Dexamethasone Blocks Sepsis-Induced Protection of the Heart from Ischemia Reperfusion Injury (44466)

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Abstract. **Previous investigations have shown that sepsis, while causing cardiac dysfunction, can protect the heart from ischemia-reperfusion injury. Sepsis-induced protection may be due to nitric oxide produced by an inducible form of nitric oxide synthase generated in response to cytokines released during sepsis. The glucocorticoid dexamethasone has been shown to inhibit the synthesis of the inducible form of nitric oxide synthase (iNOS). The goals of this study were to determine if dexamethasone would prevent sepsis-induced cardiac dysfunction and sepsis-induced protection of the heart from ischemia-reperfusion injury. In this experiment, rats were made septic by injecting *Escherichia coli* into the dorsal subcutaneous space. Control rats were injected with sterile saline. At the time of surgery, some of the control and septic animals were injected intraperitoneally with dexamethasone (3 mg/kg). The next day, 24–26 hr after injection of the first dose of *E. coli*, animals were anesthetized, and hearts were removed and studied in the isovolumic beating-heart preparation. Left ventricular end diastolic pressure was set to 5 mmHg, and left ventricular pressure was measured continuously throughout the protocol. Left ventricular developed pressure (LVDP) was used as an index of LV function. After stabilization, hearts were made globally ischemic for 35 min and then reperfused for 25 min. As has been shown previously, sepsis depressed LVDP but also protected the heart from further depression of LVDP by ischemia and reperfusion. Dexamethasone prevented both sepsis-induced cardiac dysfunction and sepsis-induced protection of the heart from ischemia-reperfusion injury. In addition plasma nitrite/nitrate levels were not different from control levels in the dexamethasone-treated septic rats whereas levels were elevated in the septic animals. The dexamethasone mediated abrogation of sepsis-induced cardiac dysfunction and protection during ischemia-reperfusion injury may be due to suppression of nitric oxide production.** [P.S.E.B.M. 2000, Vol 223]

Sepsis is the number one cause of mortality in intensive care units today (1). Two important factors in the pathophysiology of sepsis are the vascular disturbances and the myocardial depression (2) resulting from the interaction between the host defense mechanisms and the invading/contaminating bacterial elements (1). Despite antibiotic therapy and intensive care support, septic patients still have a 35%–60% mortality (3). The pathophysiological mechanisms of the host/bacteria interaction must be elucidated so that treatment regimens can be developed to reduce the high mortality of this syndrome.

Attempts to study the pathophysiology of sepsis have often involved animal models. Gram negative bacteria have been shown to induce sepsis and septic shock in animals thus resulting in a number of cardiovascular sequelae (4–6). Rats receiving *Escherichia coli* in the dorsal subcutaneous space exhibit a depression of cardiac function (6). Sepsis-induced cardiac dysfunction is apparent *in vitro*, both in terms of peak systolic pressure development and cardiac output, over a wide range of left ventricular volumes (7). Although there is cardiac depression induced by sepsis, at the same time there is a seemingly paradoxical protection of

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the heart from ischemia-reperfusion injury. When hearts from septic animals are made globally ischemic for 35 min and then reperfused for 25 min, there is complete recovery of left ventricular developed pressure to levels comparable to the preischemic levels (8). This recovery is not seen in the hearts of control animals. Under similar ischemic conditions, control hearts recover only 80% of the preischemic function.

Preconditioning is a type of protection of the myocardium from ischemia-reperfusion injury. Preconditioning is generally induced by several short replicates of ischemia followed by reperfusion, which results in protection of the myocardium from a longer ischemic episode. This protection has been shown to be present for only a few hours after the preconditioning stimulus and then to reappear 24 hr after the initial preconditioning (9, 10). Recently, Bolli and coworkers have shown that late preconditioning to ischemic injury involves nitric oxide as both a trigger for and a mediator of the late preconditioning (9, 10). Inhibition of nitric oxide production prevents preconditioning-induced protection of the heart from ischemia. Thus, there is evidence for a potential role for nitric oxide in the sepsis-induced protection of the heart from ischemia-reperfusion injury that occurs the day after the induction of sepsis.

