The Treatment of Endotoxin Shock by Beta Adrenergic Blockade

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DURING the past few years there has been a progressive increase in the incidence of gram negative infections. Shock, a not infrequent complication of this sepsis, still has a mortality of more than 50% in hospitalized patients despite all known methods of treatment.[1] The results of the treatment of endotoxin shock in animals have also been poor. In the Oklahoma Shock Tour[8] many investigators treated endotoxin shock using the methods which they specifically advocated including vasodilators, vasoconstrictors, corticosteroids, aldosterone, anticoagulants, low molecular weight dextran, blood, splanchnic denervation, celiac blockade and adenosine triphosphate. The overall results showed no significant difference in survival rates between the controls and the treated animals.

Berk and associates previously suggested that in shock of various causations excessive beta adrenergic stimulation in the pulmonary and splanchnic areas results in hemodynamic changes eventually leading to irreversible shock and death.[2, 3] These authors also demonstrated a significant increase in survival rates of dogs bled into hemorrhagic shock and treated with beta adrenergic blockade.[4] The treated dogs showed much improved hemodynamics and did not show the pathological changes consistent with excessive beta adrenergic stimulation in the pulmonary and splanchnic areas seen in the control dogs.

The purpose of this paper is to show the effect of beta adrenergic blockade on endotoxin shock in the dog.

Methods

Ninety healthy mongrel dogs, isolated for 3 weeks, weighing between 6 and 25 Kg. were anesthetized by the intravenous administration of thiamylal sodium (Surital) 15 mg./Kg. A cuffed endotracheal tube was inserted and ventilation accomplished by a positive pressure respirator using room air. The minute volume was determined by a ventilation graph for laboratory mammals by Kleinman and Radford.* The adequacy of ventilation was monitored by periodic measurements of oxygen and carbon dioxide partial pressures and adjusted accordingly during the control period.

Nonocclusive polyethylene catheters were placed in both femoral veins and arteries. One vein was used for infusion of drugs and the other for withdrawal of samples for glucose analysis. Via one artery mean arterial pressure was measured by a strain

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gage manometer and continuously recorded. From the other artery samples were withdrawn for the measurement of oxygen and carbon dioxide pressures and pH, these measurements being made on 47 dogs, 20 in group 1, 19 in group 2, and 8 in group 3 during the control period and for 6 hours following the endotoxin injection or until the dog awakened. A catheter was placed into the superior vena cava through a fore-leg vein for the measurement of central venous pressure. An electrocardiogram was continuously recorded.

Oxygen partial pressures were measured by a Clark type polarographic electrode; carbon dioxide pressures by a Severinghaus type electrode; and pH by a blood electrode. The instruments were calibrated periodically and measurements made at 37 degrees centigrade. Oxygen pressures were corrected to a pH of 7.40. Plasma glucose concentration was measured by the Ortho-Toluidine method.

Intramuscular injections of Kanamycin Sulfate 7.5 mg./Kg., and Procaine Penicillin G, 1,000 units/Kg. were given pre- and postoperatively.

Heparin sodium, 2 mg./Kg., was given intravenously and time was allowed for the stabilization of all parameters. The dogs were then injected rapidly through the femoral vein with 0.5 mg./Kg. endotoxin, *Escherichia coli* lipopolysaccharide (Difco Laboratories 0127:B8). One to 2 mg./Kg. of protamine sulfate were given when necessary depending upon bleeding from the incisions. The dogs were observed until breathing was spontaneous and satisfactory without the respirator and were then returned to their cages. Those that lived for 24 hours were considered survivors and were sacrificed at 72 hours. Postmortem examinations, both gross and microscopic, were carried out on all dogs.

The dogs were divided into the following groups:

Group 1. Thirty-six dogs were in this untreated group.

