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Endotoxin Shock: Prevented by Naloxone in Intact but Not Hypophysectomized Rats (41810)

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Abstract. Previous studies established that naloxone reverses hypotension in endotoxin, hemorrhagic, and spinal shock. We studied endotoxin shock in hypophysectomized (Hx) rats, which have little circulating β-endorphin. Hx or intact rats received surgically implanted jugular catheters for drug injection and aortic catheters for arterial blood pressure (MAP) recording. On the second day after implantation, rats were pretreated with either naloxone or saline. Two minutes later each rat received endotoxin. Following endotoxin, all rats showed a brief biphasic hypertensive–hypotensive response followed by stabilization of MAP near baseline. Within 20 min, all Hx rats, regardless of pretreatment, and the saline-treated intact rats, showed progressive hypotension ($P < 0.005$). Only the naloxone-pretreated intact rats maintained a stable MAP. Plasma endorphin measured at 20 min was undetectable in Hx rats in contrast to intact rats ($P < 0.001$); plasma corticosterone levels were likewise suppressed in the Hx rats ($P < 0.01$). Thus (1) naloxone protected only the rats with an intact pituitary–adrenal–sympathetic system, and (2) pituitary endorphin is not required to generate endotoxin shock in hypophysectomized rats.

Naloxone reverses the depressant effects of opiates (1) and similarly reverses the depressant effects of endogenous opioid compounds (2–4). Naloxone reversibility of almost any cardiovascular or psychomotor response is considered evidence of participation of endogenous opioid compounds in the pathogenesis of that response. The endogenous opioid β-endorphin is thought to be involved in the pathogenesis of the hypotension associated with a variety of conditions because of reversibility by naloxone, including endotoxic and hemorrhagic shock in dogs and rats (5–8), spinal shock in cats (5, 9), and clonidine-induced hypotension in spontaneously hypertensive rats (SHR) (10). Additional evidence is the finding that plasma from dogs made hypotensive by hemorrhage or endotoxin contains markedly elevated levels of β-endorphin (11, 12).

Recent work of Holaday *et al.* (7) showed that intracerebrally administered naloxone would not reverse the hypotension of hemorrhagic shock in hypophysectomized rats, whereas intact rats responded to intracerebral naloxone. They concluded that intracerebral opiate receptors were probably of major importance in the generation of hypotension in the hemorrhagic shock model. Since β-endorphin, like other pituitary derived substances, disappears from the circulation after hypophysectomy (13), we also used hypophysectomized rats as model to determine whether circulating β-endorphin should be considered necessary for the pathogenesis of endotoxic shock. We reasoned that naloxone would have no effect in ameliorating hypotension in rats without circulating endorphin.

Materials and Methods. The effect of hypophysectomy and naloxone treatment on endotoxin-induced hypotension was studied in four groups of rats ($N = 4$ to 5 per group) in a 2×2 design.

Animals. Seven-week-old hypophysectomized and intact male Sprague–Dawley rats were obtained from King Laboratories (Oregon, Wisc.). They were housed three to a cage before surgery and individually after surgery at 25°C on a 12-hr light–dark schedule, and allowed standard laboratory chow and tap water. Because of the higher mortality of the hypophysectomized (Hx) rats from respiratory infections, tetracycline was added to the drinking water of all rats when infections were prevalent in the colony.

Preparative surgery. Arterial and venous catheters were surgically implanted 2 days before the experiment. To accomplish this, rats were anesthetized with sodium pentobarbital (50 mg/kg ip) prior to surgery. Hx rats were pretreated with dexamethasone sodium phosphate 250 μg/kg ip 1 hr prior to anesthesia.

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The abdominal aorta was exposed via an abdominal incision and cannulated with a polyethylene catheter (Intramedic PE-10 at the tip) so that the tip of the catheter was below the level of the renal arteries. The tubing was brought out anteriorly, threaded subcutaneously, then sutured in an interscapular position with a small exteriorized loop. The tubing was filled with a heparin solution and heat sealed. The jugular vein was cannulated with Silastic (Dow) tubing; the external end was tunneled into the interscapular position, filled with heparin, and the end knotted and sutured in place with an exteriorized loop. On the day following surgery, Hx rats received hydrocortisone sodium succinate 0.1 mg im at 0800 and 1700, and L-thyroxine 2.0 μg im at 0800.

