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Enhanced vascular effects of cyclic GMP in septic rat aorta

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MCKENNA, THOMAS M. *Enhanced vascular effects of cyclic GMP in septic rat aorta.* Am. J. Physiol. 254 (Regulatory Integrative Comp. Physiol. 23): R436-R442, 1988.—The modulation of vascular function by guanosine 3',5'-cyclic monophosphate (cGMP) in sepsis was examined in isolated rat aortas. Basal cGMP content was similar in aortas from sham-operated [3.6 ± 0.8 (SE) pmol cGMP/mg protein] and from septic (3.2 ± 1.0) rats. Acetylcholine-induced increases in cGMP content were significantly greater in aortas from sham (109.7 ± 31.1) than aortas from septic rats (42.1 ± 10.6). Maximal contractile performance by aortas from septic rats was impaired whether contractions were induced by the α_1 -receptor agonist norepinephrine (497 ± 49 mg tension/mg tissue vs. sham 749 ± 43) or by KCl depolarization (265 ± 31 vs. sham 613 ± 79). Aortas from septic rats also exhibited a rightward-shifted dose response to norepinephrine. Inhibition of endogenous cGMP production by myoglobin or methylene blue treatment disproportionately improved responses by aortas from septic rats to both norepinephrine and KCl. In contrast, exposure of aortas to exogenous 8-bromo-cGMP engaged exaggerated vasodilatory responses in tissue from septic animals. Aortas from sham and septic rats contracted equally to stimulation by phorbol 12,13-dibutyrate. These findings indicate that reduced vascular contraction in sepsis is not mediated by altered cGMP levels, but rather that enhanced sensitivity to effects of cGMP may contribute to the disorder.

sepsis; phorbol; endothelium-derived relaxing factor; vascular contraction

TREATMENT OF RATS with endotoxin or induction of sepsis via a polymicrobial peritonitis causes a state of vascular hyporesponsiveness to several contraction agonists, including norepinephrine (NE), vasopressin, and angiotensin II (9, 27). The disordered in vivo vascular responses persist when vascular tissue is examined in vitro; isolated rat aortas manifest diminished contractile response to the prostaglandin endoperoxide analogue U-46619 and to NE (17, 24). The reduced response to NE is at least partially mediated by vascular endothelium, because removal of endothelium from isolated aortic tissue from septic rats returns contractions toward normal (17).

A potential mediator of the effect attributable to the endothelium, in sepsis, is endothelium-derived relaxing factor (EDRF). EDRF is released in response to many agents, including acetylcholine, ATP and ADP, bradykinin, histamine, and thrombin (see Ref. 10 for review).

EDRF causes vasodilation by activation of soluble guanylate cyclase; the activated enzyme in turn catalyzes the synthesis of 3',5'-cyclic monophosphate (cGMP), (20), which has vasodilatory actions. Spontaneously released EDRF can decrease the net resting tone in rat aorta (16).

Ferrous hemoproteins (hemoglobin and myoglobin) inhibit endothelium-dependent vasodilation, presumably by binding EDRF as it diffuses through the extracellular space between endothelium and smooth muscle (14, 15), thereby preventing activation of guanylate cyclase. Direct inhibition of guanylate cyclase by methylene blue, which may act by inactivating a heme prosthetic group associated with the enzyme, will also inhibit cGMP synthesis and vasodilation in response to EDRF (14, 20).

In the present study, aspects of the relationship between vascular contraction and cGMP-mediated vasodilation in the septic state were examined. Basal and stimulated cGMP production, as an index for EDRF release or vascular cGMP productive capacity, and the modulation of receptor (NE)- and KCl-induced contraction by altered intracellular cGMP activity were measured in a rat model for experimental sepsis.

MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats (Taconic Farms, Germantown, NY) weighing 250–350 g were used in all experiments. Rats were made septic by a cecal ligation and puncture (CLP) method as described by Wichterman et al. (31), with a modification that an additional ligation of the cecal ileocolic vascular bundle was performed. Cecal ligation and puncture reliably induces positive blood cultures in all rats subjected to this procedure (31). Sham-operated rats had the cecum exposed, manipulated, and returned to the peritoneal cavity without additional trauma. All surgery was performed under 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane) anesthesia. Animals were killed by decapitation 24–48 h postsurgery. Criteria for selection of animals deemed septic for use in this study included listlessness, piloerection, hyperpnea, and nasal and circumorbital discharges.