Nitric oxide has also been proposed as a possible mediator of sepsis-induced cardiac dysfunction (1). During sepsis, cytokines, and other factors cause nitric oxide levels to rise *via* induction of a nitric oxide synthase (1, 11). Lipopolysaccharide, IL-1β, TNFα, INFγ, and IL-6 have all been shown to induce iNOS mRNA and activity in cardiac myocytes (12). Since iNOS is a source of nitric oxide, it can supplement the normal nitric oxide levels produced by constitutive nitric oxide synthase (cNOS) (1, 13). Thus, if nitric oxide is the mediator of cardiac functional alterations during sepsis, then it is conceivable that suppression of iNOS could prevent some of the sepsis-induced cardiac changes.

Dexamethasone is a glucocorticoid that has been shown to inhibit iNOS production by repressing gene expression, inhibiting protein synthesis, and decreasing iNOS protein stability (14, 15). The present experiment was designed to assess the effects of dexamethasone during sepsis by monitoring cardiac function before ischemia and recovery of cardiac function after 35-min ischemia and 25-min reperfusion. In addition blood nitrite/nitrate levels were measured at the time of sacrifice because they can be used as an index of nitric oxide levels.

Materials and Methods

Surgery. Male Sprague Dawley rats (271–397 g) from Charles Rivers (Wilmington, MA) were stabilized for at least 1 week in the Medical Center animal care facility prior to use. The LSUMC Institutional Animal Care and Use Committee approved the protocols for this project. On the day of surgery, rats were anesthetized with ketamine:xylazine (40:5 mg/kg) *via* an intramuscular injection. A polyethylene catheter was inserted into the dorsal subcutaneous space after the skin had been cleared from the underlying tissue. The rats were weighed, and 3 mg/kg dexamethasone was administered intraperitoneally to those rats that were in the dexamethasone groups. There were four experimental groups: control, control with dexamethasone, septic, and septic with dexamethasone. Thirty minutes after surgery (generally around 10 AM), 0.1 ml *Escherichia coli* (containing $\approx 10^9$ *E. coli*) was injected into the catheters of the septic groups, and sterile saline was injected into the catheters of the control groups. After surgery, the rats were put into individual cages and provided with water and no food. Five hours after the surgery, the temperature was measured on some of the animals, by inserting a catheter containing a thermistor into the colon. Temperature was displayed on an Edwards cardiac output computer (Santa Ana, CA). Also, 5 hr after the surgery, the injection of *E. coli* was repeated for the septic groups.

Heart Preparation. The day after surgery colonic temperatures were again measured. The animals were anesthetized by injection of sodium pentobarbital (35 mg/kg) intraperitoneally. The abdomens were opened, and aortic blood samples were taken. Plasma was used for analysis of nitrite/nitrate by the Griess method after deproteinization with zinc sulfate and conversion of nitrate to nitrite with nitrate reductase (16). Immediately after thoracotomy, the hearts were removed and placed in cold Krebs Henseleit bicarbonate buffer. The individual hearts were then hung on a cannula from a water-jacketed Langendorff apparatus (8). A balloon attached to a size-4 dual lumen catheter was inserted into the left ventricle *via* the left atrium and attached securely within the hearts. The balloon volumes were increased to set the left ventricular end diastolic pressure to $\approx$ 5 mmHg as measured by a P23 ID transducer and recorded by a Grass polygraph (Warwick, RI). If the intrinsic rate of the hearts was below 300, they were paced at 300 beats per minute with a Grass stimulator.