Experimental procedure. Two days later, between 1000 and 1400 and at least 17 hr after the last injection of hydrocortisone, the rats were lightly restrained in a warmed plastic holder for blood pressure studies. After 5–10 min of baseline quiet, mean arterial blood pressures (MAP) were recorded from the aortic catheter using a Gould–Statham model P23dB pressure transducer, a Hewlett–Packard 8805C pressure amplifier, and a Hewlett–Packard 7702B chart recorder. Instruments were calibrated with a mercury manometer; sensitivity was estimated to be <2 mm Hg. Pulse pressure tracings were electronically averaged to a mean arterial pressure. After stable baseline records were obtained, rats were randomly assigned to receive either naloxone 1 mg/kg (0.4 mg/ml, Endo Laboratories, Wilmington, Del.) as a bolus over 5 sec via the jugular catheter, or an equivalent volume of 0.9 saline, given at time zero. This dose was chosen as it has been suggested as an optimum bolus dose in endotoxin-treated rats (5). At 2 min the rats received endotoxin (Lipopolysaccharide B, Boivin method, E. coli 0111:B4, control 704385, Difco Laboratories, Chicago, Ill.) 50 mg/kg (LD_{85}; 20 mg/ml in 0.9 saline) as a bolus over 5 sec. The catheters were flushed after each injection. A continuous blood pressure record was made over the first 3 min of the study, including both injections. Thereafter, a 5-sec record was made at 4, 5, 10, 15, and 20 min into the study.

At 20 min, each rat was removed from the holder, decapitated, and blood collected in a heparinized tube. Blood was held on ice, centrifuged, and the plasma stored at −20°C for hormone determinations. Completeness of hypophysectomy was verified by inspection of the base of the brain and skull.

Hormone determinations. Plasma corticosterone was assayed by RIA using the method of Keith *et al.* (14). Briefly, the ether-soluble fraction of plasma was suspended in buffer and incubated with ^{3}H-corticosterone plus antiserum. The free corticosterone was precipitated with charcoal–Dextran, and the supernatant, containing antibody-bound corticosterone, was counted by liquid scintillation spectrometry. Sensitivity was 0.5 μg/dl of plasma.

Plasma β-endorphin was assayed by RIA using the method of Allen *et al.* (15) with a sensitivity of 10 pg/ml of plasma. Briefly, plasma β-endorphin was adsorbed to Vycor glass (Corning) and eluted with 30 ml acetone. The acetone eluates were dried, reconstituted in buffer, and β-endorphin measured by a nonequilibrium RIA using antiserum which reacts with β-endorphin and β-lipotropin on an equimolar basis. Recovery of added β-endorphin averaged 60%; standards were carried through the assay procedure.

Analysis. Blood pressure in each rat was expressed as a percentage of baseline for analysis. The significance of the experimental treatment on the change in MAP from baseline was determined in each group by the analysis of simple effects. Differences among means at 20 min were determined by the Newman–Keuls test (16).

Results. *Effect of hypophysectomy.* Hx rats had no visible pituitary tissue at autopsy. Baseline arterial pressures were significantly less in the Hx rats compared to intact rats, confirming previous observations (7); however, in both groups of rats, MAPs recorded from the aorta were substantially higher than previously reported blood pressures recorded with tail arterial catheters (Table I).

Circulating β-endorphin was absent in the Hx rats, confirming previous observations of the pituitary origin of plasma endorphin (13). Likewise, the pituitary–adrenal axis was markedly depressed in these rats as indicated by the very low plasma corticosterone concentrations (Table II). There was no effect of naloxone versus saline pretreatment on these values.

TABLE I. MEAN ARTERIAL BLOOD PRESSURES AND WEIGHTS OF HYPOPHYSECTOMIZED AND INTACT 7-WEEK-OLD RATS

	Baseline MAP (mm Hg ± SEM)	Weight (g ± SEM)
Hypophysectomized (9)	86.6 ± 4.0	176.6 ± 2.8
Intact (8)	112.9 ± 2.7	241.6 ± 10.2
P (two-tailed *t* test)	<0.01	<0.01

Note. *N* in parentheses.

Blood pressure responses. There was no significant effect of either naloxone or saline alone on MAP in either Hx or intact rats. With the injection of endotoxin, all groups demonstrated a similar rapid transient biphasic hypertensive–hypotensive response, which began during the last part of the injection and ended as a discrete trough 6–15 sec after the end of the bolus. Figure 1 shows representative tracings of this response, which did not appear after injections of saline or naloxone. Pilot experiments clearly showed that this event did not require a prior injection to "volume load" the animal. Following this event, MAPs in all groups returned to near baseline for the next 3 min, being slightly but not significantly lower in the Hx rats (94 vs 98 of baseline). Subsequent recordings demonstrated differing effects of endotoxin in the four groups: Hx rats, regardless of pretreatment condition, had a steady decline in MAP

TABLE II. PLASMA CORTICOSTERONE AND ENDORPHIN LEVELS IN INTACT AND HYPOPHYSECTOMIZED ENDOTOXEMIC RATS PRETREATED WITH SALINE OR NALOXONE

	Corticosterone (μg/dl ± SEM)	β-Endorphin (pg/ml ± SEM)
Hypophysectomized		
Saline-treated (4)	1.3 ± 0.1	<10[a]
Naloxone-treated (5)	1.5 ± 0.1	<10[a]
Intact		
Saline-treated (4)	5.9 ± 0.5	456 ± 30
Naloxone-treated (4)	5.8 ± 0.3	433 ± 107
P	<0.01[b]	<0.001[c]

Note. *N* in parentheses.

