The Heart as a Target Organ in Systemic Allergic Reactions

COMPARISON OF CARDIAC ANAPHYLAXIS IN VIVO AND IN VITRO

By Norine Capurro and Roberto Levi

ABSTRACT

The purpose of this investigation was to define and quantitatively evaluate cardiac anaphylaxis in vivo. Guinea pigs, passively sensitized with graded amounts of rabbit antipenicilloyl antibody, were anesthetized, ventilated, and challenged intravenously with a constant amount of antigen (anaphylaxis in vivo). In other experiments, guinea pig hearts were excised, perfused in a Langendorff apparatus, and challenged (anaphylaxis in vitro). During in vivo anaphylaxis, sinus rate increased 10-30 beats/min, conduction arrhythmias occurred in 15 of 22 experiments, and ventricular fibrillation was seen in 8 of 22 experiments. Tachycardia and arrhythmias began approximately 20 seconds after antigen administration and were accompanied, but not preceded, by respiratory and pressor changes. During in vitro anaphylaxis, sinus rate increased 70-110 beats/min, coronary flow rate decreased 2-22%, conduction arrhythmias occurred in 21 of 31 experiments, and ventricular ectopic activity was seen in 13 of 31 experiments. Tachycardia and arrhythmias began approximately 15 seconds after antigen administration. Sinus tachycardia, atrioventricular conduction block, increased ventricular automaticity, and histamine release were characteristic features of cardiac anaphylaxis in vivo and in vitro. Both in vivo and in vitro, the intensity of the cardiac reaction depended on the amount of antibody used in passive sensitization. Our results clearly indicate that the heart reacts as a target organ in systemic anaphylaxis of the guinea pig.

KEY WORDS: guinea pig anaphylaxis, isolated heart anaphylaxis, anaphylactic cardiac histamine release, drug allergy, histamine release

Anaphylaxis of the isolated guinea pig heart is a specific crisis in cardiac function characterized by histamine release, tachycardia, arrhythmias, and decreased coronary flow (1, 2). These studies of cardiac anaphylaxis in vitro prompted us to investigate whether the heart undergoes such a reaction during systemic anaphylaxis. Cardiac involvement in systemic hypersensitivity reactions has not been clearly defined. Cardiac disturbances during systemic anaphylaxis in the guinea pig have been variously interpreted as a secondary reaction to the asphyxia produced by bronchospasm (3, 4), the result of ischemia produced by impaired coronary circulation (5), or a manifestation of direct participation of cardiac tissue (6).

The purpose of the present investigation was to define the cardiac reaction as a consistent feature of systemic anaphylaxis in the guinea pig and to evaluate quantitatively the intensity of this reaction.

Methods

ANTIGENS

Benzylpenicilloyl-protein conjugates (BPO-protein) were chosen as antigens. Benzylpenicillin was coupled to guinea pig gamma globulin (GPGG) and to bovine gamma globulin (BGG) according to the method of Levine (7). The number of haptenic groups per carrier molecule was estimated by the penamaldate method (8). BPO<sub>16</sub>-GPGG was used for sensitization and BPO<sub>32</sub>-BGG was used for challenge to ensure specificity of response for the BPO determinant.

GRADED CARDIAC ANAPHYLAXIS

Guinea pigs were passively sensitized with graded amounts of rabbit antisera and challenged with a constant amount of antigen. This approach was adopted to permit quantification of cardiac anaphylaxis over the widest range of responses to antigen challenge. Rabbit anti-BPO was prepared according to the method of Levine (7). The rabbits were immunized with BPO-GPGG-Freund's adjuvant, and the sera from several
bleedings were pooled. The gamma globulin fraction of the pooled antisera contained 3.0 mg/ml of specific (anti-BPO) antibody protein by quantitative precipitation (9) with BPO-BGG.

In Vivo Experiments.—Male Hartley guinea pigs weighing between 300 and 400 g were passively sensitized by intraperitoneal injection of 0.3, 0.9, or 3.0 mg of rabbit anti-BPO. Twelve hours later, these guinea pigs were anesthetized with sodium pentobarbital (35-50 mg/kg, ip). A tracheal cannula was inserted and connected to a respirator (Harvard Apparatus, model 608). Artificial ventilation (constant stroke rate of 40/min, stroke volume of 1 ml/100 g body weight plus an equivalent of the dead space) was maintained throughout the stabilization and experimental periods. A side arm of the tracheal cannula was connected to a pressure transducer (Statham, model P23AA). The output (an indirect measurement of bronchial resistance) was displayed on one channel of a two-pen recorder (Dynograph, model 542). The carotid artery was cannulated and connected to another pressure transducer. Blood pressure tracings were displayed on the second channel of the pen recorder. Standard four-limb electrocardiographic tracings were displayed on an oscillograph (Texas Instruments, model P2). After a stabilization period of 30 minutes, 5 mg of BPO-BGG (antigenic challenge) was rapidly injected intravenously via a cannula inserted in the jugular vein.