Ischemia/Reperfusion. The hearts were stabilized for $\approx$ 5 min, and coronary flow was measured. The hearts were then made globally ischemic for 35 min by occluding the tubing from the Langendorff column to the heart. Pacing was stopped during ischemia. Hearts were then reperfused for 25 min, and pacing was resumed after the hearts achieved a regular rhythm. Coronary flow was measured during the preischemic period, every 30 sec during the first 5 min of reperfusion, and every 5 min for the remaining 20 min. Left ventricular pressure was measured continuously and recorded with a Grass polygraph. The left ventricular end diastolic pressure was subtracted from the systolic pressure for reporting of left ventricular developed pressure (LVDP) that was used as an indicator of cardiac function.

Statistical Analysis. Mean, standard error of the mean, and sample numbers are reported. The preischemic and postischemic values were analyzed statistically with paired *t* tests or analysis of variance (ANOVA) with repeated measures. Comparisons between groups were made with a *t* test whereas comparisons among the groups were

made with ANOVA. In reporting statistical significance, outliers were discarded if they were more than the mean plus three times the standard deviation.

Results

In some animals, colonic temperature was determined at ≈ 5 hr after the first injection of *E. coli* (Fig. 1). Control animals had a normal temperature at 5 hr as did control animals treated with dexamethasone. Septic animals had an elevated temperature by 5 hr, whereas septic rats that received dexamethasone 30 min before the first dose of *E. coli* did not have an increased temperature. Temperatures at 24 hr were elevated in both septic groups when compared with the control groups. Animals in the dexamethasone-treated septic group had a temperature comparable to the septic group at 24 hr in spite of the apparently slower development of fever as suggested by the normal temperature at 5 hr.

Nitrite/nitrate levels are reported in Figure 2 for all animals from which a blood sample could be obtained at the time of sacrifice. The septic group had an elevated nitrite/nitrate level when compared with both control groups and the septic group treated with dexamethasone. In the septic group of animals that were treated with dexamethasone, the sepsis-induced rise in nitrite/nitrate was not seen.

The volume of the water injected into the balloon to achieve an end diastolic pressure of 5 mmHg was measured. The volume of water needed to achieve this end diastolic pressure is an index of ventricular compliance. The mean ± standard deviation and number for each group was as follows: control = 0.12 ± 0.02 ml (n = 5), control with dexamethasone = 0.11 ± 0.01 ml (n = 6), septic = 0.07 ± 0.01 ml (n = 6), and septic with dexamethasone 0.10 ± 0.02 ml (n = 7). Although the lower volume required in the septic group would suggest that there was a tendency for a decrease in compliance in sepsis, analysis of variance showed no difference among the four groups.

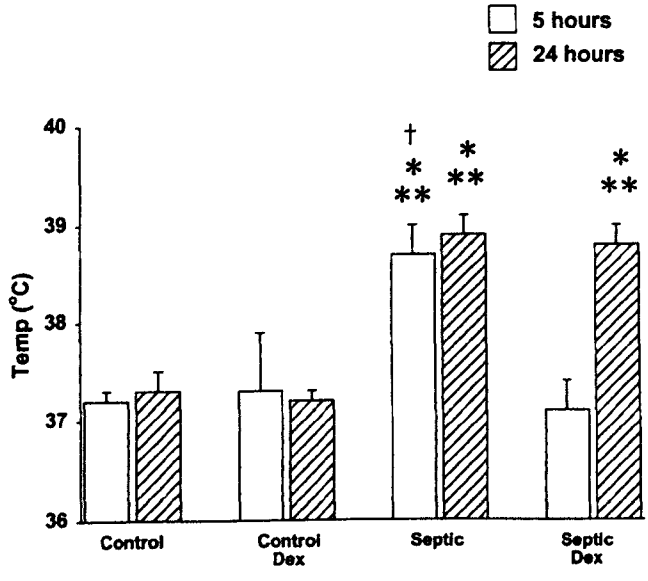

Figure 1. Colonic temperature 5 hr and 24 hr after surgery in the Control (n = 4), Control with Dexamethasone (Dex) (n = 5), Septic (n = 7), and Septic with Dex (n = 7) groups; *P < 0.05 different from Control within the postsurgery time group; **P < 0.05 different from Control with Dex within the postsurgery time group; †P < 0.05 different from Septic with Dex within the postsurgery time group. The figure displays the 24-hr temperatures of only the animals from which 5-hr temperatures were obtained.

