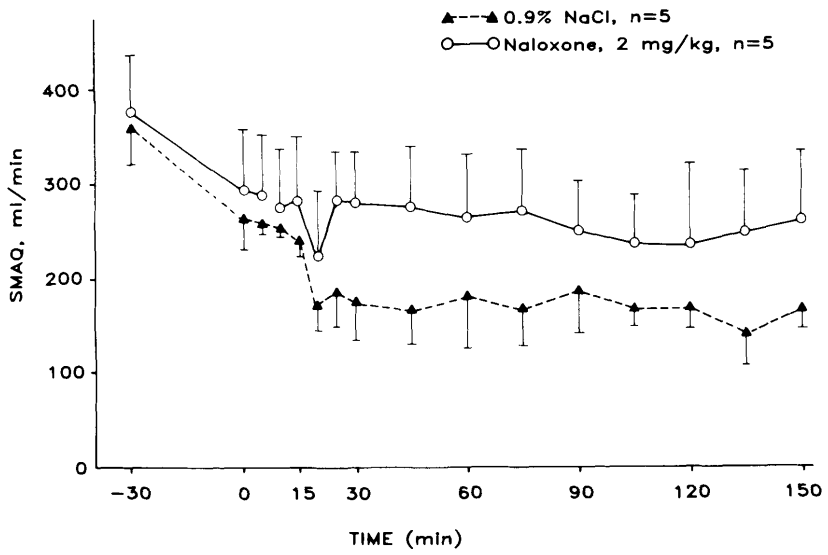

FIG. 6. Naloxone pretreatment better maintains superior mesenteric arterial blood flow (SMAQ, ml/min) after endotoxin, 0.1 mg/kg iv.

vascular resistance rose after endotoxin similarly in both naloxone and saline groups.

Survival. One dog in each group died about 2 hr after 1.5 mg/kg endotoxin. The naloxone pretreatment reduced lethality at both 24 and 72 hr with this batch of endotoxin from 80 to 20% ($P = 0.09$). Naloxone improved 24-hr survival from 0 to 60% in animals receiving 0.1 mg/kg endotoxin ($P = 0.08$). Moreover, only 1 of 10 naloxone pretreated dogs developed the bloody diarrhea which was found in all 10 control dogs ($P = 0.00006$).

Metabolic changes. Naloxone pretreatment resulted in significantly less acidosis than con-

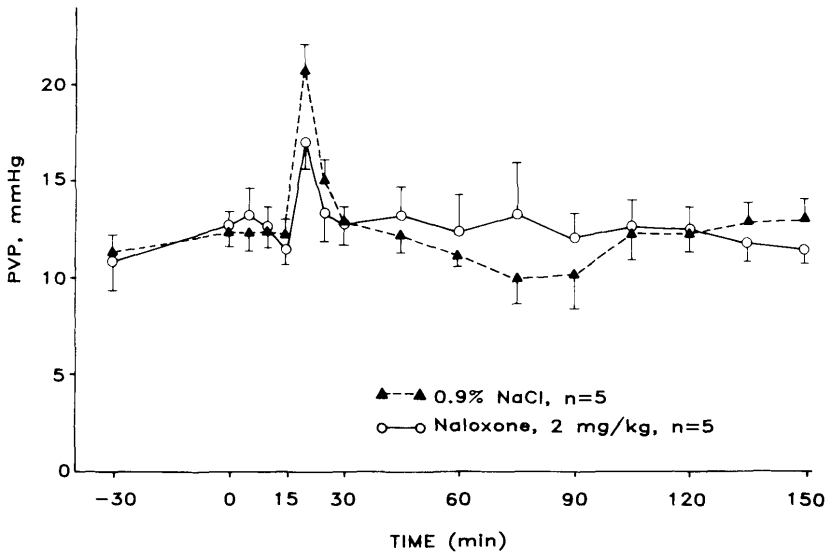

FIG. 7. Naloxone pretreatment blunts the increase in portal venous pressure (PVP, mm Hg) after endotoxin, 0.1 mg/kg iv.

