Complement activation-related cardiac anaphylaxis in pigs: role of C5a anaphylatoxin and adenosine in liposome-induced abnormalities in ECG and heart function

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Szebeni, János, Lajos Baranyi, Sándor Sávay, Michael Bodó, János Milosevits, Carl R. Alving, and Rolf Bünger. Complement activation-related cardiac anaphylaxis in pigs: role of C5a anaphylatoxin and adenosine in liposome-induced abnormalities in ECG and heart function. Am J Physiol Heart Circ Physiol 290: H1050–H1058, 2006.—Cardiac anaphylaxis is a severe, life-threatening manifestation of acute hypersensitivity reactions to allergens and drugs. Earlier studies highlighted an amplifying effect of locally applied C5a on the process; however, the role of systemic complement (C) activation with C5a liberation in blood has not been explored to date. In the present study, we used the porcine liposome-induced cardiopulmonary distress model for 1 characterizing and quantifying peripheral C activation-related cardiac dysfunction; 2) exploring the role of C5a in cardiac abnormalities and therapeutic potential of C blockage by soluble C receptor type 1 (sCR1) and an anti-C5a antibody (GS1); and 3) elucidating the role of adenosine and adenosine receptors in paradoxical bradycardia, one of the symptoms observed in this model. Pigs were injected intravenously with different liposomes [Doxil and multilamellar vesicles (MLV)], zymosan, recombinant human (rhu) C5a, adenosine, and the ensuing hemodynamic and cardiac changes (hypotension, tachy- or bradycardia, arrhythmias, ST-T changes, ventricular fibrillation, and arrest) were quantified by ranking on an arbitrary scale [cardiac abnormality score (CAS)]. There was significant correlation between CAS and C5a production by liposomes in vitro, and the liposome-induced cardiac abnormalities were partially or fully reproduced with zymosan, rhuC5a, adenosine, and the selective adenosine A1 receptor agonist cyclopentyl-adenosine. The use of C nonactivator liposomes or pretreatment of pigs with SCR1 or GS1 attenuated the abnormalities. The selective A1 receptor blocker cyclopentyl-xanthine inhibited bradycardia without influencing hypotension, whereas the A2 blocker 4-[2-(7-amino-2-(2-furyl)-1,2,4]triazolo[2,3-a]1,3,5]triazin-5-ylamino]ethyl)phenol (ZM-24135) had no such effect. These data suggest that 1) systemic C activation can underlie cardiac anaphylaxis, 2) C5a plays a causal role in the reaction, 3) adenosine action via A1 receptors may explain paradoxical bradycardia, and 4) inhibition of C5a formation or action or of A1-receptor function may alleviate the acute cardiotoxicity of liposomal drugs and other intravenous agents that activate C.

adenosine receptors; allergy; anaphylactoid reactions; bezold-jarisch reflex; electrocardiography; cyclopentyl-xanthine; hemodynamic changes; hypersensitivity reactions; pseudoallergy

CARDIAC ANAPHYLAXIS is one of the most severe, potentially lethal manifestations of immediate allergy from a variety of allergens and drugs. It involves major abnormalities of cardiac electrical conductance and ventricular function, leading to heart rate changes with conductance blocks, arrhythmias, ventricular fibrillation and arrest, acute circulatory failure, and, occasionally, death (6, 12, 30, 34, 51). As for its mechanism, activation of mast cells in the heart is a key underlying process (17, 18, 23, 24, 27, 28), although details of the reaction remain poorly understood. One of its controlling factors recognized in the past is the anaphylatoxin C5a, whose intracorony administration was shown to amplify the electrical and mechanical response of isolated guinea pig heart to a variety of allergens (7, 8, 17, 18). However, in the absence of information on the site and the amount of C5a formation during classical allergic reactions in vivo and the trafficking of anaphylatoxins into and within the heart, the physiological relevance of cardiac responses to locally applied C5a remains questionable. Another unsolved question in this area is whether C5a plays a role in the adverse cardiac effects of some complement (C)-activating drugs, where prior exposure to the drug or to its components and, hence, sensitization cannot be established. Examples for the latter phenomenon include the acute cardiac disturbance caused by the anticancer drugs paclitaxel (Taxol) (5, 19, 21, 32, 33, 50), liposomal amphotericin B (Ambisome) (1), liposomal daunorubicin (DaunoXome) (10), and liposomal doxorubicin (Doxil) (2, 49). Taxol activates C via the emulsifier of paclitaxel, Cremophor EL (42, 48), whereas liposomal drugs do so via their phospholipid bilayer capsule (38, 41).