The hemodynamic state of the animals was not monitored before death. However, Wichterman et al. (31) found mean systemic blood pressure of septic rats to be no different from controls until immediately before death; others also showed that base-line blood pressure

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of unanesthetized septic rats remains within the normal range 48 h after the CLP procedure (9).

Measurements of aortic contraction. The thoracic aorta was dissected immediately after death, the adventitia removed, and two to four rings of 3.5 mm in length were cut in a jig. Deendothelialized tissue was prepared, prior to sectioning into rings, by perfusion of the isolated aorta with 1–2 mg/ml sodium deoxycholate in Krebs-Ringer bicarbonate buffer (KRB) for 20 s, followed by a 5-min wash with KRB (mmol composition: 118 NaCl, 4.7 KCl, 1.3 CaCl₂, 1.2 MgSO₄·7H₂O, 1.2 KH₂PO₄, 25.0 NaHCO₃, and 11.7 glucose) (pH 7.4) while bubbled with 95% O₂–5% CO₂.

Each ring was mounted between two stainless steel hooks in a 10-ml jacketed organ bath and maintained in KRB at 37°C. One hook was stationary, and the other was attached to an isometric force transducer (Kulite Semiconductor BG-10). Tension was recorded on a Gould-Brush 2400 chart recorder. All rings were subjected to a conditioning protocol that included 1) equilibration to a resting tension of 3 g for 30–45 min (preliminary experiments showed this to be the optimal length-tension relationship for ring contraction); 2) application of NE (10⁻⁷ M) with acetylcholine (ACh, 10⁻⁶ M) added at maximal tension to confirm the functional presence or absence of the endothelium (10); and 3) an additional dose of NE (10⁻⁷ M). The rings were flushed between each conditioning treatment with KRB until a stable base-line tension was reattained. The rings were removed, blotted, and weighed after completion of the experiments.

Blockade of EDRF by myoglobin. Reduced myoglobin was prepared by the procedure described by Martin et al. (15) in which myoglobin is reduced by treatment with sodium hydrosulfide (Na₂S₂O₄, at a 10-fold molar excess) and the mixture dialyzed against distilled water. Aortic rings from each rat were always tested in pairs, with one ring treated with 50 μM myoglobin and the other ring a control for treatment effects. Conditioned ring pairs (from CLP, sham, and nonoperated control rats) were treated with or without myoglobin 10 min before and throughout the measurement of contractile responses to stepwise cumulative additions of NE (10⁻¹⁰–10⁻⁵ M) to the organ bath.

Contractile response to phorbol 12,13-dibutyrate and modulation of NE-induced contraction by methylene blue and 8-bromo-cGMP. Four rings from the same aorta were prepared from each CLP or sham-operated rat. All rings were conditioned before measuring contractile responses. Each ring was tested with a different procedure: 1) a ring with intact endothelium was contracted in a cumulative, stepwise manner with NE (10⁻¹⁰–10⁻⁵ M), then methylene blue (10⁻⁵ M) was added during maximal contraction and any additional contraction recorded; 2) a ring with intact endothelium was contracted with a maximally stimulatory dose of phorbol 12,13-dibutyrate (10⁻⁶ M); 3) a deendothelialized ring was treated with methylene blue (10⁻⁵ M) 10 min prior to and throughout NE (10⁻¹⁰–10⁻⁵ M)-induced contraction; and 4) a deendothelialized ring was treated with both methylene blue (10⁻⁵ M) and 8-bromo-cGMP (10⁻⁴ M) 10 min prior to and throughout

NE (10⁻¹⁰–10⁻⁵ M)-induced contraction.