TABLE 1. *Comparison of Propranolol Dose, Infusion Period, Time Given after Endotoxin Versus Survival*

Dose Propranolol (mcg./Kg.)	Infusion Period (minutes)	Time Given After Endotoxin (minutes)	No. Dogs	No. Lived
300	120	5	6	5
150	3	5	3	3
150	3	60	3	2
200	3	15	1	1
250	3	60	6	4
500	3	60	1	1
750	3	60	2	2
1,000	3	15	2	1
1,000	3	60	1	1
1,500	3	15	2	1
1,500	3	30	1	1
1,500	3	45	1	1
1,500	3	60	3	2

Group 2. The 32 dogs in this group, beginning 5 to 60 minutes after endotoxin injection, were given intravenous propranolol * by an infusion pump either at a rate of 2.5 μg./Kg. for 120 minutes, or received propranolol over a 3-minute period, the doses ranging from 150 to 1,500 μg./Kg. (Table 1). Ringer's solution was infused until the arterial pressure rose to, or the central venous pressure rose above control values, the average amount being 40 ml./Kg. Sodium bicarbonate was given intravenously in small intermittent doses based on the arterial pH, the average total amount being 3 to 5 milliequivalents per kilogram. The following drugs were given to counteract the undesirable effects of propranolol as previously outlined by the authors [4]: Fifty per cent dextrose in water was infused periodically depending on the blood sugar values, an average of 20 ml./Kg. being given. Five per cent dextrose in water was given by subcutaneous clysis before the dogs were returned to their cages, an average of 28 ml./Kg. being given. Atropine, 0.02 mg./Kg. was given intravenously when the pulse rate fell significantly

* Inderal®, Ayerst Laboratories.

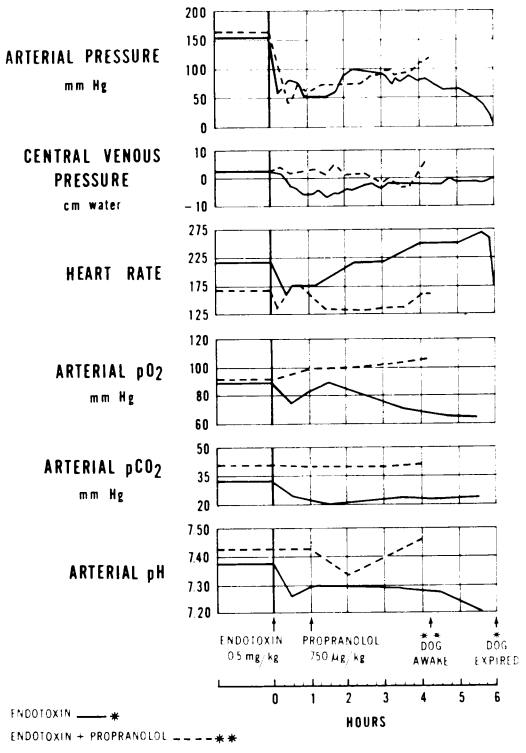

FIG. 1. Hemodynamic and biochemical changes occurring in typical dogs given endotoxin alone, group 1, and endotoxin and propranolol, group 2.

below control values. Five dogs were given calcium chloride, 10 to 20 mg./Kg. to improve myocardial contractility. Ten dogs received caffeine and sodium benzoate, 72 mg./Kg., when during the recovery period their level of consciousness decreased.

Group 3. Twenty-two dogs were in this group. They received the same drugs as group 2, except propranolol was omitted. The average amounts given of 50 per cent dextrose in water was 10 ml./Kg. and of Ringer's solution was 80 ml./kilogram.

Results

Survival. In group 1, 7 of 36 dogs lived giving a 19.4 per cent survival rate. Twenty-five of 32 of the dogs of group 2 survived, a 78.2% survival rate, and of the dogs in group 3, 6 of 22 survived, a 27.2% survival rate. A statistical comparison of these results using tests of equality of proportions and chi square showed a significant difference among all groups, P being <0.001. The number of dogs in each propranolol time-dose group was too small to be statistically significant.

Hemodynamic Changes. The dogs of groups 1 and 3 following the injection of endotoxin showed a rapid fall in arterial pressure lasting 30 to 60 minutes, the pressure then gradually returning to normal or subnormal levels. Following this there was a progressive decline in the pressure until death. The dogs that were given endotoxin and then treated with propranolol showed hemodynamic patterns that were quite different from those given endotoxin alone. These dogs had the early hypotensive phase but the second phase was absent (Fig. 1).