We reported previously that intravenous injection of a small amount of large multilamellar vesicles (MLV) (43, 47) or of Doxil (44) in pigs induces dramatic hemodynamic changes via C activation in peripheral blood. The clinical picture included cardiac abnormalities typical of anaphylaxis, suggesting that the model may have utility in the elucidation of the role of systemic C activation in this syndrome. Accordingly, the specific goals of the present study were 1) to characterize the cardiac electric and functional changes during liposome-induced peripheral C activation; 2) to develop criteria for quantitative assessment of cardiac abnormalities in vivo; 3) to explore the role of C5a in the changes and potential benefits of C5a blockade; and 4) to elucidate the roles of myocardial adenosine release and adenosine A1 and A2 receptors in para-

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doxical bradycardia, an intriguing phenomenon observed in our model that we report here for the first time.

MATERIALS AND METHODS

Materials

Chemicals and drugs. Dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, AL). Zymosan, Nω-cyclopentyl-adenosine (CPA), recombinant human C5a (rhuC5a), and adenosine were from Sigma Chemical (St. Louis, MO). 4-2-[7-Amino-2-(2-furyl)]1,2,4]triazolol[2,3-a][1,3,5]triazin-5-ylamino]ethylphosphonol (ZM-241385) was from Tocris (Ellisville, MO), and 8-cyclopentyl-1,3-dipropyl-3,7-dihydro-1-purine-2,6-dione (CPX) was generously provided by Dr. Ray Olsson, Department of Medicine, University of Florida (Tampa, FL). Recombinant soluble C receptor type 1 (sCR1), murine anti-porcine C5a (GS1, Chemicon, Temecula, CA), and the antibodies for the pig C5a ELISA were provided by Avant Immunochemicals (Needham, MA), Dr. Gregory L. Stahl (Boston, MA), and Professor Otto Goetze (Heidelberg, Germany) as detailed previously (45, 47).

Liposomes. The source, preparation, and detailed characterization of Doxil and synthetic, large MLV and unilamellar vesicles (LUV) consisting of DMPC, DMPG, and Chol (50:5:45 mol ratios) were described in detail in previous studies (43, 44, 47).

Experiments Using Pigs

The Institutional Animal Care and Use Committee approved the procedures described, which was conducted in compliance with the Animal Welfare Act and adhered to the principles stated in the current Guide for the Care and Use of Laboratory Animals. Yorkshire swine in the 25- to 40-kg range were sedated with intramuscular ketamine (500 mg), followed by intubation or tracheotomy, and anesthesia with isoflurane using a Narkomed 28 (North American Drager, Telford, PA) anesthesia machine. Inspired O2 and end-tidal CO2 tensions were maintained at 21 volume percentage and 35–40 mmHg, respectively. Continuous acquisition and analysis of ECG and hemodynamic data were done with the use of a personal computer and in-house data acquisition and analysis software DataLyser (developed in Labwindows CVI), compatible with a National Instruments (Austin, TX) data acquisition card. The data were acquired at 250-Hz sampling rate.

ECG recording was started at 1–2 min before injection of test substances and lasted for 20 min of postinjection. Heart rate was calculated from the R-R intervals, obtained from 10 consecutive QRS complexes on the ECG. Measurement of T-wave amplitude and ST-segment alterations were done by cursor operation. Other details of hemodynamic analysis, including equipment, placement of catheters, and measurement of systemic and pulmonary arterial pressure surces (SAP and PAP, respectively), cardiac output (CO), and end-tidal PCO2 were described previously (43, 47).

Liposomes, zymosan, C5a, adenosine, and CPA were administered into the jugular vein as bolus injections (within about 5–10 s), using PBS as vehicle. Each injection was followed by 5–10 ml of PBS wash. CPX and ZM-24135 were administered by infusion 10–15 min before the liposome injections. CPX was initially dissolved in ethanol, which was then diluted 200-fold in saline. The C inhibitor sCR1 and GS1 were applied as described earlier (47).