[a] 10 pg/ml was the lower limit of the assay. All hypophysectomized rats (except one naloxone-pretreated rat with a level of 30 pg/ml) had no detectable circulating endorphin.

[b] Two-tailed *t* test, hypophysectomized vs intact.

[c] Fisher's exact test, hypophysectomized vs intact.

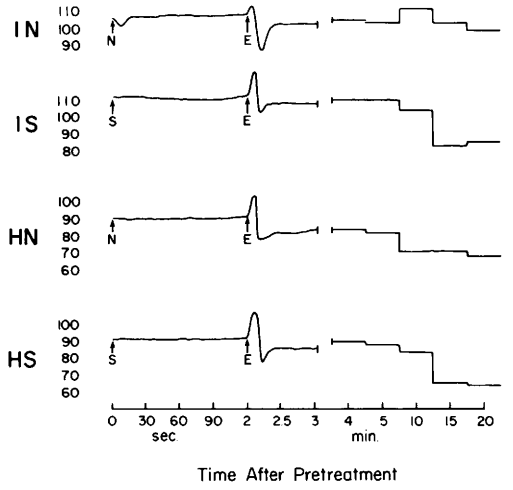

FIG. 1. Representative MAP recordings from one member of each treatment group. Rats were injected with 2.5 ml/kg of saline (S) or naloxone (N) at time 0. At 2 min rats were injected with endotoxin (E) (2.5 ml/kg). I, intact; H, hypophysectomized. The ordinate is mean arterial pressure in mm Hg. The nonlinear abscissa was necessitated by the switch to an intermittent record after 3 min. The brief decrease in MAP after naloxone injection in rat IN was not significant and was not seen in other members of that group.

over the recording period, reaching a value significantly different from baseline at 20 min (MAP 78 of baseline or 68 ± 5.2 mm Hg). In contrast, intact rats demonstrated a protective effect of naloxone pretreatment. Naloxone pretreated intact rats maintained their MAPs at >96 of baseline (NS) following the initial biphasic response; however, saline-pretreated intact rats demonstrated the same significant fall in MAP over time as did the Hx rats, reaching 80.5 of baseline at 20 min (absolute MAP 95 ± 4.1 mm Hg) (Fig. 2). Newman-Keuls analysis confirmed the difference in the mean of the naloxone-pretreated intact group from the other three groups ($P < 0.01$).

Discussion. The objectives of the present study were to examine the hypotensive effect of endotoxin shock in hypophysectomized rats and to determine if it could be prevented by naloxone. Several important findings are presented: first, hypophysectomized rats do exhibit a hypotensive response to endotoxin; second, as predicted, naloxone was not effective in preventing it. A third unexpected finding was the transient hypertensive/hypotensive

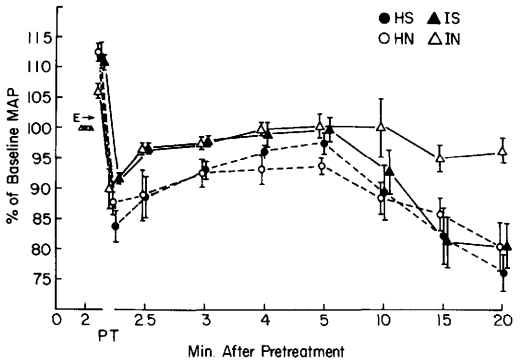

FIG. 2. Mean arterial pressure after endotoxin injection in each group. Values are expressed as percentage of baseline. Rats were injected with endotoxin (E) 2 min after pretreatment with either naloxone or saline; P and T refer to postinjection peak and trough, which occurred at slightly different times in each animal. Analysis of simple effects demonstrated a significant ($P < 0.005$) fall in MAP over 20 min in all groups except IN. I, intact; H, hypophysectomized; S, saline pretreated; N, naloxone pretreated. Groups HN, HS, and IS could not be shown to differ from one another at 20 min (Newman-Keuls test, $P < 0.01$). Vertical bars indicate standard error. Note the nonlinear abscissa.

response in all groups immediately after endotoxin injection.

The finding that hypophysectomized rats exhibit a hypotensive response to endotoxin was not unexpected given the fragile nature of these animals, but it could not have been predicted from the hypothesis that β-endorphins are of central importance in the pathogenesis of shock.