Modulation of KCl-induced contraction by methylene blue and 8-bromo-cGMP. Four rings with intact endothelium were prepared from CLP or from sham-operated rats. After the rings were conditioned, two rings were contracted with NE (10⁻¹⁰–10⁻⁵ M) to provide comparison to contractions induced with KCl. The remaining two rings were contracted with a high KCl KRB buffer (100 mM, produced by equimolar KCl for NaCl substitution). Methylene blue (10⁻⁵ M) was added to the organ baths of both rings contracted with KCl after maximum contraction was attained, and any additional increments in tension were measured. A dose of 8-bromo-cGMP (10⁻⁴ M) was then added to the baths of rings treated with KCl and methylene blue, and decreases in tension were measured until stable values were observed.

Measurement of aortic ring cGMP levels. Three rings were prepared from each CLP or sham-operated rat. The rings were placed in 10 ml KRB (bubbled with 95% O₂–5% CO₂) at 37°C and allowed to equilibrate for 45 min. One ring received no treatment and was removed and processed after equilibration. The remaining two rings were subjected to NE (10⁻⁷ M) for 5 min, then one of the two rings was removed and processed. The final ring, while still exposed to 10⁻⁷ M NE, was treated with ACh (10⁻⁶ M) for an additional minute before processing. The rings were processed by rapidly blotting excess KRB buffer, freezing in liquid N₂, and storage at –80°C. The frozen tissue was homogenized in 1 ml ice-cold 6% trichloroacetic acid, and after centrifugation, the supernatants were chromatographed on Dowex 50W-X8 (H⁺) ion exchange columns and the eluants lyophilized. The lyophilized residues were assayed for cGMP by radioimmunoassay utilizing a New England Nuclear kit (Boston, MA). Ion exchange chromatography and cGMP assay followed protocols provided by the manufacturer. The pellets were solubilized in 1 N NaOH, neutralized with concentrated HCl, and protein content was determined with a Bio-Rad (Richmond, CA) protein assay kit utilizing bovine serum albumin as standard. Cyclic GMP is presented in picomoles per milligram protein after correction for extraction losses and column blanks.

Statistical analysis. Aortic ring contractile performance to cumulative NE doses was characterized by integrating the tension developed by aortic rings in response to NE: i.e., mg tension/mg tissue vs. ln [NE] M. EC₅₀ values (concentration of agonist causing a half-maximal contraction) were calculated by linear regression after logit-log transformation of dose responses. Tests for differences between EC₅₀ values were based on mean log values. Comparisons of paired and independent mean treatment effects were by the appropriate Student's *t* test (28), with probabilities of 0.05 or less accepted as significant. Myoglobin treatment effects were analyzed by the variance test for homogeneity of the binomial distribution in a 2 × 3 contingency table (28). All data are expressed as means ± SE.

Drugs. Norepinephrine bitartrate, 8-bromo-cGMP, phorbol 12,13-dibutyrate, methylene blue, and equine myoglobin (type 1) were obtained from Sigma (St. Louis, MO). Acetylcholine chloride and sodium deoxycholate



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were obtained from Calbiochem Behringer (La Jolla, CA).

RESULTS

Vascular responses to NE in sepsis. The CLP procedure reproducibly suppressed isolated aortic function in each of the studies in which responses to NE were measured. Rings from septic rats, when untreated by other agents, manifested diminished contraction during maximal NE stimulation and, generally, an increase in EC_{50} values (Table 1 and Figs. 1 and 2). Preliminary experiments indicated that NE doses $>10^{-5}$ M always produced less tension than 10^{-5} M, presumably by activation of β_1 -receptors.

Effect of EDRF blockade by myoglobin treatment. Myoglobin (50 μ M) frequently enhanced conditioned aortic ring resting tension in a manner similar to that described by others (16). The increase in tension above the original base-line tension by rings from control rats averaged 133 ± 30 mg tension/mg tissue wt; $n = 9$. Those from sham-operated rats increased 90 ± 27 ; $n = 9$. Those from CLP rats increased 123 ± 38 ; $n = 11$. The increases in tension were not different between the three experimental groups (analysis of variance). Aortic rings from septic animals exhibited significantly enhanced contractile responses to NE in the presence of myoglobin; this effect was not seen in similarly treated rings from control or sham-operated rats (Fig. 3, variance test for homogeneity of the binomial distribution; $P < 0.05$). EC_{50} values for NE during myoglobin treatment were calculated utilizing any increase in base-line tension due to myoglobin as the new base line for the NE dose-response curve. Myoglobin treatment decreased EC_{50} values for all treatment groups (Table 1; $P < 0.001$), mimicking the responses seen in mechanically deendothelialized rat aortas (17).

