Histamine Release in the Dog After Leukocyte Lysate Injection

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Mixed leukocyte suspensions, containing 10⁴ leukocytes per ml, were prepared from dog peripheral blood by dextran sedimentation. Lysates of these suspensions, prepared by sonification, were administered intravenously to the autologous dogs. The plasma histamine levels were increased to an average value 320% above the control values (P < 0.001). The individual elements in the mixed leukocyte suspensions were separated by sucrose gradient and glass bead column fractionation procedures, and purified suspensions of platelets (no other formed elements present), lymphocytes (95 to 100%), and granulocytes (90 to 98%) were obtained. Lysates of these suspensions all had significant histamine-releasing activity when administered intravenously to dogs. Although leukocytes and platelets from other species are known to harbor mediators of histamine release, this study provides the first demonstration of the histamine-releasing activity of dog leukocytes and platelets. Many of the studies of leukocyte and platelet histamine-releasing factors have employed in vitro systems leaving the in vivo role of these factors in doubt. The present studies provide evidence that leukocyte and platelet histamine-releasing factors have in vivo activity.

Histamine, a potent biological amine, has been implicated in the mediation of several important phenomena including inflammation, allergic reactions, and shock (11). Histamine is stored largely in the granules of mast cells and circulating basophils or platelets, and its release from these stores may be important in initiating immunological injuries and the inflammatory response. Accordingly, the mechanisms responsible for histamine release have received a great deal of attention in recent years, and various materials, including cationic proteins (12), antigens (6), and compounds such as 48/80 (4), have proven to be potent histamine-releasing agents.

Cationic proteins from human platelets have been shown to release (8) histamine from rat mast cells. In previous studies (5), we demonstrated that extracts of human platelets and of purified human lymphocytes and granulocytes released histamine from human leukocytes in vitro. The present studies were initiated to determine if leukocyte extracts could release histamine in vivo in the intact dog.

MATERIALS AND METHODS

Leukocytes were prepared for use in this study as follows. Blood was drawn through 18-gauge needles into heparinized syringes and transferred to polypropylene centrifuge tubes containing one part sedimentation fluid and two parts blood. The sedimentation fluid contained 3% dextran (molecular weight, 188,000) in 2.5% glucose and normal saline with 20 units of heparin/ml or 0.017 M ethylenediaminetetra-acetic acid (EDTA) as anticoagulant. After mixing, the erythrocytes were allowed to sediment two-thirds the length of the tube at room temperature. The resulting platelet and leukocyte-rich supernatant fluid was aspirated and centrifuged at 150 × g for 10 min at room temperature (model SP/x, Ivan Sorvall, Inc., Norwalk, Conn.), and the pelleted leukocytes and platelets were used as outlined below.

Purified suspensions of platelets were prepared as follows. A 150-g platelet-leukocyte button, prepared as above, was washed in tris(hydroxymethyl)aminomethane (Tris)-albumin (6) buffer and resuspended in a final volume of 5 ml of Tris A. This suspension was layered over two discontinuous sucrose gradients composed of 4-ml layers of 40, 35, 30, 25, 20, and 15% sucrose in Tris A. The gradients were centrifuged at 150 × g for 6 min at 2 C in a swinging bucket rotor (model PR-2 or B-20, International Equipment Co., Needham Heights, Mass.). The leukocyte and erythrocyte-free platelet bands which formed at the tops of the gradients were aspirated and centrifuged at 2,500 × g for 30 min at 2 C. The platelet buttons were resuspended in 20 ml of cold Tris A.

Suspensions of purified lymphocytes and granulocytes were prepared by using a glass bead column technique essentially the same as that described by
Leukocyte (9), except that 35-ml plastic syringe barrels rather than glass tubing were used to prepare the glass bead columns, and Tris A was used for buffering throughout. To reduce erythrocyte and platelet contamination, the purified lymphocyte and granulocyte suspensions were each incubated at 37 °C for 20 min in a solution containing one part pH 7.4 Tris buffer and nine parts 0.83% ammonium chloride. The cells were then collected by centrifugation at 150 × g for 10 min and resuspended in Tris A at 10⁶ cells/ml.