Estimation of C5a Production by Liposomes In Vitro

The C5a-producing potency of three specified liposome preparations (MLV, Doxil, and LUV) was assessed by incubation of 5 mg phospholipid/ml liposome with heparinized (20 IU/ml) pig plasma in vitro for 15 min at 37°C. The reaction was stopped by the addition of 20 mM EDTA, followed by measurement of porcine C5a by ELISA. Details of the assay utilizing affinity purified porcine C5a, anti-hog C5a MAb T13/9, rabbit IgG against mouse IgG, and biotinylated rabbit anti-hog C5a were described earlier (14, 45). The calibration curve was linear in the 0.6–10 ng C5a/ml range.

Statistical Methods

Data are presented as typical SAP and PAP curves and ECG tracings and are means (SD) for continuous variables. For analyzing the correlation between C5a formation in vitro and heart function in vivo, we developed a scoring system to provide a semiquantitative estimate of myocardial dysfunction on a scale of 1 to 5. The definition and details of cardiac abnormality scores (CAS) are described in the legend of Table 1. The correlation between CAS and C5a production by liposomes in vitro was analyzed by calculating the nonparametric (Pearson) correlation coefficient, which considers ranks without requiring normality in the distribution of values. All statistical analyses were done with GraphPad Prism.

RESULTS

Characteristics of Liposome-Induced Cardiac and Hemodynamic Changes

Figures 1–3 show typical cardiac and systemic hemodynamic changes that followed the injection of increasing doses of Doxil or MLV in pigs. They illustrate the common, as well as the differing, features of more than a hundred reactions induced by Doxil, MLV, and a variety of other reactogenic

Table 1. Quantification of liposome-induced cardiac abnormalities in pigs

<table>
<thead>
<tr>
<th>ECG Abnormalities</th>
<th>Hemodynamic and Cardiorespiratory Alterations</th>
<th>Qualitative Description</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arrhythmia episodes and/or transient tachycardia</td>
<td>No or minimal changes in PA, SAP, CO, PAP, and PCO2</td>
<td>Minimal</td>
<td>1</td>
</tr>
<tr>
<td>Longer lasting arrhythmia with tachycardia</td>
<td>Moderate rise of SAP and reduction of PA and no or minimal changes in CO, PAP, and PCO2</td>
<td>Mild</td>
<td>2</td>
</tr>
<tr>
<td>Major arrhythmias with tachycardia and/or bradycardia</td>
<td>Initial rise followed by moderate declines in SAP, declines of PA, CO, and PCO2, and moderate rise of PAP</td>
<td>Moderate</td>
<td>3</td>
</tr>
<tr>
<td>Major arrhythmias with tachycardia and/or bradycardia and ST depression/T-wave changes</td>
<td>Dramatic and extended declines in SAP, PA, CO, and PCO2, and major rise of PAP</td>
<td>Severe</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Dramatic, extended, and irreversible declines in SAP, PA, CO, and PCO2, and maximal rise of PAP. Fatal without CPR.</td>
<td>Lethal</td>
<td>5</td>
</tr>
</tbody>
</table>

CAS, cardiac abnormality score, an arbitrary rank based on severity of ECG and associated hemodynamic and cardiorespiratory abnormalities; PA, pulse amplitude; SAP, systemic arterial pressure; CO, cardiac output; PAP, pulmonary arterial pressure; CPR, cardiopulmonary resuscitation, consisting of an administration of intravenous epinephrine (0.01–0.1 mg/kg) with or without chest compression and/or electroconversion. Grouping of symptoms was based on analysis of 111 liposome reactions triggered in a total of 63 pigs over several years of experimentation with the model. Each CAS category listed was observed at least 22 times.
liposomes. The most prominent common feature of liposome reactions was their rapid development and reversibility, with most hemodynamic and ECG alterations returning to baseline or near baseline within 15–30 min. The variable features included the direction and extent of heart rate and SAP changes, the degree of pulmonary hypertension with or without reduction of CO and PCO₂, and the nature and severity of arrhythmias. Thus Fig. 1 demonstrates a moderate reaction to Doxil, characterized by an abrupt drop of mean arterial blood pressure (MAP) (Fig. 1A) that was associated with massive pulmonary hypertension, decreased CO, and decreased PCO₂ (Fig. 1B). During the nadir of blood pressure curve, lasting for ~4 min, we observed a transient tachyarrhythmic episode followed by ST depression and T-wave elevation (Fig. 1C, curves b–d, respectively). Although the MAP did not completely return to baseline, the ECG normalized after about 12–15 min (curve e). Figure 2A presents a more severe reaction to a higher dose of Doxil, involving a deeper and longer hypotensive period compared with that presented in Fig. 1. This reaction was associated with severe bradycardia with arrhythmia with the presence of incomplete as well as complete atrioventricular (AV) blocks with asystole (Fig. 2B). Curves b–d in Fig. 2B show gradual increase of PQ interval leading to 2-to-1 AV block, suggesting that the bradycardia was not of sinus origin but rather a reflection of slowed AV conduction. Figure 3 illustrates a lethal reaction involving ventricular fibrillation and cardiac arrest within 3 min after the injection of MLV. Resuscitation of this animal with epinephrine is also documented as a sudden overshoot of MAP into the hypertensive range.