Contractile response to phorbol 12,13-dibutyrate and modulation of NE-induced contraction by methylene blue and 8-bromo-cGMP. Aortic rings with intact endothelium

exhibited prompt and robust contraction when exposed to phorbol 12,13-dibutyrate (10^{-6} M). The maximal tension generated by rings from septic and sham-operated rats were essentially identical (Fig. 1).

Aortic rings from septic rats were much more sensitive to both methylene blue and 8-bromo-cGMP than were rings from sham-operated rats. Comparison of contractile performance by rings with intact endothelium revealed that inhibition of guanylate cyclase by methylene blue (10^{-5} M) in rings from sham-operated rats caused essentially no increase in tension above that induced by 10^{-5} M NE, whereas similarly treated rings from septic rats responded to methylene blue with a 28% increase in tension (from 497 ± 49 to 637 ± 52 mg tension/mg tissue; $P < 0.01$). The initial difference in maximal tension between rings with intact endothelium from sham-operated and septic rats ($P < 0.001$) was no longer significant after the methylene blue treatment (Fig. 1).

The influence of exogenous cGMP on ring function was examined in aortic rings lacking endothelium (confirmed by no response to 10^{-6} M ACh during the conditioning procedures; data not shown) and continuously treated with methylene blue (10^{-5} M). Comparison of the responses to NE in the continuous presence or absence of 8-bromo-cGMP (10^{-4} M) indicates that exogenous cGMP can significantly suppress the maximal contraction attained by rings from both sham-operated (645 ± 50 vs. 761 ± 51 mg tension/mg tissue; $P < 0.005$) and septic (348 ± 37 vs. 609 ± 38 ; $P < 0.001$; Fig. 1) rats. Whereas exogenous cGMP significantly reduced responses to NE by rings from both sham-operated and septic rats, the suppression of maximum contraction was much greater in tissue from septic rather than sham-operated rats. The response of rings from septic rats to NE in the presence of 8-bromo-cGMP was $58 \pm 6\%$ of that of non-8-bromo-cGMP-treated rings; in contrast, 8-bromo-cGMP-treated tissue from sham-operated rats performed at $84 \pm 3\%$ of the nontreated rings (Fig. 1).

TABLE 1. Mean integrated dose and EC_{50} responses to norepinephrine by aortas from sham and septic rats

	Control	Sham	Septic	P
<i>Effects by myoglobin on norepinephrine-induced contraction</i>				
Not treated	$4,511 \pm 283$ (9)*	$3,389 \pm 345$ (9)	$2,232 \pm 300$ (11)	<0.01
	-7.02 ± 0.09 †	-6.92 ± 0.10	-6.89 ± 0.17	NS
Treated with myoglobin, 50 μ M	-7.75 ± 0.12	-7.88 ± 0.11	-7.94 ± 0.05	NS
	‡			
<i>Effects by methylene blue and 8-bromo-cGMP on norepinephrine-induced contraction</i>				
Not treated		$4,261 \pm 227$ (12)	$2,401 \pm 280$ (12)	<0.001
		-7.44 ± 0.06	-6.80 ± 0.19	<0.01
Deendothelialized		$10,291 \pm 669$	$7,878 \pm 425$	<0.01
+ methylene blue, 10 μ M		-9.13 ± 0.13	-8.93 ± 0.10	NS
Deendothelialized		$3,968 \pm 313$	$2,078 \pm 190$	<0.001
+ methylene blue, 10 μ M		-7.64 ± 0.06	-7.58 ± 0.10	NS
+ 8-bromo-cGMP, 100 μ M				
<i>Reference norepinephrine-induced contractions for comparison with KCl-induced contractions</i>				
Not treated		$3,576 \pm 156$ (7)	$2,151 \pm 304$ (7)	<0.01
		-7.24 ± 0.08	-6.78 ± 0.12	<0.01

Values are means $\pm$ SE. *Mean integrated dose response (expressed as area: mg tension/mg tissue $\times$ $\ln$ [NE] M); † Mean log EC_{50} (M); ‡ Integrated dose response cannot be calculated due to myoglobin effect on base-line tension.