The group 3 dogs required twice as much Ringer's solution as did the group 2 dogs to maintain their arterial pressure, the former receiving 80 ml./Kg., the latter 40 ml./Kg.

There was a significant difference among the three groups in the arterial oxygen partial pressure following the endotoxin injection (Table 2). On comparing the lowest oxygen pressure between 4 and 6 hours, the dogs of group 2 had significantly higher per cent of control values than those of groups 1 and 3; and group 3 values were significantly higher than group 1. In group 1 the arterial oxygen pressures fell progressively below control levels following the endotoxin, those that died having a significantly greater fall than those that lived. In group 2 there was a gradual increase above control values of the oxygen pressures reaching a peak between 4 and 6 hours. The oxygen pressures did not change significantly in group 3. All of these differences were found to be statistically significant, $p < 0.001$, by means of the non-parametric analysis of variance using chi square. In group 3 there was an increase in the oxygen pressure of those that lived, but this was not statistically significant.

TABLE 2. *Comparison of Arterial Oxygen Pressures of Dogs Receiving Endotoxin and Different Treatments*

			Mean ± S.D. Arterial pO_2 mm. Hg, 37° C., pH 7.40 Figures in Parentheses Indicate Per Cent of Control					
			Control			4 to 6 Hours After Endotoxin		
Group	No. Dogs	No. Lived	All	Lived	Died	All	Lived	Died
1	20	5	83.0 ± 10.5	87.2 ± 16.0	81.6 ± 8.2	70.9 ± 14.9 (85.4)	84.8 ± 11.0 (97.0)	66.2 ± 13.2 (81.0)
2	19	15	81.3 ± 9.6	82.1 ± 10.2	78.5 ± 7.2	90.3 ± 15.34 (111)	91.8 ± 13.4 (112)	85.0 ± 22.8 (109)
3	8	2	88.0 ± 4.0	91.5 ± 3.5	86.8 ± 3.6	90.7 ± 23.9 (103)	98.5 ± 4.9 (108)	88.1 ± 27.6 (101)

Pathological Changes. The dogs of group 1 that died consistently showed gross areas of hemorrhage in the lungs, livers and spleens. The livers appeared turgid. The upper gastrointestinal tracts showed marked hyperemia of the mucosa, this being most marked in the duodenum and less in the stomach and upper jejunum. There was often blood in the stomachs and intestines. Microscopically the lungs were by far the most severely effected organ, showing Grade IV congestive changes with dilated vessels and focal to confluent hemorrhages scattered throughout the parenchyma, but particularly in the peribronchial areas. Edema was marked and there were many areas of atelectasis throughout. The livers and spleens showed congestive changes, Grade I to III, with dilated vessels and hemorrhages the latter often distorting the architecture. The upper gastrointestinal tracts showed moderate congestive changes, Grade I to II, most evident in the duodenum. In most dogs there was a loss of one or more surface cell layers exposing dilated suffused capillaries sufficiently so to account for the intraluminal hemorrhage. Severe hemorrhagic necrosis, as commonly described by most authors, was never present. The kidneys, skin, subcutaneous tissues, muscles and adrenals appeared essentially normal. The surviving dogs of group 1 showed minimal evidence of atelectasis and congestion in the pulmonary and splanchnic areas. Five out of six of these dogs showed some degree of penumonia. The remaining organs appeared normal.

The surviving dogs of group 2 showed nothing or minimal congestive changes in the pulmonary and splanchnic areas. Twelve had early pneumonia this being present only in those with pulmonary changes. The kidneys, adrenals, skin, subcutaneous tissues and muscles appeared essentially normal. Of those dogs of group 2 that died, there were variable and moderate changes of congestion and atelectasis in the splanchnic and pulmonary areas of four dogs while three showed no changes in these organs. The remaining organs of all group 2 dogs appeared quite normal.

The pathological findings of the dogs of group 3 were similar to those of group 1.