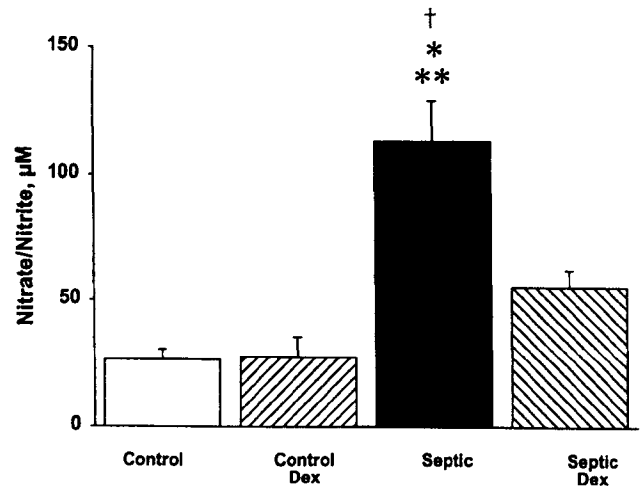

Figure 2. Blood levels of nitrite/nitrate at time of sacrifice in the Control (n = 6), Control with Dexamethasone (Dex) (n = 7), Septic (n = 9), and Septic with Dex (n = 10) groups; *P < 0.05 different from Control; **P < 0.05 different from Control with Dex; †P < 0.05 different from Septic with Dex.

The LVDP before ischemia exhibited trends in common with those reported previously (6–8) (Fig. 3). The LVDP of the septic group was decreased when compared with all the other experimental groups in accordance with the characteristic sepsis-induced cardiac dysfunction. LVDP in the hearts of septic rats treated with dexamethasone was comparable to LVDP in the hearts of control rats.

After ischemia the control, control with dexamethasone, and septic with dexamethasone groups did not recover LVDP to preischemic levels. Ischemia-reperfusion injury was not seen in the septic group in which postischemic LVDP was not significantly different from the preischemic

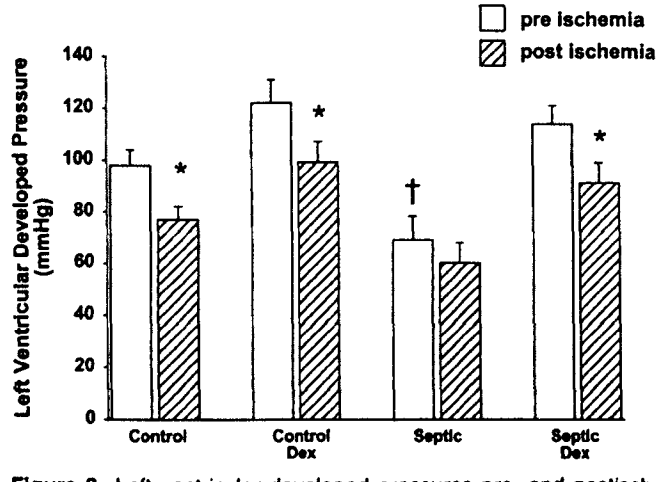

Figure 3. Left ventricular developed pressures pre- and postischemia (after 25 min reperfusion) of hearts in the Control (n = 5), Control with Dexamethasone (Dex) (n = 6), Septic (n = 6), and Septic with Dex (n = 5) groups; *P < 0.05 different from preischemia; †P < 0.05 different from Control, Control with Dex, and Septic with Dex.

LVDP. The recovery of the developed pressure of the animal hearts after 25 min of reperfusion as expressed as a percentage of the preischemic developed pressure for the groups was: control = 78.6% ± 2.5%, control with dexamethasone 80.5% ± 2.9%, septic = 90.5% ± 11.6%, and septic with dexamethasone = 79.7 ± 6.0%. The hearts of the rats in the septic group exhibited protection from the ischemia-reperfusion injury.