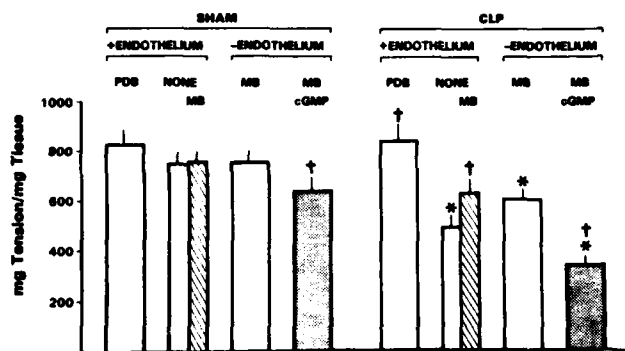


FIG. 1. Maximum contractile responses by aortic rings from sham-operated ($n = 12$) and septic (CLP, $n = 12$) rats. Four rings per rat were tested (from left to right) under following conditions. 1) Endothelium intact ring plus phorbol 12,13-dibutyrate (PDB) (10^{-6} M). 2) Endothelium intact ring plus cumulative norepinephrine (NE). This response should be taken as control response (no other treatments) against which others may be compared. Attached (■), increased tension after methylene blue (MB) (10^{-5} M) treatment. 3) Deendothelialized ring plus cumulative NE (10^{-10} – 10^{-8} M) while continuously exposed to MB (10^{-5} M). 4) Deendothelialized ring plus cumulative NE (10^{-10} – 10^{-8} M), while continuously exposed to both MB (10^{-5} M) and 8-bromo-cGMP (10^{-4} M) (■). * Significant difference in contractile responses between equivalently treated rings from sham-operated and septic rats. † Significant differences between untreated (control) and treated aortic rings from sham-operated rats or significant differences between untreated and treated aortic rings from septic rats. Data are presented as means $\pm$ SE.

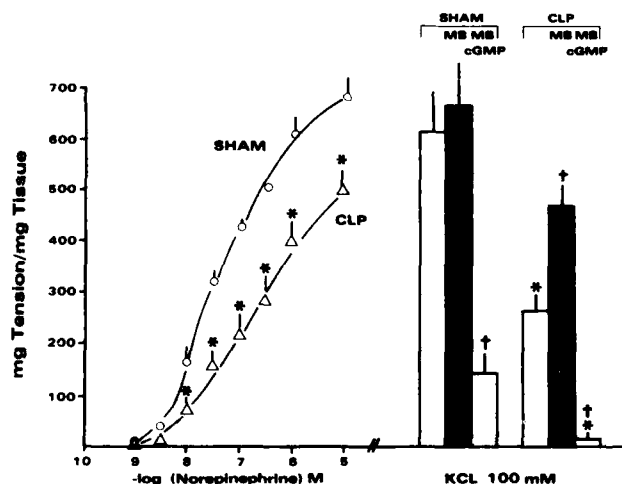


FIG. 2. Cumulative dose-response curves to norepinephrine (NE) (left) and maximum contractile responses to 100 mM KCl (right) by aortic rings from sham-operated ($n = 7$) and septic (CLP, $n = 7$) rats. Four rings per rat were tested. Two rings were exposed to cumulative NE doses, and remaining 2 rings were contracted with 100 mM KCl (□). ■, Increases in tension by KCl-contracted rings after methylene blue (MB) (10^{-5} M) treatment. ▤, Stable tension developed subsequent to exposure to 8-bromo-cGMP (10^{-4} M) by KCl- and MB-treated rings. Data are presented as means $\pm$ SE of averaged duplicate observations. Some SE bars were deleted for clarity in graphing. Symbols indicating significant differences are same as described in Fig. 1.

