Plasma Changes in Endotoxin and Anaphylactic Shock (ATP, ADP and Creatine Phosphorus)

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ABSTRACT

Decisive patterns have been demonstrated in plasma adenosine 5' triphosphate (ATP) levels in both endotoxin and anaphylactic shock which correlate with periods of low platelet counts, low arterial pressures and abnormal electrocardiograms. When these irregularities were occurring, the plasma ATP level was low; when improvement occurred, the plasma ATP level rose. Plasma ATP levels appear to be an index to the metabolic state of the animal.

The plasma creatine phosphate (CP) level showed a tendency to decrease when the ATP level dropped in anaphylactic shock, although the CP level did not recover to the same extent as the ATP level. In endotoxin shock, the plasma CP level increased on an average of six-fold. It is proposed that this rise resulted from either CP mobilization from tissues, for the purpose of replenishing the energy deficient myocardial muscle, or possible leakage from damaged cells.

Adenosine diphosphate (ADP) plasma values were measured in both anaphylactic and endotoxin shock. High initial ADP values were prone towards a more severe anaphylactic reaction and a shorter survival time in the endotoxin shock experiments.

Introduction

Adenosine 5' triphosphate (ATP) is universally accepted as the major energy source for cellular function and creatine phosphate (CP) as the energy storehouse for muscle which intercedes to replenish ATP when the supply diminishes. Cellular biochemical analyses in dogs during hemorrhagic and endotoxin shock have demonstrated the existence of an aerobic state27,43 and energy failure.26,42 The high energy compounds ATP and CP were found to be decreased during shock20 and also mechanical restriction of the blood
supply to skeletal muscle produced a reduction in the tissue content of ATP and CP. Chronic heart failure, produced in dogs by pulmonary arterial stenosis, lowered both the ATP and CP myocardial concentrations. These investigations have demonstrated the apparent importance of cellular ATP and CP levels in the maintenance of an adequate cellular function.

One agent suspected to be a prime factor in platelet aggregation is adenosine diphosphate (ADP). With the abnormal loss of ATP in shock, the accumulation of ADP is a possibility. Chen and Jorgensen demonstrated the breakdown of ATP in blood with transitory accumulations of ADP as well as AMP. Ireland and Mills demonstrated that when enough ADP was added to heparinized platelet rich plasma, under conditions that permit platelet clumping, half of the added ADP was gone after 20 minutes and AMP accumulated, then ADP rapidly disappeared to zero after 120 minutes. This prolonged ADP breakdown time could be dangerous as a constant increasing source of ADP becomes available. The accumulation of ADP in the cellular tissues, owing to the inadequate energy restoration of ATP and the possible ability of ADP to cross the cellular membrane, could lead to a dangerous level of ADP in the blood and be a stimulation to platelet aggregation.

Success has been demonstrated in treating dogs in experimental hemorrhagic shock with ATP infusions with claims that this high energy nucleotide alleviated the energy imbalance. Others have shown that ATP can possibly traverse the cellular membrane, which may justifiably explain its success in the treatment of shock. CP infusions have also been shown to increase markedly the survival rate in dogs during hemorrhagic shock. A knowledge of the plasma levels of these high energy compounds in the shock state could be helpful in determining the method or type of treatment. The plasma ATP, ADP and CP level could be an index to the energy state of body tissues and possibly give some indication of the severity of the shock condition. Periodic monitoring of these levels during their infusion may also be important since excessively high plasma ATP and CP levels could be dangerous. ATP is known to be a potent vasodilator and at excessive plasma concentrations could lead to a dangerous drop in blood pressure, and the accumulation of plasma ADP could lead to stimulated platelet aggregation and embolization. These reasons stimulated our interest in the development of clinically useful test to measure plasma ATP, ADP and CP concentrations.

Previous studies from our laboratory on anaphylactic and endotoxin shock enabled one to find the time when a low cellular energy state in rabbits may exist. In the present study, these two shock models were used to determine if any changes occurred in the plasma ATP, ADP or CP and to correlate these changes with changes in the platelet count, arterial pressure and the electrocardiogram to find meaningful patterns which could indicate the arrival of a serious metabolic problem.

Materials and Methods

New Zealand albino rabbits, maintained on a commercial rabbit diet, were used for the studies. Anaphylactic shock was produced in eight male rabbits, sensitized with 2 ml of human serum injected slowly into the marginal ear vein on two consecutive days. Shock was induced after 12 days of the initial sensitizing dose. Shock was induced after 12 days of the initial sensitizing dose. The shock dose consisted of 0.5 ml of human serum diluted to 1 ml with isotonic saline. This solution was slowly injected into the marginal ear vein over a 30 second time period. Previous to the shock dosage each rabbit was anesthetized with sodium
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Pentobarbital, 30 mg per kg of body weight.