TABLE I. ARTERIAL BLOOD GASES, pH, Hct, AND GLUCOSE RESPONSES TO NALOXONE PRETREATMENT IN CANINE ENDOTOXIC SHOCK (1.5 mg/kg *E. coli* ENDOTOXIN)

			Time (min)			
		Baseline	15	30	90	150
pH (units)	Saline	7.30 ± .02	7.33 ± .03	7.17 ± .03	7.20 ± .05	7.23 ± .05
	Naloxone	7.33 ± .03	7.35 ± .03	7.29 ± .03*	7.29 ± .02	7.32 ± .03
PO_2 (mm Hg)	Saline	83 ± 3	82 ± 4	82 ± 6	79 ± 8	85 ± 6
	Naloxone	85 ± 2	93 ± 3	92 ± 4	93 ± 7	102 ± 2
PCO_2 (mm Hg)	Saline	47 ± 4	44 ± 3	43 ± 4	41 ± 7	33 ± 3
	Naloxone	47 ± 2	40 ± 4	31 ± 2*	25 ± 4	28 ± 2
BE (mEq/liter)	Saline	−2 ± 1	−1 ± 2	−8 ± 1	−9 ± 1	−10 ± 2
	Naloxone	−2 ± 2	−2 ± 1	−10 ± 1	−11 ± 1	−10 ± 2
Hct (%)	Saline	40 ± 3	41 ± 3	51 ± 5	55 ± 4	53 ± 6
	Naloxone	42 ± 2	46 ± 2	50 ± 4	52 ± 3	56 ± 3
Glucose (mg/dl)	Saline	108 ± 5	112 ± 5	195 ± 24	114 ± 8	107 ± 9
	Naloxone	102 ± 10	117 ± 16	162 ± 23	114 ± 21	97 ± 12

Note. Values are means ± SEM. BE, base excess.
* $P < 0.02$ versus saline.

trols which became manifest after endotoxin injection (Tables I and II). Arterial PO_2 was slightly higher and PCO_2 slightly lower with naloxone, results which were more pronounced after 1.5 mg/kg endotoxin (Table I). Tachypnea due to endotoxin was slightly less with naloxone, but this difference was not statistically significant. There were no differences between saline and naloxone pretreatment in serial hematocrit, serum glucose, and calculated base excess.

Discussion. In contrast to our previous report using naloxone after endotoxin (3), naloxone pretreatment prevented the development of the bloody diarrhea typical of canine endotoxemia (10, 11). The dogs were sacrificed 24 hr after the shock period in our previous study for humane reasons because they all developed bloody diarrhea. Even so, survival rate at 24 hr was significantly enhanced by naloxone (3). Simultaneous administration of naloxone and endotoxin also leads to a higher

TABLE II. ARTERIAL BLOOD GASES, pH, AND Hct RESPONSES TO NALOXONE PRETREATMENT IN CANINE ENDOTOXIC SHOCK (0.1 mg/kg *E. coli* ENDOTOXIN)

			Time (min)			
		Baseline	0	30	60	90
pH (units)	Saline	7.29 ± .03	7.32 ± .02	7.32 ± .02	7.25 ± .03	7.25 ± .06
	Naloxone	7.36 ± .01	7.37 ± .04	7.36 ± .03	7.36 ± .04**	7.33 ± .01
PO_2 (mm Hg)	Saline	79 ± 4	83 ± 4	80 ± 6	86 ± 6	76 ± 7
	Naloxone	84 ± 3	83 ± 6	80 ± 6	88 ± 3	92 ± 7
PCO_2 (mm Hg)	Saline	41 ± 1	36 ± 1	35 ± 1	32 ± 1	35 ± 3
	Naloxone	43 ± 3	36 ± 2	37 ± 2	34 ± 2	29 ± 1
Hct (%)	Saline	47 ± 2	47 ± 2	49 ± 2	52 ± 2	54 ± 2
	Naloxone	49 ± 3	52 ± 3	52 ± 3	53 ± 3	49 ± 1

** $P < 0.05$ versus saline.
Values are means ± SEM.

survival rate and a prolonged survival time as shown by Raymond *et al.* (4). The 2 animals surviving 96 hr in their study, moreover, demonstrated no gross pathological changes in the hepatosplanchnic organs, kidney, heart, lungs or brain. Typical bloody diarrhea, however, developed in all of the animals dying before 96 hr regardless of treatment (11 controls and 9 treated with naloxone). The better protection from bloody diarrhea in our study might be due to any number of differences between our studies and Raymond's: timing of treatment (as we would argue based on our studies), dosage and batches of endotoxin (vide infra), or presence of anesthesia, which is important in shock and in endorphin systems (12). Data about bloody diarrhea and survival also have been obtained in a feline model. There was no significant difference in 48-hr survival (1/5 controls, 3/5 treated) or in the occurrence of bloody diarrhea (3/5 controls, 2/5 treated) when naloxone was given as a bolus to cats in shock 30 min after injection of endotoxin (13).

