Immediate Effects of Intravenous Endotoxin on Serotonin Concentrations and Blood Platelets

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Adult mongrel dogs were given a lethal dose of E. Coli endotoxin by rapid intravenous injection. Total serotonin levels in the serum fell rapidly, with the concentration in the portal vein and pulmonary artery significantly exceeding that in the femoral artery within the first minute after injection. Small rises in plasma serotonin were found in some of the animals. These changes were coincident with a sharp fall in the number of circulating platelets, with striking changes in platelet morphology, and with the initial fall in blood pressure.

The in vivo administration of gram negative bacterial endotoxins is associated with certain characteristic manifestations. These include leukopenia, thrombocytopenia, and profound vasomotor disturbances, terminating in shock. In the dog spasm of the small hepatic veins occurs, followed promptly by a rise in the portal venous pressure, with pooling of blood in the liver and intestine, and a subsequent reduction in cardiac output. Changes similar to those produced by endotoxin are also seen after injection of over 70 substances, including glycogen, and bear a remarkable resemblance to the phenomena seen in anaphylaxis.

Recent work has shown that serotonin and histamine are released from platelets by antigen antibody reactions, as well as by glycogen, in the rabbit. A histamine like substance also has been identified in dog plasma after endotoxin. Observations by Gordon and Lipton, and by Gilbert have suggested indirectly that serotonin might play a role in endotoxin shock. Because of these findings this work was undertaken to assess the direct effects of intravenously injected endotoxin on serotonin concentrations and on platelets.

Methods

Mongrel dogs, weighing between 18 and 30 Kg., were lightly anesthetized with thiopental sodium. In 9 dogs the chest was entered through the bed of the left fourth rib. A Starling pump was used to give positive pressure respiration. The lung was retracted and the pericardium opened over the pulmonary artery and outflow tract. The pulmonary artery was secured by means of a 3-0 purse string suture. The arterial wall was incised 1 to 2 cm. above the pulmonary valve, and a saline filled no. 280 polyethylene cannula was inserted into the pulmonary artery to record pressure changes. By means of a concentrically fitting cuff the catheter was firmly secured in the desired position. In a similar fashion another catheter, for sampling, was placed in the pulmonary outflow tract, extending through the valves.

A chest tube was placed in position to catch dependent drainage, the catheters were brought out between the interlobar fissure to separate stab wounds, and the chest closed. In a second group of 9 dogs the pancreatoduodenal vein was cannulated, and the catheter was extended into the portal vein. In all dogs bilateral femoral artery cannulation was done, and an additional cannula was placed in the inferior vena cava, via the common femoral vein.

Purified E. Coli endotoxin* was administered

*Generously supplied by Dr. Wesley W. Spink. The method of preparation has been described previously.
ENDOTOXIN AND SEROTONIN CONCENTRATIONS

Average changes in platelet counts and in serotonin concentrations following intravenous injection of E. Coli endotoxin (1 mg./Kg.) Pulmonary artery group.

by rapid injection into the inferior vena cava. A dose of 1 mg./Kg. was used, which was lethal within 8 hours for all of the dogs studied. Blood specimens were obtained in siliconized syringes, and timed by stop watch. Blood for plasma determinations was placed in siliconized tubes containing ethylene diamine tetraacetate (E.D.T.A.) 10 mg./10 ml. blood, as an anticoagulant. Platelet counts were done in duplicate or in quadruplicate, using Rees Ecker solution, and a direct counting technic. Blood for platelet counts and for blood smears was taken from the tubes containing E.D.T.A., but in some instances blood was obtained directly from the polyethylene catheters. Serum was obtained from blood treated by the method of Davis, and serum and plasma serotonin concentrations were determined fluorimetrically.9

Results

Because of substantial variations in the platelet counts (range 120,000 to 486,000/cu. mm.) and in serum serotonin concentrations (range 0.21 to 1.10 μg./ml.) in control specimens in different animals, those results were expressed in terms of the proportion of the control value remaining at each specific time after endotoxin.

Pulmonary Artery Group

The average changes in platelet counts and in serotonin concentrations in the first group of 9 dogs are shown in figure 1. A prompt fall in the platelet count occurred, reaching approximately 50 per cent of control levels 15 seconds after endotoxin. The average count fell to 20 per cent of control values after 60 seconds, and was essentially unchanged between 1 and 5 min. The platelet counts were not significantly different in the 2 sites, except at 30 seconds, when the difference was 12 per cent (S.E. of difference: 5 per cent).

There was a parallel, though slower fall in serum serotonin levels in the first minute after endotoxin. Serum levels in the pulmonary artery significantly exceeded those in the femoral artery, up to and including 3 min. after endotoxin. Serum levels in the pulmonary artery significantly exceeded those in the femoral artery, up to and including 3 min. after endotoxin. At 45 sec. the difference was greatest (34 per cent), being highly significant statistically (P = 0.0001). Femoral artery serotonin levels fell to 3 per cent of control levels 3 min. after endotoxin, then returned to 34 per cent of control levels one hour after endotoxin. Small increases in plasma serotonin were seen in pulmonary artery blood (range 0 to 0.11 μg./ml.) and in femoral artery blood (range 0 to 0.09 μg./ml.) reaching a maximum average value in both sites 30 seconds after endotoxin.

