Effect of Endotoxin on Hemodynamics and Norepinephrine Metabolism in the Dog (36788)

PAPINENI S. RAO, BUDH D. BHAGAT, AND DENIS CAVANAGH
(Introduced by W. Van Beaumont)

Department of Gynecology and Obstetrics and of Physiology, St. Louis University School of Medicine, St. Louis, Missouri 63104

Endotoxin shock is a syndrome resulting from sepsis due to gram-negative bacteria. Coliform endotoxins, most commonly used in inducing experimental endotoxin shock are water soluble lipopolysaccharide–protein complexes which on the death of the cell are released from the cell membrane of the invading gram-negative bacteria into the tissue of the host. Circulatory failure following the intravenous administration of coliform endotoxin in experimental animals has been reported by several investigators and this circulatory failure associated with gram-negative bacillary bacteremia is a clinical problem of increasing magnitude.

It has been suggested (1, 2) that selective vasospasm is the mediating mechanism in endotoxin shock. It is known that endotoxins release various neurohormonal agents, *viz.*, histamine, serotonin and catecholamines, all of which can be associated with an increased sympathoadrenal activity and selective vasospasm. Further, endotoxins may alter the normal response of the peripheral vasculature (resistance and/or capacitance vessels) to endogenous sympathomimetic agents. A recurrent theme in the history of shock has been the importance of the adrenal glands and the degree of sympatho-adrenal involvement in this disease process. Rosenberg *et al.* (3) noted a rise in plasma catecholamine concentration in the dog immediately after endotoxin administration. Epinephrine soon returned to control levels but norepinephrine remained slightly elevated. Likewise, Spink and his co-workers (4) reported a rise in plasma epinephrine levels with the onset of hypotension and then a gradual return to normal levels. Contrary to the results of others, these investigators found little or no elevation of norepinephrine. Heiffer, Mundy and Mehlman (5) have suggested that during the late stages of endotoxin poisoning there is evidence that the rabbit becomes refractory to increased endogenous epinephrine and norepinephrine. In the subhuman primate, we have found a significant increase in plasma norepinephrine levels at three minutes after endotoxin administration and thereafter, the levels began to fall and were near normal at 120 min (6)

The aim of the present study was to investigate further the effect of endotoxin on catecholamines in order to gain information on the mechanism involved. For this purpose the catecholamines and metabolites were measured in plasma and selected organs.

Materials and Methods. Fourteen dogs (female) of random breed with an average weight of 12.5 kg were anesthetized by intravenous administration of pentobarbital sodium (20 to 30 mg/kg of body wt). They were divided arbitrarily into two groups having seven in each group. Coliform endotoxin (Difco Laboratories, Detroit, MI) was administered intravenously as a single bolus in a dose of 2 mg/kg of body weight; while control animals received normal saline only. The endotoxic group as well as the control group were injected with the labeled norepinephrine so as to study its decay in plasma and its content in the tissues.

Hemodynamic Determinations. Pressures were measured by Statham transducers, and a Sanborn recorder (7700 series) was used for the continuous monitoring of aortic pressure, central venous pressure, renal artery flow, and electrocardiographic tracings. Aor-

tic pressure was measured by passing a polyethylene catheter along the femoral artery into the aorta to the level of the renal arteries. Central venous pressure was measured by passing a polyethylene catheter along the femoral vein into the inferior vena cava so that the tip came to rest just proximal to the right atrium. This venous catheter was used to inject lipopolysaccharide *Escherichia coli*. A square wave electromagnetic flow meter and a corresponding probe (acute) were used to measure the left renal artery flow. Renal artery pressure was measured as renal orifice pressure, and renal vascular resistance was calculated from directly measured renal blood flow. Standard electrocardiogram leads were used to record cardiac rate and rhythm. Urinary output was measured with the use of ureteral polyethylene catheters. Statistical analysis (t test) was performed according to the method described by Steel and Torrie (7).

Catecholamine studies. Estimation of ^{3}H-norepinephrine and ^{3}H-normetanephrine. *dl*-Norepinephrine-7-^{3}H (10 μCi/kg) (4.5 to 7 Ci/mmole, New England Nuclear Corporation, Boston, MA) was administered to the dogs intravenously 30 min prior to the administration of *E. coli* endotoxin. The blood samples were obtained 5 min before and 3, 5 and 30 min after the administration of endotoxin. Blood (10 ml) was drawn from the femoral artery into ice-cold tubes containing 0.1 ml of heparin sodium (1000 units/ml) and 100 μg of ascorbic acid. Centrifugation was immediately carried out at 4° for 20 min at 3000 rpm. The plasma was drawn off and was deproteinized with ice-cold 0.4 N perchloric acid.