This relative difference in sensitivity to 8-bromo-cGMP between rings from sham-operated and septic rats is significant ($P < 0.01$).

Altering aortic ring function by removing the endothelium and treatment with methylene blue (10^{-5} M) caused a marked equivalent reduction in EC_{50} values for NE by

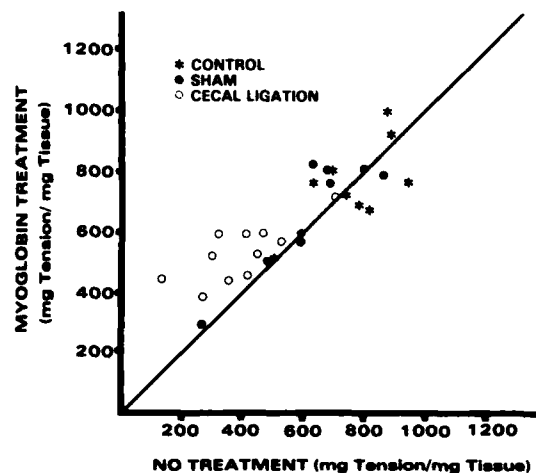


FIG. 3. Maximum contractile responses to norepinephrine (NE) by aortic rings from control ($n = 9$), sham-operated ($n = 9$), and septic (CLP, $n = 11$) rats. Paired aortic rings were tested in presence (ordinate) or absence (abscissa) of 50 μ M myoglobin. /, Isotension between ring pairs. Points above the line indicate an enhanced contractile response by myoglobin-treated number of an aortic ring pair (○, ●, *).

aortas from sham-operated and septic rats ($P < 0.001$; Table 1) in comparison to values from untreated tissue. The reduced EC_{50} values were reflected in very large integrated dose-response estimates of aortic ring performance; however, because maximal contraction by tissue from septic rats was not completely restored with these treatments (Fig. 1), a difference in performance between aortic rings from sham-operated and septic rats remained (Table 1).

Replacement of endogenous cGMP with 8-bromo-cGMP (10^{-4} M) in deendothelialized methylene blue-treated rings from sham-operated rats during NE-induced contractions prevented any significant decrease in EC_{50} . Tissue from septic rats in contrast, manifested a significant decline in EC_{50} values for NE despite the exogenous 8-bromo-cGMP (Table 1).

Modulation of KCl-induced contraction by methylene blue and 8-bromo-cGMP. The diminished vascular contraction caused by sepsis is more apparent when paired aortic rings from the same animal are maximally contracted with KCl (100 mM, 57% suppression) instead of NE (10^{-5} M, 28% suppression; Fig. 2). The responses of aortic rings precontracted by KCl to sequential application of methylene blue (10^{-5} M) and 8-bromo-cGMP (10^{-4} M) were similar to those of the NE-contracted rings. Methylene blue had little additional effect on KCl-induced contraction by tissue from sham-operated rats but caused a striking enhancement of contraction in tissue from septic animals ($P < 0.001$; Fig. 2). Aortic rings from sham-operated and septic rats were both strongly suppressed by 8-bromo-cGMP (10^{-4} M), with tension by rings from septic animals returning to baseline values (Fig. 2).

Tissue cGMP content. Basal levels of cGMP were not different in tissue from sham-operated or septic rats (Table 2). Exposure of aortic rings to NE (10^{-7} M) for 5 min caused a small nonsignificant (by paired t test) increase in cGMP content in both tissues; however, the

relative differences in cGMP response to NE produced a significant disparity in cGMP content of tissue from sham-operated and septic rats (Table 2). Acetylcholine (10^{-6} M) for 1 min induced a 30-fold increase in cGMP content in aortic rings from sham-operated rats, whereas rings from septic rats generated significantly less cGMP (13-fold; Table 2).

