Separation of Primary and Secondary Cardiovascular Events in Systemic Anaphylaxis

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SUMMARY The purpose of this investigation was to differentiate primary cardiac participation in systemic anaphylaxis from a cardiac reaction secondary to respiratory distress. Hemocyanin-sensitized guinea pigs were anesthetized with sodium pentobarbital and artificially ventilated. The chest was opened and the left ventricle cannulated. The electrocardiogram, bronchial resistance, arterial blood pressure, and left ventricular pressure and its first derivative were recorded. Following intravenous administration of antigen, the sinus rate increased by about 50-60 beats/min, left ventricular dP/dt increased by a factor of 3, and mean arterial pressure doubled. Conduction disturbances occurred in all of the experiments and ventricular fibrillation in four of six. These changes were concomitant with a 4-fold rise in bronchial resistance. To separate the cardiac and respiratory components, antigen was administered directly into the left ventricle to expose the heart to antigen before the lungs. The intracardiac challenge resulted in increases in sinus rate and left ventricular and arterial pressures quantitatively similar to changes recorded from guinea pigs after the intravenous challenge. However, all these changes preceded the rise in bronchial resistance by 60 seconds. Arrhythmias occurred as frequently as with the intravenous challenge. Our findings show that by use of an appropriate route for administration of antigen, cardiovascular and respiratory components of systemic anaphylaxis can be separated. Our data also indicate that anaphylactic cardiovascular changes can be dissociated temporally into two sets of events: an initial primary cardiac reaction caused by intracardiac release of histamine and a subsequent cardiovascular reaction secondary to systemic release of mediator.

SEVERE disturbances of cardiovascular function occur during systemic anaphylaxis in the guinea pig. Characteristic features of cardiac anaphylaxis in vivo and in vitro include sinus tachycardia, atroventricular conduction block, increased ventricular automaticity, and histamine release. The similarities between the effects on the heart of anaphylaxis in vivo and in vitro led us to the conclusion that the guinea pig heart reacts as a primary target organ in systemic hypersensitivity reactions. However, because cardiac and respiratory events occur at the same time during systemic anaphylaxis, the possibility could not be excluded that certain cardiovascular changes were secondary to the effects of bronchospasm.

The present investigation describes an experimental model in which injection of antigen directly into the left ventricle of sensitized guinea pigs permits identification of the primary cardiac effects of anaphylaxis in vivo.

Methods

Thirty-two Hartley guinea pigs of either sex were sensitized by two consecutive weekly intraperitoneal injections of 1 mg of alum-precipitated keyhole limpet hemocyanin (KLH); 15-30 days after sensitization blood samples were taken by puncture of the retro-orbital plexus, and the serum level of anti-KLH anaphylactic antibody was determined by passive cutaneous anaphylaxis (PCA). By this method of sensitization the sera gave 4-hour PCA reactions in dilutions up to 1:100.

Twenty-two Hartley guinea pigs of either sex weighing 600-800 g, previously sensitized with KLH, were anesthetized by intraperitoneal injection of sodium pentobarbital (30-60 mg/kg) and placed on a small surgical table which was thermostatically controlled to maintain body temperature at 37°C. A tracheal cannula was inserted and connected to a small-animal respirator. Artificial ventilation at a constant rate of 40/min and a tidal volume of 1 ml/100 g of body weight plus the dead space volume was maintained throughout the experiment. A side arm of the tracheal cannula was connected to a pressure transducer. The output (an indirect measure of bronchial resistance) was displayed on a pen recorder. The left carotid artery was cannulated and connected to another pressure transducer and arterial pressure was displayed on the pen recorder. The chest was opened by a midline sternotomy, and a polyethylene cannula (PE 160), connected to a pressure transducer, was inserted into the left ventricle near the apex and sutured to the myocardial wall. Left ventricular pressure (LVP) and its first derivative (dP/dt, a measure of contractility) were displayed on the pen recorder. Standard lead II electrocardiographic tracings also were recorded. After a stabilization period KLH (5 mg, dissolved in 0.5 ml of saline) was rapidly injected intracardially into 16 guinea pigs through the cannula inserted in the left ventricle, and intravenously in six others through a cannula inserted into the jugular vein.