The coronary flows were not different among the groups either before ischemia or at 25 min after ischemia (Fig. 4). By 25 min of reperfusion, the coronary flow had returned to preischemic levels for all groups with the exception of the septic group. The septic rat hearts recovered coronary flow to only 76% of the preischemic level. This suggests that recovery of coronary flow may not be required for recovery of postischemic LVDP in septic rat hearts.

Discussion

Nitric oxide was originally known to affect vascular smooth muscle contraction. More recently, it has been noted that nitric oxide may have other effects including myocardial effects. Nitric oxide causes vasodilation and may be involved in the massive vasodilation that occurs in sepsis (1, 3). In myocytes, nitric oxide and iNOS induction have also been shown to cause changes in myocardial metabolism, relaxation, and contractile function (1, 11, 12, 17–19). Dexamethasone, by inhibiting production of iNOS, may prevent the rise in nitric oxide and thereby attenuate cardiac dysfunction and vasodilation.

Several investigators have found that pretreatment of rats with dexamethasone prior to the administration of endotoxin partially (20) or completely abolishes endotoxin-induced contractile dysfunction (21). We have shown previously that dexamethasone partially alleviates myocardial dysfunction induced by gram negative bacteremia (22). In the present study, treatment of rats at the time of induction of sepsis prevented sepsis-induced cardiac dysfunction. The

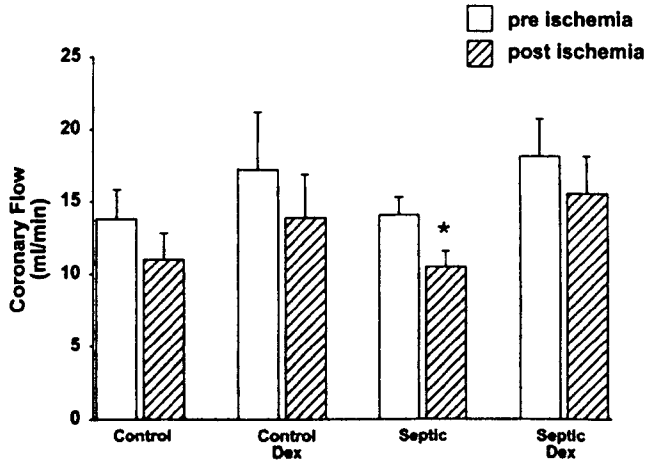

Figure 4. Coronary flow pre- and postischemia (after 25 min reperfusion) of hearts in the Control (n = 5), Control with Dexamethasone (Dex) (n = 6), Septic (n = 6), and Septic with Dex (n = 5) groups: *P < 0.05 different from Control.

apparent complete inhibition of cardiac dysfunction, in the present study, as opposed to partial inhibition, in our previous study (22), could have resulted from the use of an isovolumic preparation in the present study in which ventricular-developed pressure was measured at only one preload. Our previous study evaluated the hearts in a working heart preparation in which both pressure work and volume work were measured, and volume work may be a more sensitive measure of loss of cardiac reserve. Dexamethasone thus appears to attenuate, if not prevent, sepsis-induced cardiac dysfunction. The mechanism of protection may be due to the prevention of the induction of iNOS. The levels of nitric oxide, as estimated by the nitrate/nitrite assay, were decreased in septic animals pretreated with dexamethasone as was the cardiac dysfunction. This association suggests that nitric oxide may have a role in the cardiac dysfunction due to sepsis.

It is interesting to note that dexamethasone prevented or attenuated sepsis-induced cardiac dysfunction, cardiac protection, and the rise in plasma nitrite/nitrate levels. Although dexamethasone appeared to prevent the early development of fever in septic animals, this protection was not observed at 24 hr. Dexamethasone therefore did not completely block all of the effects of bacteremia in this model. In preliminary studies in which a 3–5 fold greater dose of bacteria was given, dexamethasone improved survival. Three out of three dexamethasone-treated septic rats survived whereas only one of three untreated septic animals survived to 24 hr. LVDP of the isolated heart of the surviving untreated septic animal was only 13 mmHg whereas it was ≈ 60 mmHg in hearts from the dexamethasone-treated rats. Thus dexamethasone may attenuate dysfunction in a severe sepsis model and prevent dysfunction in a less severe sepsis state although not all symptoms of bacteremia are equally affected.