DISCUSSION

Aortic rings from septic rats manifest impaired contractile performance in response to NE or KCl stimulation. The sensitivity to NE (EC_{50}) and maximum tension generated after NE and KCl are both adversely affected by sepsis. Sepsis-induced disorders in vascular function that could contribute to this state include 1) impaired structural or functional integrity of vascular contractile elements (23, 25) or inadequate energy stores from contraction; 2) defects in α_1 -receptor number or coupling to intracellular second-messenger systems (4, 26); and 3) altered intracellular Ca^{2+} messenger activity mediated by enhanced Ca^{2+} efflux (13), inhibited Ca^{2+} release or influx (11), or subnormal responses by Ca^{2+} -activated processes (8). Cyclic GMP-induced vasodilation in normal vascular tissue has been attributed to influences in each of these categories (11, 13, 23, 25, 26, 30).

Equivalent contraction by aortas from sham-operated and septic rats after exposure to phorbol 12,13-dibutyrate (PDB) indicates that tissue from septic animals contracts normally when stimulated by an agent that bypasses the requirement for receptor (NE)- or depolarization (KCl)-initiated contraction; it is therefore unlikely that the septic state damages contractile elements or decreases energy stores to an extent that normal contraction cannot be supported. The phosphodiesteratic hydrolysis of membrane phosphoinositides subsequent to binding of an agonist (NE) to its α_1 -adrenergic receptor yields at least two active second-messenger products, 1,2-diacylglycerol (DAG) and inositol 1,4,5-trisphosphate, both of which induce or sustain vascular contraction (3, 26). PDB substitutes for DAG in the activation of protein kinase C (PKC), a phospholipid- and Ca^{2+} -dependent enzyme (see Ref. 21 for review). The mechanism whereby activated PKC induces vascular contraction is not completely understood, but one action may involve phosphorylation of components of the actin intermediate system to produce a tonic contraction (22). The Ca^{2+} dependency for PKC activation is probably satisfied at normal intracellular resting Ca^{2+} levels in the presence of DAG or phorbols (21).

Aortic ring EC_{50} values for NE are altered after inhi-

bition of synthesis of endogenous cGMP or after augmentation of intracellular cGMP concentration by treatment with exogenous cGMP. An analogous modulation by cGMP of the competitive action of the α_1 -adrenoceptor antagonist prazosin has recently been reported in the rat aorta (1). In all cases, inhibition of cGMP action improved function of aortic rings from septic animals (Figs. 1-3 and Table 1). However, it is clear that contractile performance by rings from septic rats was not completely normalized, even in comparisons where no significant statistical difference in contraction remained after inhibition of cGMP production (i.e., rings from sham-operated and septic rats containing intact endothelium contracted with NE or KCl and then treated with methylene blue). The data may reflect a basal cGMP effect that cannot be entirely prevented by myoglobin or methylene blue treatment. Others (14) have shown that 30 μ M methylene blue cannot completely prevent an increase in cGMP in the rabbit aorta after treatment with acetylcholine.

The enhanced sensitivity to cGMP apparent in tissue from septic rats, which occurs in the presence of normal or low intracellular cGMP concentration (Table 2), implies that vasodilatory effector processes dependent on cGMP are augmented or that the contractile "drive" against which cGMP-induced vasodilation acts is reduced. Sepsis-induced reductions of ~50% in hepatic α_1 -receptor number and aortic basal- and NE-stimulated second-messenger production occur in the rat (4). Cyclic GMP treatment also inhibits accumulation of a second-messenger product of phosphoinositide hydrolysis (inositol monophosphate) and vascular contraction in the rat aorta (26). Because NE-induced phosphoinositide hydrolysis and contraction are closely correlated in the rabbit aorta (3), a similar relationship in the rat aorta could yield the diminished contraction observed in tissue from septic animals. It should be noted, however, that a sizeable α_1 -adrenergic receptor reserve exists in vascular smooth muscle cells isolated from the rabbit aorta. Receptor-coupled Ca^{2+} efflux, used as an index for NE-stimulated intracellular Ca^{2+} mobilization, decreased only 8% even when 69% of α_1 -receptors were irreversibly inactivated with phenoxybenzamine (7). In addition, KCl-induced vascular contraction does not cause phosphoinositide hydrolysis in the rat aorta (6), and at maximally stimulatory concentrations of KCl, release of catecholamines from nerve terminals in the isolated tissue contributes little to the contractile response (29). These observations, in view of the strikingly greater sepsis effect on KCl- vs. NE-induced contractions (Fig. 2), suggest that diminished contractile ability in sepsis cannot be attributed solely to α_1 -receptor-associated disorders.