Twelve unsensitized guinea pigs were anesthetized and prepared as described above. After an equilibration period histamine (30 µg/kg, dissolved in 0.5 ml of saline) was injected either intracardially (left ventricle) or intravenously (jugular vein).

Ten sensitized guinea pigs were challenged intracardially in the absence of anesthesia and artificial ventilation. Symptoms of anaphylaxis (coughing, scratching, prostration, convulsions) began within seconds of chal-
Systemic anaphylaxis in the anesthetized, ventilated guinea pig (intravenous antigenic challenge). The guinea pig had been sensitized with keyhole limpet hemocyanin (KLH). Recordings show the increase in bronchial resistance (BR), carotid blood pressure (BP), left ventricular pressure (LVP), the first derivative of left ventricular pressure (dP/dt), and changes in the lead II electrocardiogram. Under each ECC panel the number at the lower left is the time after antigenic challenge, and the number at the lower right is the sinus rate. vFib = ventricular fibrillation.

Intravenous Antigenic Challenge

Recordings from a representative experiment in which an anaphylactic reaction was elicited by an intravenous antigenic challenge are shown in Figure 1. Bronchial resistance rapidly increased, beginning 34 seconds after injection, reached a peak within 1 minute and 20 seconds, and remained relatively constant thereafter. Coinciding with the increase in bronchial resistance there were increases in blood pressure, LVP, and dP/dt that reached a peak at 1 minute and 45 seconds, and progressively declined. Within 20 seconds after injection of antigen the sinus rate began to increase and attained a maximum at 1 minute and 45 seconds. The P-R interval increased and reached a peak at 1 minute and 45 seconds. Inversion of the T wave and S-T segment elevation were observed. Ventricular extrasystoles, atrioventricular dissociation, ventricular tachycardia, and eventually ventricular fibrillation occurred.

Intracardiac Antigenic Challenge

Figure 2 shows records from a typical anaphylactic reaction caused by injection of antigen into the left ventricle. Blood pressure, LVP, dP/dt, and sinus rate rapidly increased and reached plateaus within 1 minute. Bronchial resistance began to increase 1 minute and 20 seconds after injection of antigen and reached a maximum by 3 minutes and 30 seconds. A second peak in blood pressure, LVP, and dP/dt occurred at 3 minutes and 40 seconds. Inversion of the T wave and S-T segment elevation were observed. Atrioventricular conduction was progressively impaired until atrioventricular dissociation developed; this was followed by multifocal ventricular extrasystoles and eventually ventricular fibrillation.

Comparison of Intravenous and Intracardiac Challenges

The temporal development of the changes in sinus rate and bronchial resistance elicited by intravenous and intracardiac challenge are compared in Figure 3. Whereas, following intravenous antigen, tachycardia and bronchospasm developed concomitantly up to about 75% of their maximum, with intracardiac antigen tachycardia preceded bronchospasm by more than 1 minute. Consequently, sinus rate increased and reached a peak before the onset of bronchospasm.

Results

Chemicals

KLH (A grade) was purchased from Calbiochem and was dissolved in pyrogen-free saline and filtered prior to use. Alum (aluminum hydroxide gel, Alhydrogel containing 1.3% Al₂O₃ and 2% Al(OH)₃) was purchased from Accurate Chemical & Scientific Corp., and histamine dihydrochloride, from Sigma Chemical Co.). All histamine values refer to the free base.
In this study, the development of tachycardia and bronchospasm during systemic anaphylaxis was examined. The figure shows the time course of the changes in cardiovascular and respiratory parameters during systemic anaphylaxis, elicited by intracardiac antigenic challenge in anesthetized, ventilated, hemocyanin (KLH)-sensitized guinea pigs. Points are means of changes occurring at given times (n = 16). Mean changes in blood pressure and LV dP/dt which occurred 2 minutes after antigen were significantly different from those observed at 4 minutes. Mean blood pressure, P < 0.05; dP/dt, P < 0.02.