In Vitro Experiments.—Guinea pigs were passively sensitized by intraperitoneal injection of 0.3, 0.9, 1.8, 3.0, or 9.0 mg of rabbit anti-BPO. Twelve hours later, these guinea pigs were killed by a blow to the base of the skull. The heart was excised, mounted in a Langendorff apparatus, and perfused at a constant pressure (40 cm H2O) with oxygenated Ringer’s solution at 37.5°C (2). Isometric ventricular contraction, a surface electrogram, and coronary flow rate were recorded as previously described (2). Heart rate and atrioventricular conduction time (P-R interval) were determined from the surface electrogram. Hearts were perfused for 45 minutes prior to experimentation, by which time heart rate and contractility had reached a steady state. Antigenic challenge was accomplished by rapid intra-aortic injection of 1 mg of BPO-BGG dissolved in a constant volume of warm oxygenated Ringer’s solution. A series of 2-minute samples of coronary perfusate was collected before and after the challenge with antigen for a total period of 14 minutes. Histamine was determined in the coronary perfusate by the fluorometric procedure of Anton and Sayre (10) as we have described previously (11).

HISTAMINE AND CARDIAC PARTICIPATION IN SYSTEMIC ANAPHYLAXIS

Thirty-six guinea pigs were actively sensitized by two consecutive daily intraperitoneal injections of 10 mg of BPO-GPG. Fifteen to 30 days after sensitization, 14 of the guinea pigs were killed by a blow to the base of the skull; their hearts were excised, mounted in the Langendorff apparatus, and challenged with BPO-BGG. Twenty-two guinea pigs were passively sensitized by intraperitoneal injection of 0.3, 0.9, 1.8, 3.0, or 9.0 mg of rabbit anti-BPO. Twelve hours later, these guinea pigs were anesthetized with sodium pentobarbital (35-50 mg/kg, ip). A tracheal cannula was inserted and connected to a respirator (Harvard Apparatus, model 608). Artificial ventilation (constant stroke rate of 40/min, stroke volume of 1 ml/100 g body weight plus an equivalent of the dead space) was maintained throughout the stabilization and experimental periods. A side arm of the tracheal cannula was connected to a pressure transducer (Statham, model P23AA). The output (an indirect measurement of bronchial resistance) was displayed on one channel of a two-pen recorder (Dynograph, model 542). The carotid artery was cannulated and connected to another pressure transducer. Blood pressure tracings were displayed on the second channel of the pen recorder. Standard four-limb electrocardiographic tracings were displayed on an oscillograph (Texas Instruments, model P2). After a stabilization period of 30 minutes, 5 mg of BPO-BGG (antigenic challenge) was rapidly injected intravenously via a cannula inserted in the jugular vein.

The heart was excised, mounted in a Langendorff apparatus, perfused for 30 minutes, and challenged with BPO-BGG. Fourteen hearts were quickly homogenized, and their histamine was extracted and measured by the fluorometric procedure of Anton and Sayre (10). The histamine content of 23 normal guinea pig hearts was also determined.

DRUGS

Histamine dihydrochloride was purchased from Sigma Chemical Co. All histamine values refer to the free base. Buffered sodium penicillin G was purchased from E. R. Squibb & Sons, Inc. Bovine and guinea pig gamma globulins were purchased from Miles Laboratories, Inc.

Results

GRADED CARDIAC ANAPHYLAXIS IN VIVO

Systemic anaphylaxis in the anesthetized, artificially ventilated guinea pig was characterized by a crisis in both cardiovascular and respiratory functions. In particular, sinus tachycardia, atrioventricular conduction block, increased ventricular automaticity, and increased blood pressure followed by a prolonged depressor effect were constant features of the acute systemic allergic reaction.