Discussion

In endotoxin shock in the dog there are two phases of hypotension. The early phase occurs immediately after the administration of endotoxin and lasts up to one hour, the arterial pressure then gradually returning to normal or subnormal levels. Following this there is a progressive decline in pressure to death. The first phase is thought to be due to hepatic outflow obstruction which results in hepatosplanchnic pooling, decreased venous return, and a decrease cardiac output. This constriction of hepatic

veins very likely is due to an anaphylactic reaction and can be prevented by pretreatment with phenoxybenzamine, an alpha adrenergic blocker. In most other species including man, hepatic outflow obstruction probably does not occur. This explains why alpha blockade is of no value in the treatment, as opposed to pretreatment, of shock.

The second phase of hypotension in the dog is similar to that which occurs in other species and probably represents the effects of endotoxin that are of clinical importance. The cause of the second phase has been unknown and there has been no satisfactory treatment once shock is established. The early and late phases may be superimposed, the first affecting the second, but they do not seem to be causally related.

In these studies beta adrenergic blockade prevented the second phase of hypotension when given as long as one hour after endotoxin. Also the dogs given propranolol required significantly less Ringer's solution to maintain their arterial pressure than did the dogs given endotoxin alone. Both of these observations suggest that the dogs treated with propranolol had a better total body perfusion. Since propranolol decreases myocardial contractility and rate, its beneficial effects could only have been on the peripheral circulation. This improvement can be explained by the blocking of the hemodynamic chain reaction set off by excessive beta adrenergic stimulation with the opening of multiple arteriovenous shunts (Fig. 2).

The group of dogs treated with endotoxin and then beta blockade did not show the fall in arterial oxygen pressure as did those given endotoxin alone. These differences in arterial oxygen pressure cannot be explained by changes in ventilation, pH or temperature, but can be explained by right to left circulatory shunting with bypassing of the alveolar capillary membrane in the dogs receiving endotoxin but not in those treated with propranolol. Again the beneficial effect of beta blockade must have been on the microcirculation and can best be explained by the closure of multiple pulmonary arteriovenous shunts (Fig. 2).

The dogs given endotoxin and then sodium bicarbonate and Ringer's solution did not have as large a fall in arterial oxygen pressure as those given endotoxin alone. This can be explained by the improved hemodynamics due to treatment, resulting in less endogenous catecholamine secretion with fewer arteriovenous shunts being opened.

The untreated dogs that survived the endotoxin had only a small fall in arterial oxygen as compared to those that died. This also suggests that the lack of opening of arteriovenous shunts, in this case due to biological variation, is associated with increased survival. Conversely the propranolol treated group that died had a small increase in arterial oxygen suggesting they died from a cause not associated with the opening of shunts, possibly from propranolol as previously noted.[4]

The cause of the increase in the arterial oxygen pressure in the propranolol group is not completely clear. It is possible that the thiamyal sodium caused the opening of multiple arteriovenous shunts in the pulmonary areas giving abnormally low control arterial oxygen pressures with an apparent elevation later when the effect of the anesthetic was no longer present. Pilot studies in this laboratory suggest this as do studies by Steiner and Calvin.[10] Hyperventilation as a cause of the increase in oxygen pressure is suggested by a fall in the partial pressure of carbon dioxide. However, both parameters remained stable during the one hour control period, the fall in carbon dioxide did not occur in all the dogs in which the oxygen levels increased, and most important the oxygen and carbon dioxide values did not vary together, that is the carbon dioxide fell several hours before the oxygen became elevated. Regardless of the cause of the elevation, there was a significant difference in the arterial oxy-