Using these procedures, platelet suspensions were obtained that were essentially free from other types of formed elements. Lymphocyte suspensions were 95 to 100% pure, whereas granulocyte suspensions were 90 to 98% pure. Both had very little or no platelet and erythrocyte contamination.

Mixed leukocyte suspensions, prepared by dextran sedimentation, as well as suspensions of purified platelets, lymphocytes, or granulocytes were ruptured by 5 min of sonication with a Biosonic III ultrasonic disintegrator (Bronwill Scientific, Rochester, N Y) with care to avoid heating the suspensions. All lysates and extracts were either used immediately or quick-frozen and stored at −70 °C until use. All lysates were tested for histamine-releasing activity within 1 week of preparation.

The protein concentrations of the lysates used in these studies were determined by the technique of Lowry et al. (7) with crystalline lysozyme as a standard.

Twenty mongrel dogs, weighing 15 to 25 kg, were used in this study. The dogs were anesthetized with pentobarbital, 30 mg/kg, intravenously. Endotracheal tubes were inserted, and ventilation was controlled with a Harvard constant-volume ventilator. Cardiac catheters were inserted through the femoral artery and vein to the level of the diaphragm under fluoroscopic guidance. Substances to be tested were injected undiluted in 5-ml quantities over a 10-sec interval into the inferior vena cava. Samples for plasma histamine were obtained from the aortic catheter by using heparinized plastic syringes. Aortic pressure was monitored by using a Statham pressure gauge and an Electronics for Medicine recorder. The heart rate was determined from an electrocardiogram. Plasma histamine levels, heart rates, and aortic pressures were monitored immediately before and at various intervals up to 30 min after injection of the test material.

Plasma histamine levels were determined as described previously (3). The initial plasma histamine levels ranged from 0.028 to 0.088 μg/ml, which is within the previously reported range (3). The student t test was used for statistical analyses.

RESULTS

Leukocytes were separated from 30 ml of blood from each of 10 dogs and resuspended at 10⁶ cells per ml in Tris A buffer. These suspensions contained appreciable numbers of platelets, lymphocytes, monocytes, and granulocytes as well as erythrocytes. Lysates of these dilute suspensions contained 0.6 to 1.5 mg of protein per ml, but they did not contain detectable levels of histamine. Each dog was injected with undiluted autologous leukocyte lysate, and the plasma histamine levels, aortic pressures, and heart rates were determined.

In all 10 experiments, the plasma histamine level increased significantly after the leukocyte lysate injection (Table 1). The average maximum increase in plasma histamine level for the 10 dogs was 0.144 μg of histamine per ml. Control dogs were injected with buffer alone, and they displayed only minor changes in plasma histamine level. There were no significant changes in aortic pressure or heart rates in either control dogs or dogs injected with leukocyte lysate.

Although all of the dogs injected with leukocyte lysate had appreciable increases in plasma histamine, they did not all respond in the same manner. Five dogs had a rapid rise in plasma histamine level to a peak at 2 min, whereas the remaining five dogs had a gradual increase in plasma histamine with a peak of approximately the same magnitude at 30 min (Fig. 1).

To determine which of the formed elements in the mixed leukocyte lysates was responsible for the increase in plasma histamine observed after injection, purified platelet, lymphocyte, and granulocyte suspensions were prepared. Lysates of these suspensions contained 0.7 to 1.3 mg of protein per ml, but they did not contain detect-

<table>
<thead>
<tr>
<th>Table 1. Response of dogs to autologous leukocyte lysate injection</th>
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<tbody>
<tr>
<td><strong>Material tested</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Leukocyte lysate</td>
</tr>
<tr>
<td>Buffer</td>
</tr>
<tr>
<td>P&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average maximal per cent change compared to the zero time reading, expressed as the mean ± 1 standard deviation.

<sup>b</sup> Values of P were calculated for the differences between the dogs which received leukocyte lysate and those which received buffer.
able histamine. Each lysate was administered to two homologous dogs, and the plasma histamine levels, aortic pressures, and heart rates were measured.