The animals were sacrificed 120 min after the endotoxin administration and selected organs (spleen, left ventricle, left atrium, right atrium, adrenals and kidney) were analyzed for ^{3}H-norepinephrine and its metabolites. The tissue was homogenized in ice-cold 0.4 N perchloric acid.

The protein-free supernatant solutions obtained after centrifugation were absorbed on alumina at pH 8.6. The catecholamines were eluted with 0.05 N perchloric acid as described by Anton and Sayre (8). All values

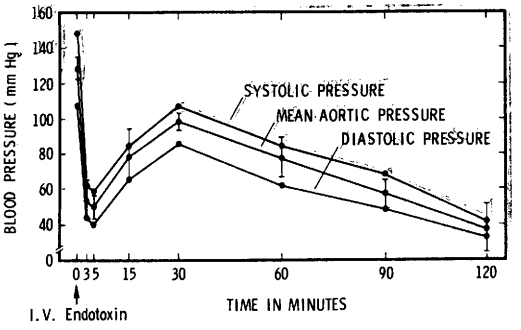

FIG. 1. The effects of intravenous administration of coliform endotoxin on the aortic pressure. Note the hypotension occurring at 3 min following the injection of endotoxin.

reported for ^{3}H-norepinephrine are uncorrected for the degree of recovery which ranged from 66 to 88% of the added counts. A 1.0 ml aliquot of the alumina eluates was counted in a liquid scintillation counter. ^{3}H-Normetanephrine was determined by adjusting the Al_2O_3 effluent and wash to pH 6.5 This was passed through a 7.5 $\times$ 1 cm Dowex-column (Dowex 50W-X4, 100–200 mesh, which has been soaked in 3 N NH_4OH overnight, and washed until it was the pH of water) and washed with 20 ml of H_2O. ^{3}H-Normetanephrine was eluted with 15 ml of 3 N NH_4OH. The elutate was partially dried and a 1 ml aliquot was counted. No correction for recovery of normetanephrine was made in final calculations.

Results; hemodynamic determinations. Aortic blood pressure. A graphic presentation of the events occurring in the systolic diastolic and mean aortic pressure following the intravenous administration of *E. coli* endotoxin is shown in Fig. 1. The pressures fell precipitously within 3 min ($p < .01$) following the administration of coliform endotoxin but reached a maximum within 5 min. During that time the mean aortic pressure fell from a control value of 129 to 50 mm Hg, depicting a drop of 62%. After this episode a recovery phase was noted and a progressive improvement in the pressures was observed over a 30 min period. The mean aortic pressure at this time was 95 mm Hg. Following this brief recovery phase the pressures fell gradually and that trend continued over the

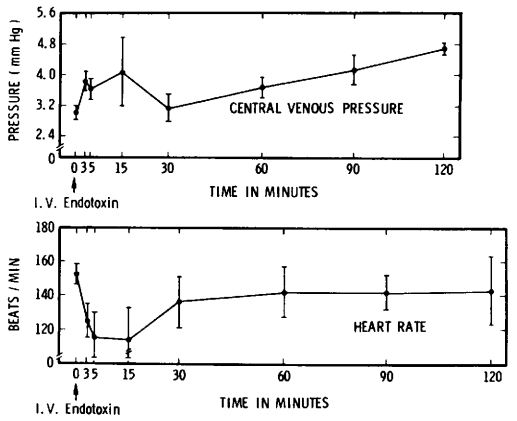

FIG. 2. Effects of intravenous injection of coliform endotoxin on central venous pressure and heart rate. The catheter was placed proximal to the right atrium. Note the sustained increase in pressure after 30 min and relative bradycardia.

120 min study period. At the end of the experiment the mean aortic pressure was 40 mm Hg.

Central venous pressure. Prior to the administration of coliform endotoxin the mean central venous pressure was 3 mm Hg. Following the administration of endotoxin it increased slightly at 3 min and returned to near normal control value at 30 min. This coincided with the recovery phase and from then on it increased progressively reaching a value of 4.9 mm Hg at 120 min. (Fig. 2).

