0031-3998/86/2008-0707$02.00/0
PEDIATRIC RESEARCH

Vol. 20, No. 8, 1986
Printed in U.S.A.

The Effect of Naloxone on the Hemodynamics of the Newborn Piglet with Septic Shock

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ABSTRACT. Naloxone has been shown to reverse the hemodynamic sequelae of experimental septic shock in adult animal models. Its effectiveness in the newborn has not been studied. To further investigate the efficacy of naloxone, we instrumented 18 piglets for continuous measurement of mean arterial pressure, mean pulmonary arterial pressure, central venous pressure, heart rate, left ventricular pressure, contractility, cardiac output, and O_2. Oxygen consumption, systemic vascular resistance, and pulmonary vascular resistance were calculated. Following a stabilization period, group B β-hemolytic Streptococci were infused over 30 min. Following the infusion, naloxone (1 mg/kg) was given followed by a continuous infusion of 1 mg/kg/h in nine treatment animals. Nine control animals were given an equal volume of saline. Both groups developed significant increases in mean pulmonary arterial pressure followed by a return to baseline. Oxygen consumption, cardiac output, contractility and mean arterial pressure decreased in both groups. Treatment with naloxone was associated with a cessation in the fall in the mean arterial pressure and the contractility. The difference in mean arterial pressure and contractility between groups was significant. The naloxone group had significantly improved 5-h survival. We speculate that naloxone may reverse some of the hemodynamic sequelae and improve survival in newborns with septic shock. (*Pediatr Res* 20: 707–710, 1986)

Abbreviations

CO, cardiac output
CVP, central venous pressure
CaO_2, arterial oxygen content
CpO_2, pulmonary arterial oxygen content
dP/dt, contractility
GBBS, group B β-hemolytic Streptococci
HR, heart rate
LVEDP, left ventricular end-diastolic pressure
MAP, mean arterial pressure
PAP, mean pulmonary arterial pressure
PVR, pulmonary vascular resistance
SVR, systemic vascular resistance
VO_2, oxygen consumption

Endogenous opioids (endorphins) may play an important role in the pathophysiology of septic shock (1). Although endorphins are elevated in stressed newborns (2), the specific role of these substances in the neonate with septic shock and the possible beneficial effect of naloxone (an opiate antagonist) therapy have not been investigated. The objectives of this investigation were to study the circulatory alterations following infusion of live GBBS in newborn piglets and to determine the efficacy of naloxone in improving the cardiovascular performance in this animal model.

Received October 7, 1985; accepted March 12, 1986.
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This research was supported by a grant from the Children's Hospital Research Foundation.

METHODS

Animal preparation. Eighteen newborn piglets (1–4 days of age) weighing 1.2–1.8 kg were anesthetized with pentobarbital (25 mg/kg intraperitoneally). Supplemental barbiturate (5–10 mg/kg intravenously) was given during the study to maintain adequate anesthesia. Rectal temperatures were maintained with electric heating pads and warming lights. An endotracheal tube was placed through a tracheotomy and the animal was ventilated with a Bourns LS 104 infant ventilator to maintain normal arterial blood gases.

Catheters were placed in the abdominal aorta (via femoral arteries), right atrium (via internal jugular vein), and left ventricle (via carotid artery). After a left thoracotomy the ductus arteriosus was ligated, a catheter was introduced into the main pulmonary artery, and an external electromagnetic blood flow transducer (Gould Electronics, Oxnard, CA) was placed around the main pulmonary artery.

Physiologic measurements. Statham p23d transducers were used for continuous measurement of MAP and phasic arterial pressure, mean PAP, and phasic pulmonary artery pressure, phasic left ventricular pressure, and CVP. LVEDP was obtained from the phasic arterial recordings. The left ventricular pressure signal was electronically differentiated with respect to time to obtain the maximum rate of rise of left ventricular pressure (dP/dt max) as a measure of cardiac contractility. The flow probe was connected to a Gould SP2202 Blood Flowmeter (Gould Electronics, Oxnard, CA) for measurement of pulmonary artery blood flow taken as equivalent to CO. All other hemodynamic variables were recorded on a Gould 2600S recorder (Gould Electronics, Cleveland, OH). CaO_2 and CpO_2 as measured by the Lex-O_2-Con analyzer (Lexington Instruments, Waltham, MD) were determined at baseline, at the end of the bacterial infusion, then hourly.