Recordings from a typical reaction are shown in Figure 1. Pulmonary resistance rapidly increased beginning 25 seconds after antigen administration. Peak resistance was reached within 1 minute and declined only very gradually thereafter. Twenty seconds after antigen administration, blood pressure increased, with the pulse pressure widening, and then declined. After 1 minute, the pulse pressure narrowed precipitously and was never regained. Following a secondary rise after 1.33 minutes, blood pressure steadily declined. Twenty seconds after antigen administration, sinus rate began to increase (Fig. 1b); it reached a peak by 1 minute (Fig. 1c) and then slowly declined over the next 8 minutes. Concomitantly, the P-R interval became progressively prolonged (Fig. 1c) until 1.25 minutes (Fig. 1d) when atrioventricular conduction block developed. Conduction block persisted, and multifocal ventricular extrasystolic activity (Fig. 1d-f) and sustained ventricular rhythms (Fig. 1g) prevailed. By 4 minutes the QRS complexes were losing voltage (Fig. 1i and j), and by 8 minutes neither the atria nor the ventricles were depolarizing in any organized fashion (Fig. 1k and l).

The extent of cardiac, as well as bronchial, changes during systemic anaphylaxis depended on the degree of sensitization. The positive chronotropic effect of anaphylaxis progressively increased as a function of the amount of sensitizing antibody (Fig. 2A). The anaphylactic impairment in atrioventricular conduction was more severe at the
higher degrees of sensitization: P-R interval prolongation was greater (Fig. 2B) and conduction block occurred more frequently (Table 1). Ventricular automaticity increased during anaphylaxis. Multifocal ventricular extrasystoles, junctional rhythms, and ventricular tachycardia occurred more frequently with increasing levels of sensitization (Table 1). The effect of anaphylaxis on blood pressure was triphasic: an initial pressor effect was followed by a secondary rise in pressure and finally by a prolonged depressor effect. The magnitude of the initial pressor effect was not significantly altered by increasing the level of sensitization (Fig. 2D). The anaphylactic increase in bronchial resistance was larger at the higher degrees of sensitization (Fig. 2C).

Few of the guinea pigs survived longer than 30 minutes after antigenic challenge, and survival was related to a low level of sensitization (Table 2). Ventricular fibrillation within 15 minutes of challenge was the terminal event in almost half (8/17) of the anaphylactic deaths (Table 2).

In control experiments, sensitized guinea pigs were challenged intravenously with 5 mg of BGG (carrier only). BGG elicited no response.

**GRADED CARDIAC ANAPHYLAXIS IN VITRO**

Hearts from sensitized guinea pigs responded to specific challenge in vitro with a crisis in cardiac function. Tracings from a representative experiment are shown in Figure 3. This heart had been excised from a guinea pig sensitized with the same amount of antibody as that given to the animal in Figure 1. The anaphylactic crisis in vitro consisted of sinus tachycardia, brief stimulation of ventricular contractile force followed by prolonged con-
Graded systemic anaphylaxis in anesthetized, ventilated guinea pigs. The relationships between the dose of sensitizing antibody and the changes in sinus rate (A), P-R interval (B), bronchial resistance (C), and mean arterial blood pressure (D) are shown. The dose of challenging antigen was kept constant at 5 mg. Points (means, x – 6–8, vertical bars = se) represent maximum changes from values immediately preceding antigenic challenge. Antigen caused a triphasic effect on blood pressure; only the initial pressor phase is plotted in D. The average initial control sinus rate was 244 ± 6 beats/min, the control P-R interval was 60 ± 1 msec, and the control mean blood pressure was 44 ± 3 mm Hg.

The severity of cardiac dysfunction depended on the degree of sensitization: the magnitude of the positive chronotropic effect increased, the incidence and duration of conduction arrhythmias increased, their onset occurred earlier, ventricular automaticity increased, and coronary flow rate was further decreased (Table 3) as the level of sensitization was increased.

As the amount of sensitizing antibody was increased, histamine release increased (Table 3 and Fig. 4). The amounts of histamine released by the hearts correlated with the increase in sinus rate (Fig. 5) and with the duration of conduction arrhythmia (Fig. 6).

In control experiments, hearts from sensitized guinea pigs were challenged in vitro with 1 mg of BGG (carrier only). BGG elicited no response.

### TABLE 1

<table>
<thead>
<tr>
<th>Amount of sensitizing antibody (mg)</th>
<th>Conduction*</th>
<th>Automaticity†</th>
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<tr>
<td>0.3</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>0.9</td>
<td>7/8</td>
<td>5/8</td>
</tr>
<tr>
<td>3.0</td>
<td>6/8</td>
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The sensitizing antibody was rabbit anti-BPO injected 12 hours prior to challenge.

* Incidence of death within 30 minutes of antigenic challenge.
† Incidence of fibrillation within 15 minutes of antigenic challenge.