Cyclic GMP-dependent phosphorylation of multiple proteins, via cGMP-dependent protein kinase, is associated with a concomitant decrease in myosin light-chain phosphorylation in the rat aorta (25). However, effects of cGMP on contractile elements may be secondary to changes in other regulatory processes, because cGMP does not influence calmodulin activation of bovine aortic vascular myosin light-chain kinase (12). Cyclic GMP

TABLE 2. Effect of norepinephrine and acetylcholine on cGMP content of aortas from sham-operated and septic rats

	Sham (n = 9)	Septic (n = 11)	P
Treatment			
None	3.61±0.76	3.19±0.99	NS
NE, 10^{-7} M	6.41±0.85	4.16±0.67	<0.05
ACh, 10^{-6} M	109.69±31.31	42.12±10.64	<0.05

Values are means ± SE in pmol cGMP/mg protein.

also does not elicit significant relaxation in chemically skinned rat aorta (18), although relaxation to cGMP has been observed in chemically skinned porcine coronary arteries (23).

Cyclic GMP reduces intracellular Ca^{2+} concentration through several mechanisms. Enhanced ATP-dependent Ca^{2+} uptake occurs in porcine thoracic aorta sarcolemmal membranes after cGMP treatment (30), and stimulation of rat aortic vascular smooth muscle cells with nitroglycerin (which activates guanylate cyclase) decreases intracellular Ca^{2+} concentration, probably by accelerated extrusion of Ca^{2+} through the sarcolemmal membrane (13). Cyclic GMP also decreases NE-stimulated Ca^{2+} influx into the rat aorta (11). Several of the mechanisms by which cGMP inhibits vascular Ca^{2+} availability could be active in sepsis; unfortunately, the current data do not indicate which is the most likely process. The potential defect in Ca^{2+} supply could also diminish receptor-induced contraction, because influx of extracellular Ca^{2+} is required for maintenance of tonic NE-induced contraction by the isolated rat mesenteric artery (5). Defects in the Ca^{2+} messenger system have been demonstrated in hepatocytes from endotoxin-treated or septic rats (8).

A caveat to the interpretation that a lesion in intracellular Ca^{2+} supply can explain the data in this study is the observation that phorbol esters and calcium act synergistically to mimic the contractile effects of neurotransmitters on dog basilar artery (2) and to enhance the Ca^{2+} sensitivity and contraction of skinned porcine coronary arteries (19). If basal levels of phosphoinositide hydrolysis (which produces DAG) provide meaningful synergism with KCl-induced increases in intracellular Ca^{2+} , then diminished basal DAG production in tissue from septic animals (4) could result in decreased maximal contraction to KCl stimulation.

The question of increased activity of cGMP-dependent vasodilatory effector processes in sepsis is unresolved. However, in view of the improved contractile function by tissue from septic rats after inhibition of cGMP action (Figs. 1-3), investigations in this area would aid in delineating which limb of the counterregulatory relation between vascular contraction and relaxation is altered in sepsis.

In summary, diminished contractile responses by aortic rings from septic rats to NE or KCl stimulation are not mediated by elevated levels of cGMP in septic tissue. If vascular tissue cGMP content is used as an index for release of endothelium-derived relaxing factor by endothelial cells, then release of this relaxing factor by aortas from septic rats is not augmented in sepsis. It may, in fact, be suppressed. Aortic tissue from septic rats is more sensitive to cGMP than is tissue from sham-operated rats; therefore inhibition of endogenous cGMP or application of exogenous cGMP produces disproportionately greater responses by rings from septic animals. Aortic rings from septic and sham-operated rats are able to contract to the same extent when treated with the PKC activator PDB; hence, intracellular energy stores and PDB-activated contractile structures are able to support normal contractions in sepsis.

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The opinions and assertions contained herein are the private ones of the author and should not be construed as reflecting the views of the US Navy, the naval service at large, or the Department of Defense. 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, Department of Health, Education, and Human Welfare (publ. no. NIH 85-23).

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