Bacterial preparation. A standard stock of serotype III GBBS (American Type Culture Collection no. 31475) was stored at −60° C in sheep blood until ready for use. The day before the study, the organism was subcultured onto sheep blood agar and incubated at 37° C for 16 to 18 h. On the morning of each study, colonies were skimmed from the surface, inoculated into Todd-Hewitt broth, and incubated for 3 h at 37° C. The broth then underwent two cycles of centrifugation (1600 × *g* for 10 min), with each cycle followed by resuspension of the bacterial pellet

in sterile, nonpyrogenic saline. A concentration of approximately 2×10^9 CFU/ml was obtained by titrating to an absorbance of 0.8 on a spectrophotometer at a wavelength of 550 nm. Viability counts of the bacterial infusate were verified with standard serial dilution and pour-plate techniques.

Experimental design. After a stabilization period of 1 h following surgery, all piglets received 15 ml/kg (corresponding to 2–4 $\times 10^{10}$ CFU/kg) of the bacterial preparation intraarterially over 30 min. Treated pigs ($n = 9$) were given naloxone 1 mg/kg at the end of the bacterial infusion followed by a continuous infusion of 1 mg/kg/h. Control animals ($n = 9$) received an equal volume of saline. Measurements were continued to a maximum of 5 h after the bacterial infusion.

At the conclusion of the experiment the small and large intestines were removed from four of the animals from each group and fixed in formalin. Sections were embedded in paraffin, stained with hematoxylin and eosin, and examined under light microscopy. The examiner was blinded as to the animal's treatment status.

Calculations and statistical analyses. SVR was calculated as follows:

$$SVR = (MAP\text{-}CVP)/CO$$

PVR was calculated as follows:

$$PVR = (PAP\text{-}LVEDP)/CO$$

VO_2 was derived from the Fick equation:

$$VO_2 = (CaO_2\text{-}CpO_2) \times CO$$

Analysis of variance for repeated measures was used to detect statistical significance within groups and between groups over time. Data taken from the record at 30-min intervals were used for the analysis. Fisher's exact test was used to compare the survival data between groups. Survival was defined as being alive at 5 h following the bacterial infusion. All variables are expressed as mean ± SEM. A p value of <0.05 was considered significant.

RESULTS

Baseline hemodynamic measurements and calculations did not differ significantly between groups. Approximately 1 h after the end of the bacterial infusion, both groups had a 20 to 30% decrease in their MAP which further declined in the control piglets, but was stabilized in the treated piglets (Fig. 1*A*). The difference between groups was significant ($p < 0.02$). The blood pressure response in the naloxone-treated pigs was not accompanied by a parallel increase in SVR. There was no significant difference in the systemic vascular resistance between groups; both groups showed an elevation during the bacterial infusion followed by a gradual return to baseline values (Fig. 1*B*).

CO was severely affected, declining by over 50% in both groups within minutes of the bacterial infusion (Fig. 1*C*). At the end of the bacterial infusion, both groups demonstrated a partial recovery in their CO which was more marked and prolonged in the naloxone-treated animals, but only transient in the controls. Although the difference in cardiac output did not reach statistical significance ($p > 0.1$), naloxone treatment tended to maintain and stabilize the CO. The fall in CO in the control group was accompanied by a profound and steady fall in cardiac contractility (Fig. 1*D*). Naloxone treatment prevented this decrease and restored cardiac contractility toward baseline ($p < 0.05$).

The pulmonary artery pressure rose significantly in response to the bacterial infusion in both groups ($p < 0.05$) and slowly returned to baseline values (Fig. 1*E*). This was unaffected by naloxone treatment. The PVR demonstrated a similar trend in both groups (data not shown). In keeping with the abrupt decline in CO, the VO_2 fell in both groups during the bacterial infusion followed by a compensatory rise (Fig. 1*F*). Although not reaching statistical significance, naloxone treatment seemed to maintain the compensatory rise in VO_2. There was no significant difference between groups for HR, LVEDP, CVP, or hematocrit (data not shown).

Representtive sections of the small and large intestine were examined by light microscopy. One of the control animals demonstrated evidence of small intestinal ischemia with confluent mucosal and submucosal hemorrhages and breakdown of mucosal crypts. Two other control piglets and one naloxone-treated piglet had less severe changes limited to congestion of the lamina propria of the small or large intestine. No severe hemorrhage or crypt disruption was evident in the naloxone-treated animals.

Naloxone significantly improved survival with seven of nine treated animals *versus* only three of nine control animals alive for at least 5 h after the end of the bacterial infusion ($p < 0.05$) (Fig. 2).

DISCUSSION

The hemodynamic changes that occur during sepsis or endotoxemia in newborn animals have only recently been examined. Endorphins may be partly responsible for these changes (3). These substances are stored in the anterior pituitary gland and share a common proopicortin precursor with ACTH (4, 5). Several investigators have documented elevations of these opioid peptides in various shock states (3). Furthermore, exogenous administration of these peptides intravenously or intracerebrally exert dramatic cardiovascular effects (3). They are thought to act on central opioid receptors to influence autonomic outflow and inhibit sympathomedullary discharge (4). However, a direct effect on opiate receptors in the heart, vasculature, and lungs has not been conclusively disproved (3).