### HISTAMINE AND CARDIAC PARTICIPATION IN SYSTEMIC ANAPHYLAXIS

Actively sensitized guinea pigs were challenged intravenously in the absence of anesthesia and artificial ventilation. Three minutes later, when respiration had ceased, the hearts were excised and subsequently challenged in vitro. The response of these hearts was compared with the anaphylactic reaction of hearts challenged in vitro only (Fig. 7). The response to antigen in vitro was greatly modified by previous systemic challenge: the sinus tachycardia was of moderate extent and duration (Fig. 8C), the slowing of the P-R interval was minimal and did not lead to atrioventricular block (Fig. 8B), the rate of coronary flow was not significantly changed from control values (Fig. 8A), and histamine release was significantly reduced (Fig. 8D).

In another series of experiments, the amount of cardiac histamine released during systemic anaphylaxis was assessed indirectly by measuring the residual histamine content of hearts from guinea pigs that had undergone systemic anaphylaxis. Residual cardiac histamine content following sys-
Anaphylaxis of the isolated guinea pig heart. The sensitization was the same as that in Figure 1. The recordings show isometric ventricular contraction (upper tracings) and surface electrograms (lower tracings). Numbers at the lower left of each section refer to time after antigenic challenge, and numbers at the lower right refer to heart rate (both atrial and ventricular rates are given during atrioventricular block). Ag = antigen, and V.T. = ventricular tachycardia.

Discussion

Our results provide both direct and indirect evidence that the heart is a target organ in systemic anaphylaxis of the guinea pig. (1) Severe disturbances in cardiac rate and rhythmicity developed during systemic anaphylaxis. (2) The electrocardiographic changes observed during systemic anaphylaxis were similar to those seen during anaphylaxis in isolated hearts. (3) The extent of the cardiac reaction in vivo and in vitro depended on the level of sensitization. (4) The reaction of the isolated heart was greatly reduced when in vitro challenge followed systemic anaphylaxis. (5) Significant amounts of histamine were released from the heart during systemic anaphylaxis.

The cardiac manifestations of systemic anaphylaxis included sinus tachycardia, progressive impairment of atrioventricular conduction leading to conduction block, and increased ventricular automaticity. Similar signs of cardiac dysfunction during systemic anaphylaxis in the guinea pig have been previously reported (4-6).

The cardiac events in systemic anaphylaxis might be interpreted as secondary reactions to the concurrent changes in bronchial resistance and blood pressure. However, the onset of cardiac changes occurred about 20 seconds after antigenic challenge, at the same time that the initial change in blood pressure occurred, and immediately before the anaphylactic increase in bronchial resistance.
Values are means ± SE; number of hearts tested is given in parentheses. Guinea pigs were passively sensitized in vivo and their hearts were challenged in vitro.

* Maximum increase from the value immediately preceding challenge.

† Average change during the 10 minutes following antigen administration from the value immediately preceding challenge.

(Fig. 1). Furthermore, the cardiac reaction in vivo very closely resembled anaphylaxis in the isolated heart. The intensity of the cardiac response, both in vivo and in vitro, depended on the amount of antibody used in the sensitization of the guinea pig (Fig. 2A and B and Tables 1 and 3). Since similar amounts of antibody were necessary for eliciting the cardiac reaction in vivo and in vitro, it would...
Relationship between histamine release and the duration of conduction arrhythmia in isolated hearts undergoing passive anaphylaxis. The abscissa indicates the amount of histamine (per wet weight of heart) released within 10 minutes after antigen administration. Each point represents a single heart. The curve was fitted by eye.

seem that the severity of the cardiac reaction in systemic anaphylaxis is truly a function of cardiac sensitization and does not just reflect a graded anaphylactic increase in bronchial resistance (Fig. 2C).

Cardiac histamine is released during hypersensitivity reactions in vitro (1) and mediates the anaphylactic changes in rate, contractility, and rhythmicity (2). Although mediators other than histamine might play a role in systemic anaphylaxis in the guinea pig, their contribution to cardiac anaphylaxis has not been established. In our system of graded anaphylaxis, the amount of histamine released in vitro depended on the level of sensitization (Fig. 4). The anaphylactic increase in the sinus rate and the duration of conduction arrhythmia correlated with the amount of released histamine (Figs. 5 and 6). Since the intensity of the anaphylactic reaction of the heart is directly proportional to the amount of histamine released in vitro, it is reasonable to assume that the amount of cardiac histamine released in vivo will similarly reflect the extent of direct cardiac participation in systemic anaphylaxis. Hearts from guinea pigs previously subjected to systemic anaphylaxis responded only very moderately to in vitro challenge (Fig. 8). The amount of histamine released was very small compared with the explosive histamine release from hearts challenged in vitro only (Fig. 8D). The most plausible explanation for this drastic reduction in histamine release is that cardiac histamine had been released during systemic anaphylaxis. This fact was verified by comparing the residual cardiac histamine content following systemic anaphylaxis with the cardiac histamine content of normal guinea pigs (Table 4). The difference between normal cardiac histamine content and post-anaphylactic cardiac histamine content represents the histamine released during systemic anaphylaxis. Since histamine can be considered to be an indicator of the anaphylactic reaction process, the extent of reduction in cardiac histamine content after systemic anaphylaxis reflects the degree of cardiac immunological reaction in vivo. The functional significance of this histamine release (1.3 μg/g) is clearly expressed in Table 3.