Endotoxin shock was produced in eight male rabbits with Difco lyophilized *Escherichia coli* (0127:B8 lipopolysaccharide). Two mg of *E. coli* endotoxin per kg of rabbit weight was dissolved in 3 ml of saline and injected into the marginal ear vein of the rabbits over a period of 90 seconds. Each rabbit was previously anesthetized with sodium pentobarbital (30 mg per kg of body weight) and because of the long duration of the experiments (up to 16 hours) additional sodium pentobarbital had to be given.

Arterial pressures and blood sampling were accomplished using 16 gauge catheters inserted into the left carotid artery of the rabbits and attached to a three way stopcock. One lead from the stopcock went to an anaroid manometer, which monitored the arterial pressure. The other opening of the stopcock was used to draw blood. Before a test sample was drawn, 2 ml of blood was first drawn into a syringe containing 2 ml of heparinized isotonic saline (2.5 units per ml) and the test sample was then drawn using a different syringe. The diluted saline blood was returned to the animal. This procedure insured a fresh sample for analysis each time.

For the extraction of ATP, ADP, and CP, blood was collected in a tube containing a final concentration of 10 mM disodium ethylenediaminetetraacetic acid (Na$_2$EDTA) to total volume of blood. Plasma contains enzymes capable of degrading both ATP$^{25}$ and CP$^{37}$ and the concentration of Na$_2$EDTA totally inhibits the breakdown of adenine nucleotides in plasma$^{20}$ as well as prevents blood coagulation. The whole blood was then centrifuged at 4000 x g for four minutes. The plasma layer was then decanted, to which an equal volume of 96 percent ethanol was added to denature enzymes.$^{20}$ The precipitate was removed by centrifugation at 12000 x g for 15 minutes and the supernatant stored at 4°C.

Electrocardiogram tracings were made.* Small areas on each of the rabbit's legs were shaved and the four heads attached to these areas using alligator clamps. Platelet counts were then determined.† Plasma ATP, ADP, and CP determinations were made‡ as previously described.$^{12,23,24}$

**Results**

The plasma ATP level in anaphylactic shock dropped significantly five minutes after the shocking dose of antigen (figures 1 and 2). This time was previously shown to be critical in the outcome of anaphylaxis. At this time the platelet count and arterial pressure are at a minimum and multiple platelet emboli were observed obstructing the pulmonary blood flow.$^{39}$ If the animal survives this period, platelet deaggregation and recovery occur. During this recovery period, the plasma ATP level gradually approached the initial level. The CP levels were found to be lower after an hour, which might represent its utilization to replenish the ATP study. The data also show an initial drop in the CP level at the critical time period of five minutes.

In anaphylactic shock the plasma ADP values indicated that the outcome was predetermined by the initial levels. Four animals were found to have a relatively high initial ADP level (figure 2) which dropped within the critical first five minutes by an average of 63 percent. This might be suspected if available plasma ADP was being utilized during platelet aggregation. Half of these animals died. On the other hand, four other rabbits exhibited low initial ADP levels (figure 1)

* A Sanborn 500 Viso Cardiette was used.
† A Becton-Dickinson Unopette 5855 Test Kit was used.
‡ A Dupont 760 Luminescence Biometer was used.
which increased after five minutes by an average of 78 percent, and all these animals survived the shock. The surviving animals showed a later trend to return to their initial levels suggesting that both high and low levels are normally maintained.

The overall average plasma ATP level changes found in endotoxin shock were related to three stages: initial shock, apparent recovery and terminal shock (figure 3). In the normal and apparent recovery stage, when the pressure, platelet count and electrocardiogram were within normal range, the ATP values were high (figures 4 and 5). During the shock stages, five minutes after injecting endotoxin and terminally, the ATP levels dropped. Ex-
experimentally, the ATP level appeared again to delineate the metabolic state of the animals as it did in anaphylactic shock. The CP level in endotoxin shock showed an unexpected pattern (figures 4 and 5). In all animals, the terminal stage plasma CP level rose several fold.

The plasma ADP pattern, which resulted during endotoxin shock, was similar to that seen during anaphylactic shock. Animals with initial ADP levels greater than 0.1 \( \mu M \) ADP (figure 5) died within an average time of 2.8 hours, while animals with initial ADP levels less than 0.05 \( \mu M \) (figure 4) survived longer to an average of 14.4 hours. The plasma ADP values of all animals which were initially high dropped five minutes after the injection of endotoxin, while the ADP values of animals which were initially low rose. This is exactly the same pattern which occurred in anaphylactic shock. The terminal ADP values were elevated an average of 2.7 fold over the initial high ADP values and 10.5 fold over the initial low ADP values. The average terminal ADP value in endotoxin shock was 0.4 \( \mu M \).