To our knowledge this finding (prevention of bloody diarrhea by naloxone) is unique. The bloody diarrhea is chiefly a problem with canine models, and one should not extrapolate this information to primates and humans. However, we are in a good position to speculate about the role of pressure and flow in the splanchnic circulation in its pathogenesis. Naloxone had no effect on portal venous pressure and superior mesenteric arterial flow when given after endotoxin (3). On the other hand, naloxone pretreatment blunted some of these responses to endotoxin so that the portal hypertension was less and mesenteric blood flow was better maintained. Exactly how naloxone modifies the endotoxin-induced hepatic venous constriction, the supposed mechanism for splanchnic congestion and bloody diarrhea (11), must remain conjectural at this point in our understanding. However, a variety of mechanisms are possible since endorphins interact with many biochemical and physiological systems (14).

The major hemodynamic finding of this study is that naloxone pretreatment attenuates the early fall in MAP, CO, and LV dP/dt max after endotoxin. We found even better cardiovascular responses when naloxone was given after the endotoxin (3) (Table III). These data again are consistent with the hypothesis that endorphins act on opiate receptors to bring about the cardiovascular consequences of endotoxic shock (1, 3). The criteria posed as crucial for the implication of endorphins and opiate receptors in the pathophysiology of any

TABLE III. SUMMARY OF EFFECTS WITH NALOXONE (N) GIVEN BEFORE (ā) OR AFTER (p̄) *E. coli* ENDOTOXIN (E AT DOSES INDICATED IN mg/kg)

	Previous study (3)		This study			
	E 0.1	N p̄	E 0.1	N ā	E 1.5	N ā
MAP	↓↓	++	↓↓	+	↓↓	+
CO	↓↓	++	↓↓	++	↓↓↓	0
LV dP/dt max	↓↓	++	↓↓	+	↓↓	+
SMAQ	↓↓	0, ±	↓↓	++		
PVP	↑↑↑↑	0, ±	↑↑↑↑	++		
PAWP						
15 min p̄ E			↑↑↑	+++	0	0
Later	↓	0	0		↓	0
MPAP						
15 mm p̄ E	↑↑	0, ±	↑↑	+	0	0
Later	↓	0	↓↓	++	↓	0
Bloody diarrhea	↑↑↑↑	0	↑↑↑↑	++++	↑↑↑↑	++++
Survival						
24 hr	↓↓↓	++	↓↓↓↓	++	↓↓↓	++
72 hr					↓↓↓	++

Note. Direction of change indicated by arrows; attenuation of effect by naloxone shown by + signs; magnitude of change relative to baseline is given by number of arrows and + signs (each worth ~ 25%). 0, no effect; ±, trend toward effect.

condition largely have been satisfied in shock (reviewed in (14)).