During ischemia and reperfusion, oxygen free radicals, including superoxide, are formed (23). Superoxide has a high affinity for nitric oxide and can combine with nitric oxide to form peroxynitrite, which is also a radical (1, 13). Scavenging of superoxide by nitric oxide could potentially attenuate cardiac injury if superoxide is the damaging agent. The elevated plasma nitric oxide levels, as reflected by the nitrite/nitrate levels, suggest that there may be a significant amount of nitric oxide available for binding of superoxide in the postischemic septic rat heart. This may be one mechanism for protection from ischemia-reperfusion injury seen in septic rat hearts; however, it has also been suggested that the nitric oxide/superoxide combination may be detrimental to tissues (13).

When the hearts of septic animals were subjected to 35 min of ischemia and then reperfused for 25 min, the hearts were able to recover left ventricular developed pressures to levels similar to the preischemic pressures. The cause of this protection from ischemia-reperfusion injury exhibited by the hearts of septic animals has not been elucidated. Perhaps hearts of septic animals recovered LVDP postischemia be-

cause ischemia was initiated from a lower pressure. However, if the pressures of the hearts of the individual septic animals were evaluated, even the hearts of animals that had preischemic left ventricular developed pressures near the normal control level were able to recover pressure close to their preischemic values. The present study shows that the hearts of the septic animals that were pretreated with dexamethasone did not exhibit protection from ischemia-reperfusion injury. Dexamethasone lowered the levels of nitric oxide in these animals, as measured by the nitrite/nitrate assay. The association between the decrease in the level of nitric oxide before ischemia and the dissolution of protection suggests that nitric oxide might also have a role in the protection of septic hearts from ischemia-reperfusion injury. This result is similar to the findings of Bolli *et al.* (9, 10) that inhibition of nitric oxide synthase can prevent late protection in the preconditioned heart.

Although this study suggested a role of nitric oxide, dexamethasone does not function solely to prevent induction of iNOS. This glucocorticoid inhibits synthesis of a number of proteins. Dexamethasone affects the synthesis and activity of many products that may have a role in cardiac function. It decreases both the free and the total activities of lysosomal enzymes such as β-glucuronidase, β-N acetyl glucosaminidase, β-galactosidase, α-galactosidase, α-mannosidase, cathepsin B and cathepsin D (24). Dexamethasone has also been shown to markedly increase osteopontin mRNA and secretion that may be a mechanism by which dexamethasone suppresses iNOS (25). Wu *et al.* suggest that glucocorticoids induce the expression of lipocortin 1 that mediates some of the anti-inflammatory effects and may mediate the inhibition of iNOS seen with dexamethasone administration (26). Dexamethasone is also a phospholipase A_2 inhibitor and has been shown to inhibit endothelin-1–stimulated arachidonic acid release in human ciliary muscle cells (27). Glucocorticoids also block conversion of arachidonic acid by cyclooxygenase into prostaglandins and suppress the production of various other inflammatory mediators (28). Thus, inhibition of iNOS induction may be only one mechanism by which dexamethasone prevents sepsis-induced protection of the myocardium.

Dexamethasone abrogated sepsis-induced cardiac dysfunction; thus, the mediators of the cardiac dysfunction may be one, or more, of the molecules of which dexamethasone prevents expression. Further research will be required to determine what combination of proteins and/or enzyme products are involved in this process. We have also demonstrated protection of hearts of septic animals from ischemia-reperfusion injury, and that dexamethasone prevented this protection. Thus, one or more of the products inhibited by dexamethasone must be involved in this protection.

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