Four animals were used as controls to determine the effect of human serum on nonsensitized rabbits. The human serum was substituted for the endotoxin injection and these same rabbits were further used for the endotoxin controls. The results are given in table I. There was no drop in the platelet count or arterial pressure during the 16 hour period.

**Figure 3.** Stages observed during *E. coli* endotoxin induced shock as evidenced by the electrocardiogram, platelet count and arterial pressure.
Neither ATP or CP showed any significant change during this time; however, the ADP level increased in all the controls over the 16 hour period. The stress of the operative procedure may have been the cause of this ADP increase. The 16 hour ADP control values are much lower than the terminal ADP values in endotoxin shock and, in this respect, the present authors believe the control ADP increase was insignificant. In addition, the early ADP rise or drop in anaphylactic and
endotoxin shock was not observed in either the initial high plasma ADP or initial low plasma ADP controls.

While the data in figures 1, 2, 4 and 5 are presented as the mean of a group of animals, the ATP, ADP and CP values given in table I are typical in that the range of variation for each point within a group never exceeded ± 10 percent. In addition, the mean coefficient of variation for the determination of 1.0 μM per liter ATP and CP were 1.5 percent and 1.9 percent, respectively. The value for an 0.1 μmole per liter ADP was 1.8 percent.

Discussion

It may be presumed from the data presented that plasma ATP measurements correlate with the energy state of the tissue. When arterial pressures, platelet counts and electrocardiograms were abnormal, in both anaphylaxis and endotoxin shock, the plasma ATP levels were low. When the animal's condition improved the plasma ATP levels rose. It is proposed that monitoring ATP plasma levels could become a clinical diagnostic tool. Past reports on increased survivals in hemorrhagic shock by ATP infusion add credence to the importance of monitoring plasma ATP levels.

The plasma CP level changes during anaphylactic shock appear to be less significant when compared to endotoxin shock. The overall trend in anaphylaxis (figures 1 and 2) shows a gradual CP decline after one hour. Endotoxin shock shows an explicit pattern (figures 4 and 5). The terminal high elevation in plasma CP in endotoxin shock may represent the mobilization of cellular CP to the myocardium in an attempt to compensate for any energy deficit. This CP is not coming from platelet release since neither CP or creatine phosphokinase have been found in platelets. The rise of CP to such a high level in the plasma leads one to assume that it is capable of crossing the cellular membrane. This would give credence to the claim of the usefulness of the intravenous infusion of CP in the treatment of shock.

Another possible source of plasma CP could be the cellular destruction of tissue by the direct effect of endotoxin or anoxia.
and loss of CP from these tissues. In this case the CP plasma level would be an index to cellular damage. The identification of the plasma levels of ATP and CP together appear to be an excellent index in determining the animals' metabolic state during experimental endotoxin shock. Low plasma ATP and CP levels in endotoxin shock appear to be not as critical as a low plasma ATP level and a high CP level. The first designates an early stage of endotoxin shock and the latter indicates the possibility of being in an irreversible stage of shock.

Plasma ADP level evaluation appears to be more complex than ATP and CP. In the aggregometer, 0.1 to 0.5 μM ADP caused reversible platelet aggregation in human platelet rich plasma. Some of the initial plasma values observed in the anaphylactic experiments were greater than 0.1 μM. High plasma ADP values (greater than 0.1 μM) do not appear to be a prerequisite for aggregation to occur since platelet aggregation occurred in all the sensitized rabbits. However, a high plasma ADP level appears to be disadvantageous since all initial low plasma level ADP (ILPL-ADP) rabbits survived, while half of the initial high plasma level ADP (IHPL-ADP) rabbits died. The drop in the plasma ADP level after five minutes (figure 2) in the IHPL-ADP rabbit is puzzling when one takes into account that the ILPL-ADP level rose at that time (figure 1).

Platelets can be aggregated by the direct effect of antibody antigen complexing. As previously stated, platelets can also be aggregated by 0.1 μM ADP in an aggregometer. Since this ADP level is exceeded in the IHPL-ADP rabbits, the ADP molecules may potentiate extra stability to the immune complexing aggregates by ADP binding on the receptor sites of the platelets. Born demonstrated that the number of platelet receptor sites for ADP is about $2 \times 10^4$ per platelet. Thus, this added stability could be adequate to hinder deaggregation or cause the formation of larger aggregates which may maintain the anoxic state long enough in the IHPL-ADP rabbits to cause metabolic irreversibility. Death in anaphylactic shock can occur in five minutes. Our experience has shown that five minutes of trachea clamp off is adequate time to kill an anesthetized rabbit causing electrocardiogram changes similar to terminal anaphylaxis (figure 6).