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Similar changes in average platelet counts to those seen in the pulmonary and femoral arteries were observed in the portal vein (fig. 2). The concentration of serotonin in the portal vein, however, exceeded the concentration in the femoral artery by 34 to 46 per cent within the first minute after endotoxin. The difference was maximal at 45 sec., and was significant at a value of $P$ less than 0.01. Platelet concentrations and serum levels rose one hour after endotoxin, and plasma levels rose in the portal vein to an average of 0.05 $\mu$g./ml. (range 0 to 0.25 $\mu$g./ml.).

Changes in Platelet Morphology

The morphology of platelets in blood samples obtained from the pulmonary artery before endotoxin, as well as 15, 30, and 60 sec. after endotoxin is seen in figures 3 to 6. Marked formation of pseudopodia and early platelet fusion occurred within 15 sec. after endotoxin (fig. 4). Clumping and fusion increased substantially within 30 sec. after endotoxin, but platelet hyalomeres remained substantially intact (fig. 5). Sixty sec. after endotoxin, however, marked fusion and loss of platelet integrity occurred, with changes similar to those of viscous metamorphosis (fig. 6). Clumping of platelets was not prevented by prolongation of the clotting time (Lee-White) to greater than one hour with heparin, and was found in all 3 vascular sites sampled in this study. Clumps of platelets were significantly reduced in number by one minute after endotoxin, and were virtually absent in smears made 5 min. after endotoxin.

Sham Procedure

A sham operation was done on 4 dogs, followed by the rapid injection of isotonic saline. Blood was removed from the femoral artery at the same time intervals, and in volumes equal to the amounts removed after endotoxin injection. In this procedure no significant change in serotonin levels occurred (fig. 7), and significant platelet clumping was not observed.

Discussion

Alterations in the concentration of serotonin in serum following endotoxin are strikingly similar to those produced in blood by anaphylaxis, and by glycogen administration in rabbits. These changes roughly parallel changes in the platelet count. Study of the immediate response to endotoxin, however, revealed certain striking discrepancies. Thus the drop in the platelet count appeared to be more sudden than the fall in serotonin levels, and pulmonary artery and portal vein serum serotonin levels significantly exceeded those in the femoral artery. Higher serum levels in the pulmonary artery probably result from release of serotonin from platelets lying in pulmonary capillaries, destruction by lung.
monoamine oxidase and subsequently lowered femoral artery concentrations. Higher pulmonary artery levels seem unlikely to be due exclusively to trapping of platelets in pulmonary capillaries, with subsequently lowered femoral artery platelet counts, because no significant difference in counts in the 2 sites was found, except at 30 seconds, when the count in the femoral artery exceeded that in the pulmonary artery. Higher portal vein serum levels may result in part from circulatory stasis, which is known to occur in the portal bed of the dog after endotoxin. It is apparent that the highest plasma levels were reached in the portal vein, however, which suggests a possible release of serotonin from the bowel. Studies now in progress indicate that such a release may occur. The finding of low plasma serotonin levels, in some cases zero, is compatible with the known difficulty in elevating plasma levels in rabbits by injection of serotonin,4 and may relate to the rapid destruction of serotonin in contact with hemoglobin, or erythrocytes, (not inhibited by iproniazid) as shown by Ling and Blum.10

Serotonin in animals and man is carried in platelets, with little, if any being present in plasma.11 Changes in platelets, therefore, are of especial interest in the interpretation of the effects of endotoxin on serotonin levels. Thrombocytopenia has been well documented 5 min. after endotoxin by Weil8 and 90 sec. after the injection of staphylococcus aureus,12 but the extreme rapidity with which the early reaction occurs has not been well documented. The agglutination of platelets by bacteria was recognized early.13 Clark and Batchelor15 have shown that platelet clumping, vesicleulation, and destruction occurs in vitro after incubation with E. Coli endotoxin. However, Stetson16 could not demonstrate clumping of platelets in vivo or in vitro due to the somatic antigen of Sh. Paradysenteriae. Nevertheless, Younger and Algire17 observed that large whitish masses appeared in circulating venous blood after injection of a polysaccharide from S. Marcescens, which they speculated were "platelet emboli, or a product of damage to the lining of blood vessels." The present study has clearly shown that the masses which they observed probably represented intravascular clumping of platelets. It is of interest that intravascular clumping of polymorphonuclear leukocytes was not seen in these studies, either separately or together with platelets, despite their known early disappearance from the circulation after endotoxin.6 It appears, moreover, that loss of platelet integrity is not necessary for serotonin and histamine release, since glycogen effects such a release in vitro6 without lysis or visible alterations in platelets.16 The sudden appearance of thrombocytopenia, platelet clumping, and liberation of histamine and
serotonin appear to be common to anaphylaxis, glycogen administration, and to endotoxin shock. The temporal relation to pressure changes which have been well documented in the dog suggests that liberation of vasoactive amines from platelets may contribute to the initial shock reaction. It seems questionable, however, that amine liberation is primarily responsible for the lethal effects of endotoxin, since glycogen is capable of effecting such a release but does not cause toxic effects in the rabbit.

Summary

The rapid intravenous injection of a lethal dose of E. Coli endotoxin in the dog produced a fall in serum serotonin concentrations and small rises in plasma serotonin levels. These changes coincided with changes in pressure in the portal vein, pulmonary and femoral arteries similar to those described by other workers. Serum concentrations in the femoral artery were significantly less than in the portal vein and pulmonary artery within the first minute after endotoxin.

A dramatic decrease in the number of circulating platelets occurred within the first minute after endotoxin, together with intravascular clumping and viscous metamorphosis of platelets. The significance of these findings is discussed.

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