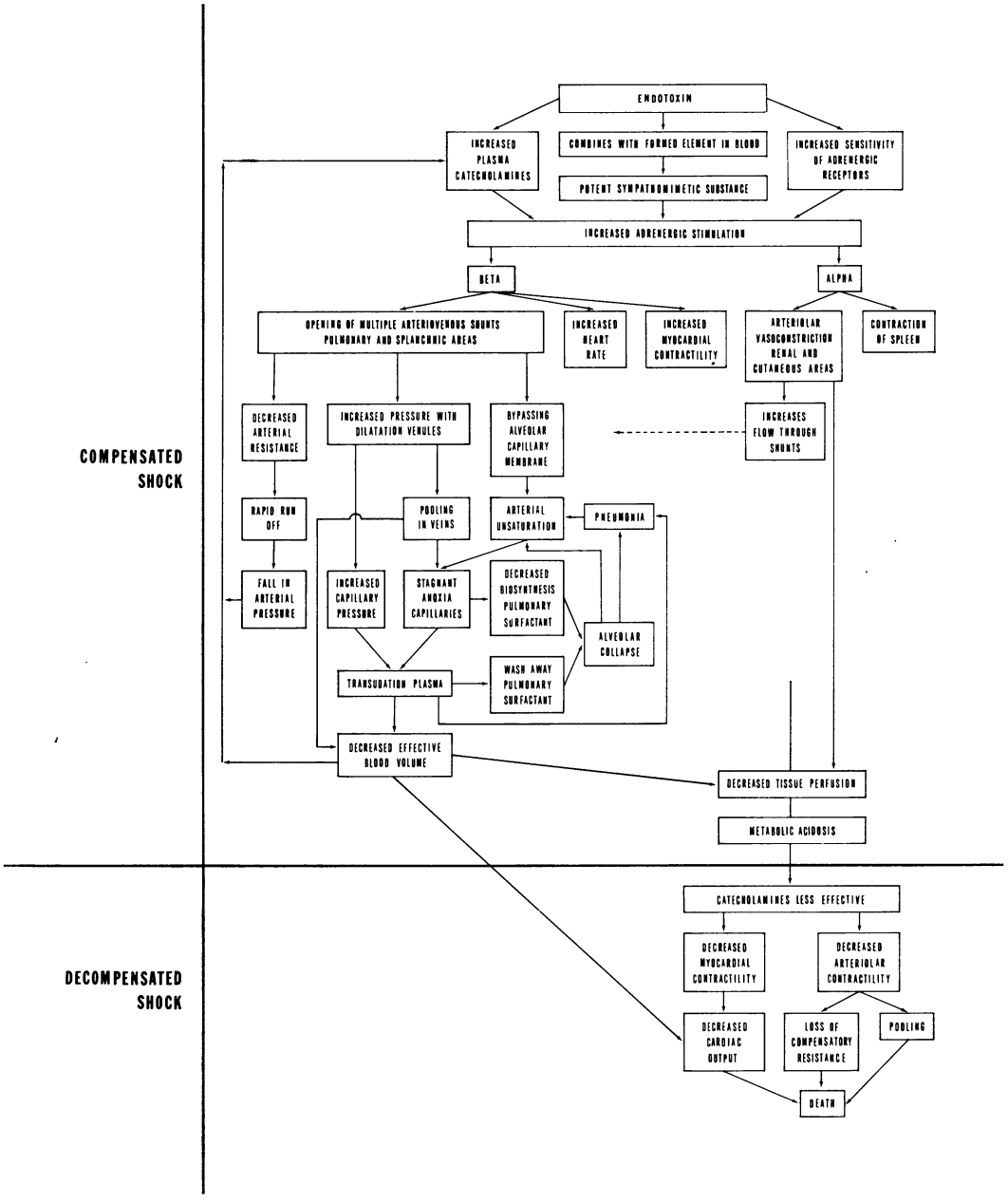

FIG. 2. Suggested hemodynamic changes resulting from endotoxin.

gen pressures following endotoxin among all groups, the propranolol group being the highest.

The dogs in endotoxin shock treated with beta blockade did not have the pathological changes of severe splanchnic and pulmonary congestion and atelectasis suggestive of excessive beta adrenergic stimulation consistently seen in the dogs given endotoxin alone. These findings again suggest the importance of beta adrenergic stimulation in the pathogenesis of shock.

Most of the surviving control dogs and about one half of the dogs treated with

propranolol developed pneumonia despite the vigorous use of antibiotics. Those that died possibly did not live long enough to develop pneumonia. Thus it is likely that endotoxin increases the animal's susceptibility to pneumonia on the basis of the atelectasis and congestive changes which it causes.