Significant effects on hemodynamics, metabolism, and survival have been found with naloxone given before (1, 6, 7), with (4), or after (1, 3, 5) endotoxin. Holaday and Faden were able to attenuate the hypotension, early bradycardia, and subsequent tachycardia due to endotoxin by naloxone pretreatment, although their major emphasis was on naloxone treatment after endotoxin (1). Survival was not prolonged by naloxone in these conscious rats. However, they were unable to evaluate CO or myocardial contractility. Three canine studies are pertinent to our results. Brüchner *et al.* blocked or attenuated the decreases in cardiac index, maximal left ventricular d*P*/d*t*, myocardial oxygen consumption, and efficiency of cardiac work as well as arterial pressure after 6 mg/kg endotoxin by pretreating anesthetized dogs with 10 mg/kg naloxone (6). Bone *et al.* blocked the fall in cardiac index and blood pressure due to 2 mg/kg endotoxin by pretreatment with naloxone 10 mg/kg 15 min before hand (7). Their results, however, are not strictly applicable to a discussion of pretreatment since they also gave naloxone, 5 mg/kg, 10 and 60 min after endotoxin. Unfortunately, neither Brüchner nor Bone reported survival results. Raymond *et al.* injected 10 mg/kg naloxone and 2 mg/kg endotoxin simultaneously (4). They showed improved hemodynamics and metabolic function in anesthetized dogs and enhanced survival in unanesthetized dogs. Thijs *et al.* (5) and we (3) have shown improved cardiovascular responses (MAP, CO, and LV d*P*/d*t* max or LV stroke work) when naloxone treatment (2 mg/kg) has been delayed after endotoxin somewhat (90 min by Thijs, 15 min by us). In addition, we have increased survival rate (converting a LD_{80} model to LD_{20}) by giving naloxone 15 min after endotoxin (3). Similar attenuation of mortality was seen in the present study. Beneficial effects of naloxone on acid-base balance resulting in less acidosis have been observed previously (3, 4). On the basis of these studies, it would be difficult to sort out which timing is best because of differences in animal models, doses of endotoxin, and the presence and type of anesthesia. The apparent advantage of pretreatment ((1, 8), this study), moreover, may not be practical in the clinical situation.

Timing of treatment with naloxone does make a difference in the cardiovascular responses as well. If one uses the statistical corrections for differences in MAP and LV d*P*/d*t* max due to naloxone pretreatment, then these cardiovascular parameters are not significantly different (after endotoxin injection) between controls and naloxone treatment. Therefore, the differences that make a difference (in survival and prevention of bloody diarrhea) are manifested early. Of course, we don't know that these early cardiovascular responses are responsible for the enhanced survival and prevention of bloody diarrhea; they may only be associated findings. In rats given endotoxin earlier treatment with naloxone resulted in better cardiovascular responses, and the increase in MAP was inversely proportional to the time delay between endotoxin and naloxone injections (8). On the other hand, even rats with naloxone-induced increases in MAP still died. Survival in our experiments was enhanced significantly even when bloody diarrhea was not prevented (naloxone treatment after endotoxin, Table III) or when cardiovascular effects were only moderate and bloody diarrhea was prevented (naloxone pretreatment before 1.5 mg/kg endotoxin, Table III). Clearly, the relationships between improved cardiovascular function and survival, and even more particularly the development of bloody diarrhea, are not established.

We have argued previously that factors other than hemodynamics are important determinants of survival since naloxone at 1 mg/kg plus 1 mg/kg/hr failed to improve survival although it did have virtually the same cardiovascular responses as naloxone at 2 mg/kg plus 2 mg/kg/hr which did improve survival (3). Similarly, Faden and Holaday thought that factors other than blood pressure led to enhanced survival since they found that naloxone-treated rats had a higher blood pressure than saline-treated control rats 5 min before both groups died (8). These factors have been reviewed (14) but are outside the scope of this discussion.

Timing of naloxone vis-à-vis endotoxin is also important in pulmonary responses. The peak MPAP and PAWP responses to endotoxin were blunted with naloxone as a pretreatment whereas naloxone after endotoxin

(3) had only a mild effect on MPAP (and none on PAWP, since it did not increase, perhaps because of a concomitant fall in CO). Almgvist *et al.* showed that naloxone treatment decreases platelet trapping in the lung due to endotoxin and that this effect was even more pronounced with naloxone pretreatment (15). The characteristic pulmonary changes in awake sheep injected with endotoxin are in two phases: an early increase in protein-poor lung lymph with a marked rise in pulmonary artery pressure and a delayed phase of increased pulmonary lymph flow with a higher lymph protein content (16). Naloxone given 30 min before endotoxin attenuates these responses (16), but delay in treatment for 1 hr after endotoxin has no effect (17). These authors conclude, since pretreatment is effective and post-treatment not, that an opiatelike substance might be released by endotoxin which results in the release of some other mediator responsible for lesions associated with endotoxic shock. Such an argument could be used to explain differences (pretreatment, working; post-treatment, not working) in cardiovascular (mesenteric and pulmonary) responses and bloody diarrhea that we have reported.