One of the most striking hemodynamic changes observed in our study was the rapid and persistent reduction in CO induced by the bacterial infusion. There were no concomitant changes in heart rate or LVEDP (a measure of preload) to account for this decline in CO. Similarly, mechanical interference with myocardial function resulting from severe pulmonary hypertension seems unlikely. The decline in CO occurred before any significant elevation in PAP and persisted despite a return of PAP to values similar to baseline. Other investigators have made similar observations (6, 7). The reduced CO most likely represents a change in the intrinsic contractile state of the heart as reflected in the reduced LV dP/dt max, which may be altered by changes in preload (8). Since LVEDP did not change significantly during the study, the reduced LV dP/dt max suggests a true decline in myocardial contractility.

Pulmonary arterial hypertensive responses during bacterial or toxin infusion have been described in piglets (6, 9, 10) and in the lamb (11). As in our study, the elevation in PAP and PVR was rapid but transient, increasing in response to bacterial or endotoxin infusion, and returning to baseline values within several minutes (6, 11). Similar responses are observed in mature animals (11–13).

Naloxone, a specific opiate antagonist with no agonist activity, has been shown to be effective in experimental septic shock using adult animals (1, 4, 14). The cardiovascular effects of naloxone are stereospecific, acting to facilitate sympathomedullary outflow through an antagonism of endorphins at sites within the central nervous system (5). An intact sympathetic nervous system and adrenal gland is required for naloxone to produce its therapeutic effects (4). A few anecdotal reports have documented the drug's effectiveness in children and adults with septic shock (16–20).

Our study confirms that naloxone attenuates septic shock and extends this observation to newborn swine. Although these animals were subjected to a relatively large and rapidly administered bacterial inoculum (a dose and rate that was necessary to consistently produce hypotension), naloxone significantly improved MAP and cardiac contractility and tended to ameliorate the decline in CO and VO_2. Lobe *et al.* (21) found evidence of gut ischemia associated with naloxone treatment of peritonitis-induced septic shock in piglets. This is in contrast to other reports showing an improvement in splanchnic blood flow and a reversal of local tissue hypoxia (22, 23). In our study, naloxone improved MAP without altering SVR, indicating an overall improvement