Additional notable features of hypotensive liposome reactions included a greater reduction of systolic pressure compared with diastolic pressure, resulting in a substantial reduction of pulse pressure amplitude (Figs. 1–3). Furthermore, as illustrated in Fig. 2, hypotension was often associated with bradycardia or bradyarrhythmia, although the physiological baroreflex response to hypotension is tachycardia. Hence, the phenomenon represents relative or paradoxical bradycardia (9, 29). Quantification of the magnitude of MLV-induced bradycardia by averaging the increase of R-R distances over baseline at maximal bradycardia (% of baseline) gave 278 ± 31% increases (mean ± SE, n = 7 pigs, 10-s sampling times; MLV, 0.1 mg/kg). We also noted that the bradycardic and arrhythmic effects of liposomes showed significant positive correlation (linear regression analysis $R^2 = 0.51$, $P = 0.005$, $n = 28$ reactions) when maximal bradycardia (% of baseline) was plotted against the SD of R-R distances at the time of peak bradycardia (SD in the 0.05- to 0.5-s range; 40 heart beats). This implies an association between increased bradycardia and increased probability of arrhythmia, another indication of nonsinus origin of bradycardia.

Fig. 1. Hemodynamic and ECG changes in a pig after bolus injection of Doxil. A: real-time tracing of systemic arterial pressure (SAP) curve. Doxil was administered into the jugular vein at 0.1 mg lipid/kg. B: time course of changes of pulmonary arterial pressure (PAP), end-tidal PCO₂, and cardiac output (CO) during same reaction. C: ECG tracings taken at time points shown in A.
Figure 4A shows an episode of major ST depression observed during severe reactions. When quantified and expressed as a function of time, the nadir of ST depression appeared 4–6 min after the injection of liposomes, i.e., with 2–3 min delay relative to the peak of hemodynamic changes (Figs. 4B and 1C, curve c). A similar analysis of the time course of T-wave elevation showed biphasic changes with peaks around 4–5 and 10–11 min (Fig. 4C).

**Quantification of Liposome-Induced Cardiac Dysfunction**

In light of the multitude and variability of liposome-induced cardiac abnormalities, it was impossible to use any of the measured ECG or hemodynamic parameters as a comprehensive index of cardiac dysfunction. We developed, therefore, a scoring system that took into consideration all cardiac electric and hemodynamic changes to differentiate among groups of symptoms with a quantitatively distinguishable level of severity. Table 1 shows the key for this classification, based on the analysis of 111 liposome reactions in 63 experiments wherein pigs were injected with Doxil, MLV, or other reactogenic liposomes. We differentiated five categories with increasing CAS in the 1–5 range, reflecting increasing severity from mild to lethal.