The combination of elements in the mixed leukocyte lysate produced the greatest histamine release, but the purified platelets, lymphocytes, and granulocytes had significant individual histamine-releasing activity (Table 2). The differences in histamine-releasing activity were not due to differences in cell or protein concentrations in the lysates. Once again, in spite of the large increases in plasma histamine, the aortic pressure and heart rate had only insignificant variations.

Intradermal injection of 0.1 ml of a lysate of 10^7 white blood cells per ml was followed by immediate wheal of 1.2 × 1.0 cm with flare but without pseudopods. Intradermal injection of 0.1 ml of lysate of 10^6 white blood cells or less was not followed by reactions greater than that which followed intradermal injection of buffer alone. After the intradermal injection of 0.1 ml of 48/80 at concentrations of 5, 2.5, or 1.25 mg/ml, immediate wheals of 1.7 × 1.8 cm were observed with increased flare and pseudopods.

**DISCUSSION**

These findings indicate that suspensions of dog peripheral blood leukocytes contain factors which induce histamine release in vivo and that this activity is associated with the platelets, lymphocytes, and granulocytes which make up these suspensions. These results complement our recent in vitro studies (5) which demonstrated that lysates of mixed human leukocyte-platelet suspensions and lysates of purified platelets, lymphocytes, and granulocytes induce histamine release from human peripheral leukocytes (5). In addition, this is the first demonstration that dog leukocyte lysates have histamine-releasing activity.

The nature of the histamine-releasing activity is obscure. Several substances are known to induce histamine release in other systems. Scherer and Janoff (12) and Nachman, Weksler, and Ferris (8) have shown that lysosomal cationic proteins from animal leukocytes and human platelets release histamine from rat mast cells in vitro. Ranadive and Cochrane have demonstrated that a purified band of protein from rabbit neutrophils releases histamine from rat mast cells and increased vascular permeability in the skin of rabbits which could be blocked with antihistamines (10). Antigens are known to induce histamine release via the reaginic antibody system (6), and rat eosinophil peroxidase has been implicated as a mediator of histamine release (2). Also compounds such as 48/80 (4), arginine or lysine-rich histones, and poly-1-lysine (10) are potent releasing agents. Although some of these substances may be responsible for the histamine-releasing activity in dog leukocytes and platelets, definitive information on the nature of this activity must await fractionation and purification of the active factors involved.

These studies are also of interest in relation to the cardiovascular effects of endogenous histamine. In a previous study (3), it was demonstrated that infusion of compound 48/80 into normal dogs was followed by prompt evaluation of plasma histamine levels and marked cardiovas-

**TABLE 2. Responses of dogs to platelet, lymphocyte, and granulocyte lysates**

<table>
<thead>
<tr>
<th>Lysate</th>
<th>Plasma histamine</th>
<th>Aortic pressure</th>
<th>Heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed leukocyte</td>
<td>338.0</td>
<td>&lt;1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Platelet</td>
<td>181.0</td>
<td>&lt;1.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>209.0</td>
<td>8.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>212.0</td>
<td>3.0</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

*Average maximum per cent change compared to the zero-time readings. Each lysate was administered to two different dogs.

![Fig. 1. Time course of histamine release after leukocyte lysate injection. Two types of histamine release curve are presented: rapid release with a peak at 2 min (●, dog 25780) and slow release with a maximum reached at 30 min (○, dog 26798). (△) Average per cent increase in aortic pressure and heart rate for the two experiments.](http://iai.asm.org/Downloaded from http://iai.asm.org/ on September 20, 2017 by guest)
cular alterations culminating in shock and acidosis. The physiological alterations observed were attributed to the effects of the liberated histamine. The average maximum per cent increase in plasma histamine in that study was 135%, whereas the mean increase in the present study was about 300%. After 48/80 infusion, the aortic pressure decreased by 71% and the heart rate decreased by 15%, whereas these variables increased by an average of 6 and 9%, respectively, after leukocyte lysate infusion. Therefore, the response obtained after compound 48/80 infusion may have been due to other pharmacological effects of 48/80 or to release of high concentration of histamine from mast cells in close proximity to blood vessels.

The limited skin reaction after intradermal injection of white blood cell lysates at concentrations 10 times higher than those used intravenously suggests that white blood cell lysates may not be highly active in releasing histamine from mast cells.

ACKNOWLEDGMENTS

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