The exact mechanism of naloxone's protection from bloody diarrhea in endotoxemia probably does not involve opiate receptors or endorphins directly since it is not protective given after endotoxin when plasma levels of endorphins are elevated (5, 7). Naloxone also has been shown to (i) stabilize lysosomal membranes, (ii) inhibit proteolysis (18), and (iii) act as a lipid antioxidant (19). Any combination of these properties might be responsible for the protection afforded by minimizing damage to cellular membranes and maintaining vascular integrity, although the concentrations required *in vitro* to demonstrate these effects are much higher (100–200 μg/ml for the first two; 165–1650 μg/ml for the third) than can be achieved by the usual dosages of naloxone given *in vivo*. Naloxone, 5 mg/kg iv, achieved serum levels of 1–2 μg/ml naloxone, and the maximum tissue level is 10 μg/ml (20, 21).

We have noted some differences in cardiovascular responses to naloxone pretreatment depending upon the dosage and batch of endotoxin subsequently injected (Table III). Perhaps some of these differences are due to subtle differences in timing or some other aspect of the experiment which escaped our notice. Irrespective of these differences, naloxone protected dogs from bloody diarrhea due to endotoxin from either batch.

Some mention needs to be made of the improved cardiovascular parameters after naloxone and before endotoxin. We had only seen a slight increase in MAP with naloxone previously (3). Our current findings may be due to naloxone reversal of some cardiovascular depression caused by the induction of anesthesia and surgical stress. It is not unreasonable to postulate that this depression is opiate-mediated since it is naloxone sensitive, stress releases endogenous opioids (22), and the cardiovascular effects of inhalation anesthetics are blocked by naloxone (23).

We noted an increase in PO_2 and a decrease in PCO_2 associated with naloxone treatment. It is possible that these results are due to naloxone-induced changes in respiratory function. Similarly, naloxone in a porcine sepsis model increases PO_2, decreases PCO_2, and decreases pulmonary vascular resistance without a change in the wet/dry lung weight ratio (24). Such regional effects certainly warrant more investigation as do the other regions of interest in endotoxic shock, especially cerebral and hepatosplanchnic. Perhaps differential effects of naloxone on vasculature in these regions (directly or indirectly by modulation of effects due to endotoxin and catecholamines as well as other mediators) will clarify the results obtained.

Previous reports have shown that naloxone has no effect on HR (3). Indeed the depression of HR seen with one batch of endotoxin in this study was not affected by naloxone administration, although naloxone pretreatment in conscious rats attenuates the bradycardia and subsequent tachycardia due to endotoxin (1, 8). The differences in HR responses, of course, may be due to differences in animal species or, more likely, the presence of anesthesia in our models since anesthetics make an important difference in opiate system responses (12). For example, tachycardia and hypertension after intravenous leu-enkephalin injection are converted in the presence of pentobarbital anesthesia to bradycardia and hypotension (25).

Despite some conjectures and problems of interpretation, the observation that naloxone,

given with or before endotoxin, prevents the development of bloody diarrhea is notable. Although the relevance of this observation to the clinical treatment of human shock is perhaps lessened by the fact that primates do not develop bloody diarrhea after endotoxin, early treatment with naloxone is required for the best responses (8) and has been shown to be important in clinical septic shock (26). Finally, our results underscore the need for careful documentation of timing of treatment as well as dose and batch of endotoxin in this model.

Mrs. Pat Piper typed the manuscript, and the work was supported by Department of Defense (U.S. Army) Contract DAMD 17-81-C-1177.