Heart rate. Following endotoxin injection bradycardia occurred immediately and the heart rate fell from 152 to 126 beats/min at 3 min, to 116 beats/min at 5 min ($p < .05$) and was 113 beats/min at 15 min. Thereafter it increased but remained below normal levels (Fig. 2).

Renal artery flow. The renal artery flow fell precipitously from a control value of 124 to 17 ml/min within 3 min ($p < .01$) following the intravenous administration of coliform endotoxin (Fig. 3). There was a brief partial recovery in which the renal flow improved to 51 ml/min at 30 min and was 56 ml/min at 60 min. After this brief recovery period, however, the renal artery flow fell progressively over the 2 hr study period and was 26 ml/min at 120 min. The increase in renal resistance showed a peak at 10 min and returned to near control value at 60 min. Although there was a general rise after this it was not marked (Fig. 3).

Urinary output. The urinary output fell from a control value of 10 to 2 ml/hr within the first 30 min following the intravenous administration of coliform endotoxin. Oliguria persisted and was pronounced as the experiment progressed, reaching a value of 0.6 ml/hr at 120 min.

Neurohormonol studies. Plasma ^{3}H-norepinephrine and ^{3}H-normetanephrine. These values were estimated prior to and at 3, 5 and 30 min after the administration of coliform endotoxin. Since in control animals the values of plasma ^{3}H-norepinephrine at 20 min after its administration did not change significantly during the following 60 min period, therefore it was considered more appropriate to express the values at 3, 5 and 30 min after shock as percentage of those found prior to the administration of endotoxin. With the fall in blood pressure following the endotoxin administration plasma norepinephrine levels fell to 63% of control value, at 5 min norepinephrine levels had improved to 90% of the control value and remained essentially unchanged thereafter. In contrast, plasma ^{3}H-noremetanephrine showed a steady increase and was 148, 192 and 202% of control value at 3, 5 and 30 min, respectively (Fig. 4).

Organ distribution of ^{3}H-norepinephrine and ^{3}H-normetanephrine. Radioactive norepinephrine and normetanephrine was estimated in the spleen, ventricle, left and right atrium, adrenals and kidney for controls as well as for animals in endotoxic shock. The results show that ^{3}H-norepinephrine concentration in the atria, ventricles and kidney remained relatively unchanged, whereas, the levels in the spleen and adrenals were significantly increased when compared with those of the control animals. An increase in the ^{3}H-normetanephrine level in organs from endotoxic animals was evident when compared with levels in the organs from control animals with the exception of the adrenals (Fig. 5).

Discussion. Endotoxin shock is the most common type of septic shock seen in the pregnant woman. The hypotensive state is

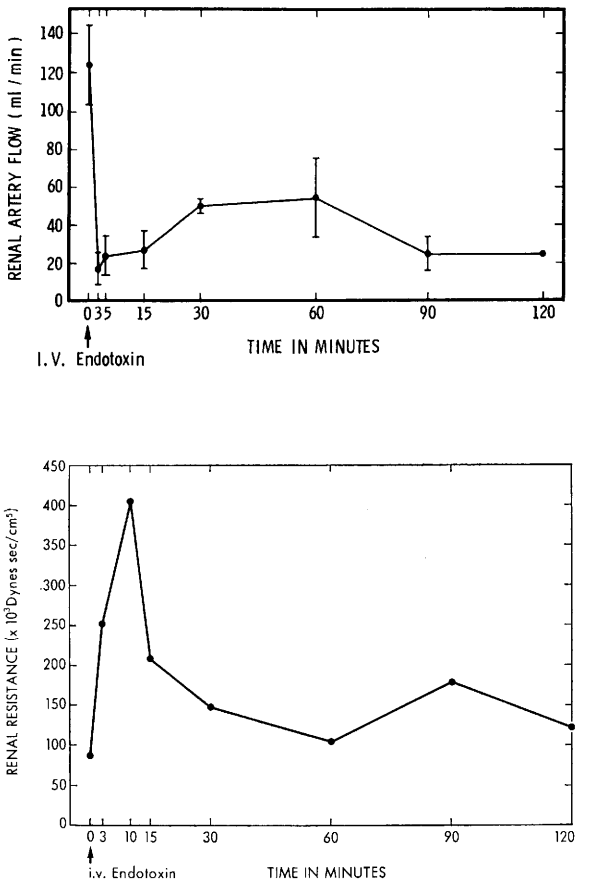

FIG. 3. Effect of coliform endotoxin on renal artery flow and renal resistance. A precipitous fall in the renal artery flow within 3 min following the intravenous administration of coliform endotoxin was a consistent finding. It should be noted that this is not independent of the aortic pressure, also note the transient recovery phase followed by a progressive fall.

not associated with blood loss, but may result from a reduction in blood volume, cardiac failure, failure of the peripheral vessels to maintain their normal tonus or a combination of these factors. In any event a disparity exists between the circulating blood volume and the capacity of the vascular bed (6).