In these studies in endotoxin shock the lungs were by far the most severely effected organ, the gastrointestinal tracts being the least effected. This is in contrast to most studies of endotoxin shock in the dog in which the intestines are said to be the most effected organ showing severe hemorrhagic necrosis of the mucosa. These observations suggest that the primary target organ in the dog is the lung and that the hepatosplanchnic pooling is only contributory. Consistent with this are the findings of Hinshaw[7] and Evans[6] who showed in animals in endotoxin shock that intestinal pathological changes are not necessary for death.

In man the lung very likely is the main target organ. The onset of gram negative shock is often heralded by unexpected tachypnea, cyanosis and arterial unsaturation often not helped by a respirator and 100% oxygen. The authors have noted several patients who died from gram negative sepsis in shock whose postmortem examinations showed pulmonary changes identical to those noted in these studies. Rosen[9] has stated that in gram negative shock there is a decrease in venous return in all species. It is possible that in man there could be significant pooling in the hepatosplanchnic area due to the opening of arteriovenous shunts and still not show the pathological changes characteristic of the dog with its peculiar outflow obstruction.

These studies have shown that dogs in endotoxin shock treated with beta adrenergic blockade not only had improved hemodynamic patterns and increased survival rates but also lacked pathological changes consistent with marked beta adrenergic stimulation, as compared to untreated dogs in endotoxin shock. These findings demonstrate the importance of excessive beta adrenergic stimulation in the pathogenesis of septic shock and add further support to the Beta Theory previously suggested by the authors.[4] In shock of various causations there is excessive adrenergic receptor stimulation. In the pulmonary and splanchnic areas beta adrenergic stimulation causes the opening of multiple arteriovenous shunts with a sudden fall in resistance, a rapid arterial runoff and a fall in arterial pressure. The arterialized blood enters the thin walled venules distending them, causing pooling there and stagnant anoxia in the capillaries. There is a loss of plasma from the vascular bed due to capillary anoxia and increased capillary hydrostatic pressure. In the lungs this transudation can wash away pulmonary surfactant leading to alveolar collapse at end expiration.[5] The hypoperfusion causes injury to the type II alveolar cells, the cells responsible for the biosynthesis of pulmonary surfactant, and this also lead to alveolar collapse. Arterial unsaturation results from shunting of the poorly oxygenated pulmonary blood into the arterial circulation due both to the opening of the multiple arteriovenous shunts and to the alveolar collapse and edema.

This dynamic concept of shock explains the seemingly contradictory findings of patients in septic shock. In order to compensate for the hemodynamic chain of events set off by the opening of the arteriovenous shunts, the cardiac output must increase; otherwise early death results. In early septic shock in patients with a good myocardium, there is a hyperdynamic circulation with a high cardiac output, a high central venous pressure, a large blood volume and decreased total peripheral resistance. However, later in shock as the myocardium fails or as the effective blood volume decreases, the cardiac output falls, the total peripheral resistance increases, and the

central venous pressure falls, the latter depending on the relative proportions of left and right heart failure. Rational treatment depends on an understanding of the dynamic concept of shock.

Summary

These studies have shown significant differences in hemodynamic patterns, survival rates, and postmortem findings between dogs given endotoxin and dogs given endotoxin followed by beta adrenergic blockade with propranolol. These differences were present when beta blockade was begun up to one hour after the endotoxin.

The dogs treated with propranolol did not show the second hypotensive phase characteristic of dogs in endotoxin shock, required much less intravenous fluid to maintain the arterial pressure, and showed significantly higher arterial oxygen pressures at 4 to 6 hours. Correlating with these findings the dogs treated with beta blockade had significantly improved survival rates, and showed little of the severe congestive changes in the pulmonary and splanchnic organs consistent with excessive beta adrenergic stimulation and characteristic of the dogs given endotoxin alone. It was suggested that the effect of endotoxin on the lung sets the stage for the development of pneumonia.

These findings offer some insight into the pathogenesis of endotoxin shock and add further support to the Beta Theory of Shock.

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