A decrease in coronary flow rate and a transient stimulation of contractile force followed by a prolonged contractile failure, in addition to sinus tachycardia and arrhythmias of both conduction and automaticity, were characteristic of cardiac anaphylaxis in vitro. In the intact guinea pig, only the electrocardiographic changes were measured; however, since the electrocardiographic changes in systemic anaphylaxis were similar to those in isolated heart anaphylaxis, it can be assumed that reduction of coronary flow rate and contractile
Isolated heart anaphylaxis and its modification by previous systemic challenge. The experimental design is shown in Figure 7. Time courses of change in coronary flow rate (A), prolongation of the P-R interval (B), increase in sinus rate (C), and the rate of histamine release (D) following isolated heart challenge are shown. Solid circles = averages (vertical bars = SE) for 14 hearts challenged in vitro only, and open circles = averages for 8 hearts challenged in vitro following systemic challenge. Abscissas: Time from injection of antigen. Ordinates: In A–C, changes from values immediately preceding antigenic challenge, and, in D, histamine release measured over 2-minute intervals. In B, the curve is interrupted during conduction arrhythmia. Average values ± se for control sinus rate, P-R interval, and coronary flow rate were 231 ± 7 beats/min, 60 ± 1 msec, and 5.1 ± 0.4 ml/min for the hearts challenged in vitro only and 258 ± 5 beats/min, 61 ± 1 msec, and 5.0 ± 0.3 ml/min for the hearts challenged in vitro following systemic challenge.

Values are means ± se; the number of observations is given in parentheses. FSA = fatal systemic anaphylaxis. Unanesthetized, nonventilated, actively sensitized guinea pigs were challenged intravenously with penicillin antigens. The amount of histamine released was calculated by subtracting the residual histamine content from the normal histamine content.

The severe consequences of the pulmonary reaction in guinea pig anaphylaxis, especially when it is unopposed by mechanical or pharmacological means, cannot be denied. However, acute cardiac dysfunction could easily be a contributing factor in anaphylactic death. The frequency of ventricular fibrillation (Table 2) in the ventilated guinea pig supports this view.

Electrocardiographic changes ranging from transient ischemia to severe rhythm disturbances have been recorded during anaphylaxis of the rabbit (13), monkey (14), and man (15–17). Thus, our failure also occurred in the systemic reaction. It is highly probable that a decrease in coronary flow rate accompanied the arrhythmias during systemic anaphylaxis, since reduced coronary flow in anaphylaxis in vitro is caused by conduction arrhythmia (2) and since arrhythmias of all types which lead to rapid or irregular ventricular rhythms have been shown to impair coronary circulation in intact animals (12). The complete loss of pulse pressure (Fig. 1) during systemic anaphylaxis indicates that cardiac output was markedly reduced. A decrease in cardiac output could have resulted from acute contractile failure, cardiac arrhythmias, or, most likely, a combination of the two.

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<th>Normal histamine content (μg/g)</th>
<th>Residual histamine following FSA (Mg/g)</th>
<th>Amount of histamine released (μg/g)</th>
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<tr>
<td></td>
<td>(23)</td>
<td>2.89 ± 0.21</td>
<td>1.27 ± 0.21</td>
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Circulation Research, Vol. 36, April 1975

Circulation Research, Vol. 36, April 1975
conclusion that the guinea pig heart reacts as a target organ during systemic anaphylaxis can tentatively be extrapolated to other species.

Acknowledgment

We are grateful to Dr. Dieter H. Sussdorf and Dr. Gregory W. Siskind for assistance in the preparation of antigens and antibodies.

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The heart as a target organ in systemic allergic reactions: comparison of cardiac anaphylaxis in vivo and in vitro.
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Circ Res. 1975;36:520-528
doi: 10.1161/01.RES.36.4.520

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