The time course of all cardiovascular and respiratory changes which occurred during anaphylaxis elicited by intracardiac challenge are shown in Figure 4. Not only the sinus rate, but also blood pressure and dP/dt increased and reached a peak prior to the onset of bronchospasm. Whereas bronchial resistance maintained a plateau at its maximum increase, sinus rate, dP/dt, and blood pressure reached a peak at 1 minute and 30 seconds, then declined and increased again to a second peak by 4 minutes. The second peak was attained after bronchial resistance had reached its maximum level. After the second peak, dP/dt and blood pressure rapidly decreased, whereas sinus rate and bronchial resistance did not decline.

Anaphylactic release of histamine

In another series of experiments, the amount of cardiac and pulmonary histamine released during systemic anaphylaxis was assessed indirectly by measuring the residual histamine content of hearts and lungs from guinea pigs that had undergone systemic anaphylaxis. Residual cardiac and pulmonary histamine content following intravenous antigenic challenge was significantly less than the histamine content of hearts and lungs from normal guinea pigs (Table 1). Thus, during fatal systemic anaphylaxis, 41% of cardiac histamine content and 78% of pulmonary histamine content were released.

Cardiovascular and respiratory events following the intravenous or intracardiac administration of histamine

In Figure 5 the effects of intravenous administration of histamine (30 μg/kg) on sinus rate, dP/dt, blood pressure, and bronchial resistance are compared with the effects of histamine injected intracardially. In both cases histamine caused increases in all four parameters; however, the time course of the changes varied with the route of administration. Within 15 seconds of the intravenous administration of histamine, bronchial resistance rapidly increased, attained a peak at 30 seconds, and declined exponentially thereafter. The rate of rise in sinus rate, dP/dt, and blood pressure was slower than that of bronchial resistance, and peak levels were reached 1 minute and 30 seconds after the injection. Following intracardiac injection of histamine, bronchial resistance increased only moderately and...
TABLE 1  Release of Cardiac and Pulmonary Histamine during Systemic Anaphylaxis

<table>
<thead>
<tr>
<th></th>
<th>Residual histamine</th>
<th>Normal histamine content</th>
<th>Histamine release (%)</th>
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<tbody>
<tr>
<td></td>
<td>after fatal</td>
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<td></td>
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<tr>
<td></td>
<td>systemic anaphylaxis (μg/g)</td>
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<td></td>
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<tr>
<td>Heart</td>
<td>2.45 ± 0.17 (10)</td>
<td>4.16 ± 0.21 (23)</td>
<td>41</td>
</tr>
<tr>
<td>Lungs</td>
<td>5.58 ± 1.09 (10)</td>
<td>25.59 ± 2.36 (23)</td>
<td>78</td>
</tr>
</tbody>
</table>

Values are means ± SE; the number of observations is given in parentheses.

Unanesthetized, nonventilated, sensitized guinea pigs were challenged intravenously with keyhole limpet hemocyanin (KLH). Histamine release was calculated by subtracting the residual histamine content from the normal histamine content and this difference is expressed as a percent of the normal histamine content.

with a delayed onset, as compared to intravenous administration. However, sinus rate, dP/dt, and blood pressure increased prior to the onset of bronchospasm; in fact, the peaks in dP/dt and blood pressure occurred at 17 seconds, prior to the onset of bronchospasm. Blood pressure and dP/dt rapidly declined, and by 45 seconds increased again to a second peak which occurred between 60 and 90 seconds. This was followed by a gradual decline in these values over the next few minutes. Cardiac conduction disturbances and arrhythmias, similar to those observed after antigenic challenge, occurred following either intravenous or intracardiac administration of histamine.

Discussion

Systemic anaphylaxis in the guinea pig is a dramatic reaction which involves respiratory distress and cardiovascular collapse. These events are mediated by a massive release of histamine from cardiac and pulmonary stores. Although the heart directly participates as a target organ in systemic anaphylaxis, the cardiac contribution is obscured by the pulmonary reaction. The reason for this is that following intravenous challenge, the pulmonary circulation is exposed to antigen before the coronary circulation. Hence, respiratory distress is superimposed on cardiac dysfunction. Therefore, we attempted to separate cardiac and respiratory changes by administering the antigen into the left ventricle so as to reverse the sequence of its distribution and expose the heart to antigen before the lungs. Our findings clearly demonstrate that, by intracardiac antigenic challenge, (1) cardiovascular and respiratory components of systemic anaphylaxis can be separated, and (2) cardiovascular changes can be temporally dissociated into two sets of events, an initial primary cardiac reaction probably caused by the release of histamine from the heart, and a subsequent cardiovascular reaction secondary to systemic release of mediator.