Since endotoxin shock is associated with an increase in sympathetic nervous activity, the prominent physiologic disturbances in shock have been quite logically attributed to vasoconstriction. In the present study, a precipitous fall in the aortic blood pressure within 3 min following the intravenous administration of coliform endotoxin is a consistent finding. There was a recovery phase followed by a progressive fall during the 2 hr study period. Similar findings have been reported by other investigators (9–12).

As the aortic pressure fell, the pulse pressure also fell from a control value of 41 to 18 mm Hg within the first 3 min. Although there was a trend towards improvement during the first 60 min of the experiment, a gradual fall was evident during the later part. This, of course, is expected since the associated adverse condition becomes more marked with the length of time the animal is in shock.

Intravenous injection of endotoxin induces relative bradycardia in the dog. The heart rate fell to 76% of control within 15 min but was 96% at 120 min. In contrast, in our studies on the subhuman primate (6) only

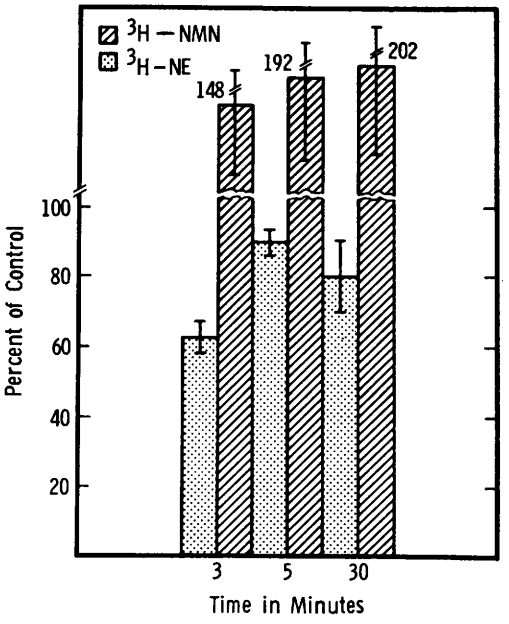

FIG. 4. Effects of intravenous administration of coliform endotoxin on the plasma norepinephrine and normetanephrine. Animals were given ³H-norepinephrine (10 μg/kg) intravenously. The experimental animals were injected with coliform endotoxin 30 min after the labeled catecholamine and were killed 120 min thereafter. The animals treated with ³H-norepinephrine but not with endotoxin, killed at the same time served as controls. Note the increase in normetanephrine while the norepinephrine levels remained low. Each bar indicates the mean ± SEM of seven experiments.

sinus tachycardia was observed. This difference is probably due to differential species response to endotoxin (13). This could also be due to direct toxic effect of endotoxin on the heart.

Immediately after injecting endotoxin, the central venous pressure showed an increase. There was a transient return to near control value at 30 min but from then on there was a progressive rise in the central venous pressure, suggestive of increased resistance in the pulmonary circulation and the reduced myocardial activity. The improvement in the renal artery flow at 30 min probably is a reflection of the improvement in the systemic pressure.

The changes in the renal artery flow correlate well with the changes in the aortic pressure, the main difference being that the recovery phase in the renal artery flow was prolonged up to a 60 min period. Urinary output fell almost immediately following the injection of coliform endotoxin and oliguria persisted during the entire experimental period. The oliguria associated with endotoxin shock is perhaps due to a reduction in aortic pressure, resulting in decreased perfusion pressure. Apparently, it is not due to a specific effect of endotoxin on the renal vasculature as it is in the subhuman primate (14).