1. Holaday JW, Faden AI. Naloxone reversal of endotoxin hyotension suggests role of endorphins in shock. Nature (London) **275**:450–451, 1978.
2. Faden AI, Holaday JW. Opiate antagonists: A role in the treatment of hypovolemic shock. Science **205**:317–318, 1979.
3. Reynolds DG, Gurll NJ, Vargish T, Lechner RB, Faden AI, Holaday JW. Blockade of opiate receptors with naloxone improves survival and cardiac performance in canine endotoxic shock. Circ Shock **7**:39–48, 1980.
4. Raymond RM, Harkema JM, Stoffs WV, Emerson TE. Effects of naloxone therapy on hemodynamics and metabolism following a superlethal dosage of *Escherichia coli* endotoxin in dogs. Surg Gynecol Obstet **152**:159–162, 1981.
5. Thijs LG, Balk E, Tuynman HARE, Koopman PAR, Bezemer PD, Mulder GH. Effects of naloxone on hemodynamics, oxygen transport, and metabolic variables in canine endotoxin shock. Circ Shock **10**:147–160, 1983.
6. Brückner JB, Faber du Faur JV, Danner R. Naloxone in the treatment of endotoxin shock. Brit J Anesth **53**:111P–112P, 1981.
7. Bone RC, Jacobs ER, Potter DM, Hiller FC, Wilson FJ Jr. Endorphins in endotoxin shock. Microcirculation **1**:285–295, 1981.
8. Faden AI, Holaday JW. Naloxone treatment of endotoxin shock: Stereospecificity of physiologic and pharmacologic effects in the rat. J Pharmacol Exp Ther **212**:441–447, 1980.
9. Wallerstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. Circ Shock **8**: 503–517, 1981.
10. Reynolds DG, Swan KG. Intestinal microvascular architecture in endotoxic shock. Gastroenterology **63**: 601–610, 1972.
11. Lillihei RC, Longerbeam JK, Bloch JH, Manax WA. Hemodynamic changes in endotoxin shock. In: Mills LC, Moyer JH, Eds. Shock and Hypotension: Pathogenesis and Treatment (The Twelfth Hahnemann Symposium). New York, Grune & Stratton, pp442–462, 1965.
12. Holaday JW, Loh HH. Neurobiology of β-endorphin and related peptides. In: Li CH, Ed. Hormonal Proteins and Peptides. New York, Academic Press, Vol. 10:pp203–291, 1981.
13. Koyama S, Santiesteban HL, Ammons WS, Manning JW. The effects of naloxone on the peripheral sympathetics in cat endotoxin shock. Circ Shock **10**:7–13, 1983.
14. Gurll NJ. Endorphins in endotoxic shock. In: Proctor RA, Ed. Handbook of Endotoxin. Amsterdam, Elsevier Biomedical, Vol 2(Hinshaw LB, Ed., Pathophysiology of Endotoxin):pp299–337, 1984.
15. Almqvist P, Kuenzig M, Schwartz SI. Effect of naloxone on endotoxin-induced platelet sequestration. Surg Forum **32**:304–306, 1981.
16. Traber DL, Thomason PD, Blalock JE, Smith EM, Adams T, Sziebert LA, Traber LD. Action of an opiate receptor blocker on ovine cardiopulmonary responses to endotoxin. Amer J Physiol **245**:H189–H193, 1983.
17. Sziebert L, Thomson PD, Jinkins J, Rice K, Adams T, Henriksen N, Traber LD, Traber DL. Effect of naloxone treatment on the cardiopulmonary response to endotoxin in sheep. Adv Shock Res **10**:121–128, 1983.
18. Curtis MT, Lefer AM. Protective actions of naloxone in hemorrhagic shock. Amer J Physiol **239**:H416–H421, 1980.
19. Koreh K, Seligman ML, Flamm ES, Demopoulos HB. Lipid antioxidant properties of naloxone in vitro. Biochem Biophys Res Commun **102**:1317–1322, 1981.
20. Pace NL, Parrish RG, Lieberman MM, Wong KC, Blatnick RA. Pharmacokinetics of naloxone and naltrexone in the dog. J Pharmacol Exp Ther **208**:254–256, 1979.
21. Ngai SH, Berkowitz BA, Yang JC, Hempstead J, Spector S. Pharmacokinetics of naloxone in rats and in man. Anesthesiology **44**:398–401, 1976.
22. Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C. β-Endorphin and adrenal corticotropin are secreted concomitantly by the pituitary gland. Science **197**:1367–1369, 1977.
23. Arndt JO, Freye E. Opiate antagonist reverses the cardiovascular effects of inhalation anesthesia. Nature (London) **277**:399–400, 1979.
24. Mamazza J, Hinchey EJ, Chiu RC-J. The pulmonary effects of opiate blockade in septic shock. J Surg Res **36**:625–630, 1984.
25. Sander G, Giles T, Kastin A, Kaneish A, Coy D. Leucine-enkephalin: Reversal of intrinsic cardiovascular stimulation by pentobarbital. Eur J Pharmacol **78**: 467–470, 1982.
26. Groeger JS, Carlon GC, Howland WS. Naloxone in septic shock. Crit Care Med **11**:650–654, 1983.

Received January 23, 1984. P.S.E.B.M. 1987, Vol. 184.
Accepted November 14, 1986.