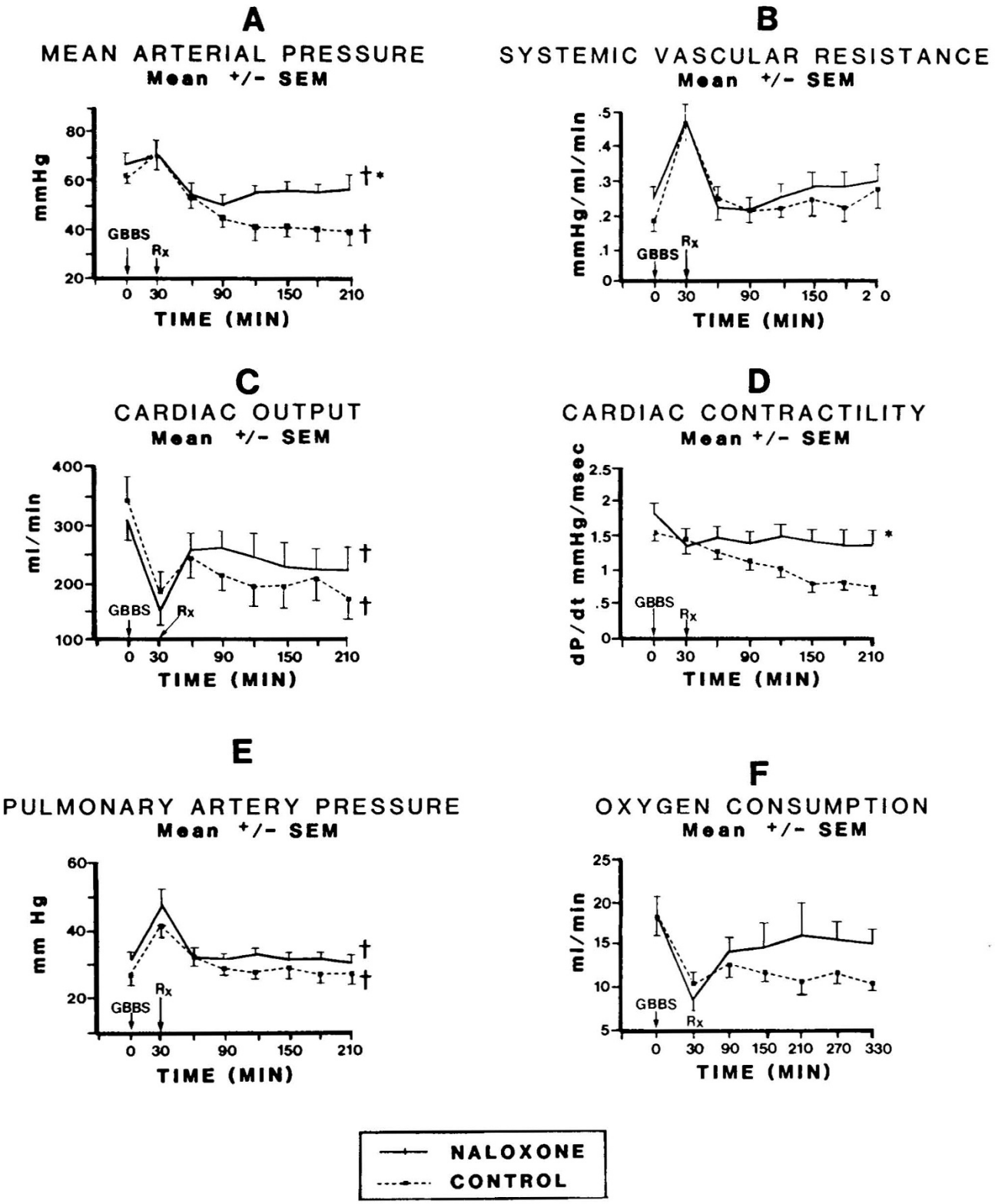

Fig. 1. Hemodynamic changes in the control and naloxone-treated groups: *A*, MAP; *B*, SVR; *C*, CO; *D*, cardiac dP/dt; *E*, PAP; *F*, VO_2. + denotes significant difference with respect to time; * denotes significance between groups; and, *Rx* denotes treatment with either naloxone or saline

in peripheral perfusion. This is supported by the histologic studies of the animals' intestinal tract. None of the naloxone-treated animals had evidence of hypoxic-ischemic changes.

Several caveats are in order regarding naloxone therapy in septic shock. Although animal studies and anecdotal reports in humans attest to the drug's effectiveness, recent controlled trials in adult humans have not been promising. Groeger *et al.* (24) found that only 50% of patients with septic shock responded to a 0.3 mg/kg dose of naloxone with an elevation of blood pressure and SVR and no change in CO. All of the responders were given naloxone before prolonged hypotension and acidosis had developed, a clinical luxury that is rarely present in neonatal sepsis. Recent reports have demonstrated that naloxone in similar doses had no significant effect on mean blood pressure, CO, or survival in septic patients (25, 26). Furthermore, adverse reactions after naloxone therapy including severe hypotension, pulmonary

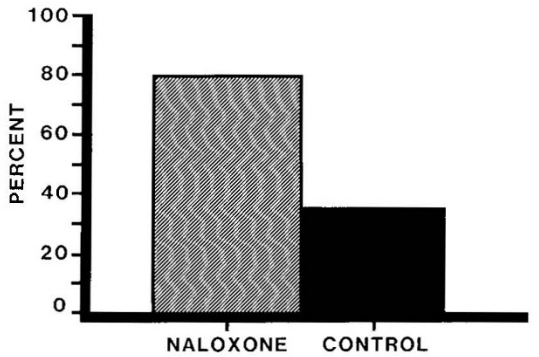

Fig. 2. Survival of the naloxone and control groups. The difference is significant ($p < 0.05$).

edema, and seizures have been reported (26, 27). Although shown to be safe even if given in massive doses to normal volunteers (28), naloxone may have undesirable consequences in the critically ill patient.

Species differences in opiate receptors or cardiovascular physiology may account for the inconsistent results of naloxone therapy in humans as compared to animals (29). Nevertheless, the piglet's cardiovascular system is physiologically similar to the human neonate (30) and the appropriateness of comparing this septic shock model to the human newborn condition has been addressed (9). Further comparisons are hampered by the lack of knowledge regarding the ontogeny of the various opiate receptors in the human newborn or piglet. Nevertheless, this model suggests that naloxone's binding to opiate receptors may be important in improving the cardiovascular performance during sepsis in newborns. Further investigations are warranted prior to initiating this as routine therapy.

Acknowledgments. The authors thank Mario Marcon, Ph.D., for his technical assistance, Mrs. Dawn Penn for her secretarial assistance, and Richard E. McClead, M.D. for his careful review of this manuscript.

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