Whereas the development of sinus tachycardia is not influenced by the route of antigen administration (Fig. 3), the development of bronchospasm is greatly delayed following intracardiac challenge. Thus, cardiac and respiratory components of anaphylaxis can be separated.

Following intracardiac antigen, two sets of maxima are clearly evident in the time course of the changes in contractility and arterial pressure (Fig. 4). Since the first set of peaks occurs before the onset of bronchospasm, it most probably reflects the intracardiac histamine release which increases both rate and contractility, with the consequent increase in cardiac output and arterial pressure. Following distribution of antigen to the lungs, histamine is massively released in the pulmonary and general circulation, leading to bronchospasm, increased peripheral resistance, and increased cardiac contractility. Although the second peaks in arterial pressure and dP/dt coincide, blood pressure begins to increase prior to the increase in contractility. Thus, the second increase in contractility could be due to the cardiac effect of circulating histamine; or it could result from a histamine-induced increase in afterload; or it may...
be caused by a combination of both mechanisms. However, the involvement of other mediators cannot be excluded. The progressive decrease in pressure and contractility which follows the secondary peaks is indicative of the severe cardiac dysfunction which consistently characterizes the later phase of anaphylaxis and comprises ventricular failure and arrhythmias. The sustained elevation of bronchial resistance probably results from the combined effects of the various mediators of pulmonary anaphylaxis [i.e., histamine, slow-reacting substance of anaphylaxis (SRS-A), and prostaglandins] and possibly from an increased bronchomotor tone caused by a vagal reflex mechanism. The prolonged sinus tachycardia reflects the high sensitivity of the sinoatrial node to circulating histamine, with the possible contribution of catecholamines released from the adrenals.

The intracardiac injection of histamine causes changes in cardiovascular and respiratory parameters that are qualitatively similar to those observed during anaphylaxis elicited by intracardiac antigenic challenge. As with antigen, two sequential peaks in blood pressure and contractility are observed (Fig. 5). Following administration into the left ventricle, histamine immediately gains access to the coronary circulation and stimulates contractility (dP/dt), rate, and consequently blood pressure. Histamine is then distributed systemically and recirculates into the heart. This causes the second peaks in arterial pressure and contractility. The onset, magnitude, and duration of the effects caused by the intracardiac administration of histamine or antigen are quantitatively different (Figs. 4 and 5). This simply reflects differences in distribution and accumulation of histamine at the various effector sites following immunological release or exogenous administration. Thus, the comparison between the effects of intracardiac injection of histamine and intracardiac antigenic challenge substantiates our hypothesis that the two subsequent peaks in pressure and contractility observed after intracardiac challenge represent the effects of histamine sequentially released from cardiac and pulmonary stores as a function of antigen distribution. Our hypothesis is further supported by the fact that the intravenous injection of histamine causes changes in cardiovascular and respiratory parameters (Fig. 5) that are qualitatively similar to those caused by intravenous antigen (Fig. 1). Thus, anaphylactic cardiac dysfunction results from a double immunological insult, both cardiac and pulmonary, and histamine is a major mediator of this reaction.

Cardiac arrhythmias invariably occurred during anaphylaxis, and ventricular fibrillation was the most common cause of death (Figs. 1 and 2). Release of histamine is probably responsible for the arrhythmias encountered during cardiac anaphylaxis.1-3 These arrhythmias tend to impair coronary flow and this, in turn, compromises myocardial perfusion and probably sustains the existing dysrhythmia.4-6 A further cause of poor myocardial perfusion is the coronary-constricting effect of prostaglandin 

In conclusion, our results clearly delineate the cardiac involvement in systemic hypersensitivity reactions and demonstrate the relevance of immunologically induced cardiovascular collapse.

References
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