The fact that naloxone was effective in preventing endotoxin-mediated hypotension in rats with intact pituitaries supports previous observations of Faden, Holaday, and coworkers. The absence of effect of naloxone in the hypophysectomized rat was originally reported in the hemorrhagic shock model (7), and is here extended to the specific situation of endotoxin shock. These data are also in agreement with work that Faden and Holaday presented in preliminary form (17). However, the hypothesis that β-endorphin is necessary for the pathogenesis of hypotension is not supported, because hypotension is easily generated in hypophysectomized rats lacking β-endorphin.

Endotoxin has a multitude of effects in the intact organism, some of which are immediate and others of which develop over hours (18). Endotoxin localizes within platelets and endothelial cells within minutes after injection, and is involved in the release of tissue factors, procoagulants, collagenase, prostaglandins, and pyrogens. It is involved in activating the complement cascade, leukocytes, and generating fever (18). The ultimate result to the organism is hypoglycemia, respiratory failure, diffuse intravascular coagulation, renal failure, circulatory collapse, and death. The release of β-endorphin in the animal experiencing endotoxic shock no doubt contributes to the fall in blood pressure; treatment with naloxone may allow animals with intact homeostatic counterregulatory mechanisms to normalize blood pressure temporarily. While most studies have failed to demonstrate any beneficial effect of naloxone on survival of endotoxemia beyond several hours (19), at least one recent report has shown such an effect on dog 7 days after endotoxin insult (20).

Comparison of two animal models which do not respond to naloxone during hypotension provides evidence for the mechanism of action of naloxone. Hypophysectomized and adrenalectomized (21) rats are similar in that both conditions result in fragile animals with low resting blood pressures, decreased plasma epinephrine, and increased susceptibility to shock. They differ in that hypophysectomized rats have absent circulating endorphin and ACTH, while adrenalectomized rats have markedly elevated levels of these hormones. The sensitivity of these animals to shock and their lack of ability to normalize blood pressure with the administration of naloxone thus most likely represents their loss of pressor counterregulatory agents (e.g., epinephrine) rather than the absence of or the overabundance of a depressor agent, endorphin. Although we did not measure plasma catecholamines in this study, this interpretation is in agreement with a recent *in vivo* observation by Mannelli *et al.* (22) that naloxone is capable of stimulating the release of catecholamines by a pheochromocytoma, a neoplasm derived from sympathetic nervous tissue, and supports the general hypothesis that endorphins modulate the release of catecholamines. Further substantiation of an interaction with catecholamines has been shown by Kayama *et al.* (23) who observed a correlation between pre-

ganglionic splanchnic nerve firing and naloxone treatment in endotoxemic cats.

It is also of interest that a variety of other single agents have been found to be capable of ameliorating some of the manifestations of endotoxin shock, among them, α-adrenergic blocking agents (24), glucocorticoids (25–27), diphenhydramine (28), indomethacin (29), and TRH (30). This reflects the complex nature of endotoxic shock; there is likely no single predominant pathophysiologic mechanism in either the generation of or the recovery from endotoxin shock.

The final point regards the specific pattern of endotoxin-induced hypotension. We describe a brief biphasic change in blood pressure in rats immediately after endotoxin injection, followed by recovery, then slow decline over the next 10–20 min, of which the latter phase may be ameliorated with naloxone in the intact rat. Although this early biphasic change may be observed in representative previously published blood pressure recordings, it has not been emphasized (31). That we reproducibly saw this early fluctuation may have resulted from our use of the technique of direct measurement of aortic blood pressure which might allow the detection of transient changes in central pressures which are not reflected in recordings from more peripheral (e.g., tail artery) vessels. The cause and significance of this phenomenon is not apparent and will require future clarification.

Our studies were done on lightly restained rats, a state known to activate endogenous opioid systems. While it is possible that this restraint may have increased the stimulated levels of β-endorphin, it seems unlikely that it would affect the observation that naloxone does not protect hypophysectomized animals, or the conclusion that pituitary opioids are unnecessary for the introduction of endotoxin hypotension.

In summary, the present study supports previous observations of the efficacy of naloxone in ameliorating endotoxin-induced hypotension in the intact rat, and supports and extends previous findings that naloxone is of no value in attenuating hypotension in hypophysectomized rats. The findings are in agreement with the hypothesis that the beneficial effect of naloxone in the intact rat is mediated through the sympathetic nervous system. We conclude that endotoxin-stimulated release of plasma endorphin is part of the multifactorial pathogenesis of hypotension in intact rats, but that β-endorphin is not necessary for the generation of endotoxin-induced hypotension in hypophysectomized rats.

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