Of all the homeostatic mechanisms initiated by acute hypotension, the most important would appear to be an intense activation of the sympathoadrenal system. Increased release of epinephrine from the adrenal medulla and norepinephrine from the postganglionic sympathetic nerve endings constitute the most immediate and most effective protective mechanism. When ³H-norepinephrine

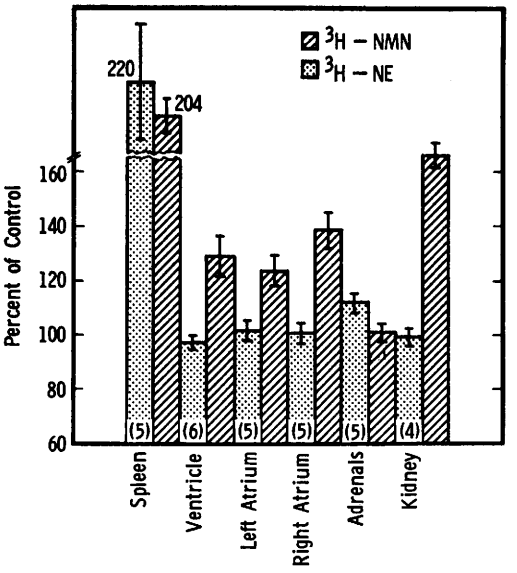

FIG. 5. Norepinephrine and normetanephrine in selected organs, 120 min after the injection of coliform endotoxin. Note the significant increase of norepinephrine in the spleen and adrenals while the concentration in the atria, ventricles and kidney remained relatively unchanged. An increase in the normetanephrine level was evident in all the organs tested except adrenals. The results are expressed as percentage of control. The number of experiments is indicated in parentheses in each bar.

is injected intravenously, it is taken up by the axonal membrane of the sympathetic nerve endings retained in storage vesicles, where it gradually equilibrates with endogenous norepinephrine stores and is released in response to nerve stimulation.

In this study with the fall in blood pressure following endotoxin administration, plasma ^{3}H-norepinephrine levels fell to 63% of control value. Five minutes after endotoxin injection the norepinephrine levels had risen to 90% of the control value, while blood pressure has decreased further. Thirty minutes after endotoxin administration, the plasma norepinephrine levels remained essentially unchanged. While plasma norepinephrine levels fell, there was a marked increase in normetanephrine levels, suggestive of increased release and metabolism of norepinephrine. It is also possible, that the large increase in norepinephrine found in spleen and kidney may be due to the extremely poor circulation to those organs during endotoxin shock. The spleen undergoes marked shrinkage during shock.

Norepinephrine (labeled) concentration in the atria, ventricles and kidneys remained relatively unaltered, whereas, the levels in the spleen and adrenals were significantly increased. There was also an increase in ^{3}H-normetanephrine level in all these organs suggesting an enhanced catabolism of labeled amine. It seems that norepinephrine released from the sympathetic nerves may cause vasoconstriction which then traps the hormone in the vascular bed. This may allow the discharged norepinephrine to return to the sympathetic nerves resulting in normal or elevated levels in organs (*e.g.*, spleen and adrenals) and lower levels in the plasma.

The enhanced uptake of ^{3}H-norepinephrine may be due to the effect of endotoxin on the permeability of the neuronal membrane resulting in increased extraction of norepinephrine and a decrease in plasma norepinephrine. It has been suggested (15) that splenic nerve and adrenal gland may have mechanisms and requirements for norepinephrine uptake, storage and distribution which differ from other tissue (*i.e.*, heart, brain, etc.). Thus in the animals receiving endotoxin some of the requirements for uptake and distribution may be markedly altered by the direct or indirect action of endotoxin.

Summary. Intravenous administration of *E. coli* endotoxin to anesthetized dogs caused a precipitous fall in arterial blood pressure and renal artery flow within 3 min. With the fall in blood pressure, plasma ^{3}H-norepinephrine fell to 63% of control levels. Five minutes after endotoxin administration, the ^{3}H-norepinephrine level increased to 90% of the control value. Thirty minutes after endotoxin administration, blood pressure had risen to 75% of control levels and ^{3}H-norepinephrine remained unchanged. ^{3}H-Norepinephrine concentration in the atria, ventricle and kidneys remained unchanged, but the levels in the spleen and adrenals were significantly increased. In all cases ^{3}H-normetanephrine levels increased suggestive of increased release and metabolism of catecholamines. It is suggested that endotoxin exerts certain changes in cellular permeability resulting in increased extraction of norepinephrine from the circulating blood.

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