The circulating ADP of the ILPL-ADP rabbits may not be initially high enough to cause any appreciable ADP binding to platelet receptor sites. Once the platelets have almost totally aggregated, the ADP level may become insignificant and the rise in observed ADP in the ILPL-ADP rabbits could be due simply to the existing anoxic state. Circulation may still be adequate for cellular ADP leakage causing the rise in plasma ADP. In the IHPL-ADP rabbits and the ADP level may have decreased, owing to ADP utilization in platelet binding. This level does not rise in the critical time of five minutes because of severe circulatory blockage, whereby cellular ADP leakage was not adequately picked up by the blocked capillaries. After a time, ADP levels approach pre-existing normals in both types of survivals as the circulation and metabolism improved.

A more than coincidental similarity exists between the ADP levels in initial endotoxin and anaphylactic shock. Again, all the IHPL-ADP rabbits in endotoxin shock showed a plasma ADP decline in the first five minutes while the ADP level in the ILPL-ADP rabbits rose (figures 4 and 5). Endotoxin alone has been shown to be capable of aggregating platelets. The IHPL-ADP rabbits died within a much shorter time when compared to the ILPL-ADP rabbits. Thus, a pathway similar to that of anaphylaxis could possibly explain these results (figure 7). The IHPL-ADP initial aggregation may be caused by both the direct effect of endotoxin and the potentiating effect of ADP, while in the ILPL-ADP rabbits initial
platelet aggregation may have occurred mainly from the direct effect of the endotoxin alone. Large aggregates could have formed in the IHPL-ADP rabbits, owing to greater adhesions between platelets, causing the plugging of larger vessels. Ischemia could be more pronounced in these rabbits causing early severe metabolic disturbance leading to the earlier deaths.

In endotoxin shock, the plasma ADP level gradually increases after the first hour (figures 4 and 5). It has been established \(^2,28,47\) with human plasma that 1 to 2 \(\mu M\) ADP was necessary to obtain irreversible platelet aggregation in an aggregometer with platelet procoagulant liberation. Such a high ADP level was never found in any of our experiments. Constantine \(^8\) found that only 0.3 \(\mu M\) ADP in platelet rich guinea pig plasma was necessary for irreversible platelet aggregation to occur in the aggregometer. This plasma value was exceeded in the terminal phase of endotoxin shock in our study where irreversible platelet aggregation has been determined. \(^23,39\) Other investigators have found that the aggregation effect of ADP can be potentiated by additional factors. For example, adrenaline potentiates the effect of ADP upon platelet aggregation. \(^46\)

When a dose of ADP was unable to cause irreversible aggregation itself, an irreversible wave was produced upon the addition of a nonaggregating amount of cephalin. \(^9\) Endotoxin was observed to have a direct effect on the vessel wall with disruption of the endothelial lining and exposure of collagen. \(^16\) Platelet aggregation on collagen was shown to be irreversible. \(^15\) Therefore, plasma ADP levels alone may not be the decisive platelet releasing agent in irreversible endotoxin shock, but a combination of factors (ADP, adrenaline, lipids, exposed collagen, etc.) could be responsible for the irreversible platelet aggregation that occurs. Our studies, have shown that the plasma ADP level does not reach the "so-called" critical level of 1 to 2 \(\mu M\) needed for irreversible aggregation in humans but does exceed the value found in guinea pigs. The increased plasma ADP level in terminal endotoxin shock could play an important role in the secondary irreversible platelet aggregation. This effect could be increased with the synergistic help of other sources.

There are several ramifications of these experiments which may have relevance to human subjects. For example, atherosclerotics have an increased sensitivity to

**Figure 6.** Comparison of electrocardiograms during trachea clamp-off (A) and terminal anaphylactic shock (B).
AFDP. Thus, monitoring the plasma ATP, ADP and CP levels may be of benefit to such individuals. It may be of value to monitor periodically the plasma ADP level in stored blood, since high levels of ADP may develop and prove detrimental if given in transfusions. It is known that artificial systems such as kidney dialyzers and lung oxygenators become impeded by platelet aggregates, although the exact cause has not been established. Perhaps a contributing factor is increased levels of ADP or CP resulting from hemolysis owing to mechanical shear of the red cells.

Periodic sampling of blood levels of ATP, ADP and CP during open heart surgery may give an indication of the metabolic state of the patient. In this respect, a 50 fold drop in plasma ATP and CP concentrations of a coronary by-pass patient just prior to death has been reported by us. 24

References