**The Role of C5a in Liposome-Induced Cardiac Dysfunction**

**Correlation between in vitro C5a production and cardiac dysfunction in vivo.** To explore the role of C5a in the cardiac changes in our model, in one of three types of experiments we selected three liposome preparations that had substantially different in vivo reactogenicity, quantified the cardiac dysfunction they caused using CAS (Table 1), and correlated the CAS values with the C5a-producing efficacy of these vesicles in pig serum in vitro. The three liposomes selected for these studies were MLV, Doxil, and LUV (see MATERIALS AND METHODS) that were previously reported to cause strong, intermediary, and no hemodynamic side effects in pigs, respectively (43, 44, 47). In addition, we also used zymosan, a C-activating yeast cell membrane extract, which also caused major hemodynamic changes in pigs (47). As shown in Table 2, C5a production by matched amounts of liposomes and zymosan showed significant correlation with CAS ($P < 0.05$), supporting the notion that C5a plays a causal role in the cardiac abnormalities caused by these agents. In addition, the finding that the nonliposomal C activator zymosan also caused cardiac changes similar to those caused by high doses of Doxil or MLV provided evidence that the reactions were not due to a property unique to liposomes.

**Cardiac effects of C5a.** In the second series of experiments aimed at exploring the relationship between systemic C5a liberation and cardiac dysfunction, we injected pigs with increasing doses of rhuC5a using a dose range that was previously reported to cause hemodynamic abnormalities in various animals (15, 20). As shown in Fig. 5A, 330 ng/kg rhuC5a, which raised the baseline C5a level in pig blood [30–40 ng/ml (45)] by 30–40%, led to a mild (CAS 2) reaction with transient reduction of pulse pressure and slight, reversible hypertension.
In an effort to elucidate the mechanism of liposome-induced paradoxical bradycardia, we examined the heart rate and blood pressure responses of pigs after intravenous administration of adenosine, CPA, MLV, Doxil, and zymosan all caused paradoxical bradycardia, i.e., decreased both the heart rate and MAP. CPX alone had a tachycardic effect and converted the bradycardic effect of exogenous adenosine was shown to intensify the allergen-induced anaphylactic crisis in isolated, perfused guinea pig hearts (7, 18), leading Del Balzo et al. to suggest that C activation functions as an amplification system in cardiac anaphylaxis. However, the physiological relevance of the latter information is not clear without evidence that allergen-induced C activation, which is usually mild and may be abrogated by other mediators and vasoactive molecules (22–24). Thus C5a was shown to intensify the allergen-induced anaphylactic crisis in isolated, perfused guinea pig hearts.

**Mechanism of Paradoxical Bradycardia**

In an effort to elucidate the mechanism of liposome-induced paradoxical bradycardia, we examined the heart rate and blood pressure responses of pigs after intravenous administration of liposomes, adenosine, or the selective adenosine A1 receptor agonist CPA alone or in combination with CPX or ZM-24135, drugs representing selective A1 and A2 receptor antagonists, respectively (11). Adenosine is known to cause bradycardia with peripheral hypotension (4, 26, 53), and, as shown in Figure 6A, these changes could be reproduced with an intravenous bolus of 0.3 mg/kg adenosine under our conditions. Figure 6B shows that the bradycardic effect of exogenous adenosine was linear in the 0.1–0.6 mg/kg dose range. As shown in Table 4, adenosine, CPA, MLV, Doxil, and zymosan all caused paradoxical bradycardia, i.e., decreased both the heart rate and MAP. CPX alone had a tachycardic effect and converted the bradycardic effect of all above reaction triggers into considerable tachycardia. Importantly, however, the hypotensive effects of these agents were not, or were minimally, inhibited by CPX, implying selective inhibition of bradycardia. ZM-24135, the selective A2 blocker, had no such effects as CPX, suggesting that bradycardia was mediated primarily by cardiac adenosine A1 receptors. Taken together, these data suggest that acute adenosine release within the heart could explain paradoxical bradycardia via A1 receptors.

**DISCUSSION**

**Cardiac Anaphylaxis and the Role of C**

Cardiac anaphylaxis, part of an acute and complex multisystem reaction, is the most severe manifestation of hypersensitivity to a variety of allergens, including food, pollens, venoms, and, importantly, certain drugs. Its pathomechanism involves activation of cardiac mast cells in the coronary arterial intima, and perivascularly, in close proximity to myocytes (22–24). As for the role of C, cardiac mast cells express high-affinity receptors for C3a and C5a whose triggering by anaphylatoxins induces the release of a variety of inflammatory mediators and vasoactive molecules (22–24). Thus C5a was shown to intensify the allergen-induced anaphylactic crisis in isolated, perfused guinea pig hearts (7, 18), leading Del Balzo et al. to suggest that C activation functions as an amplification system in cardiac anaphylaxis. However, the physiological relevance of the latter information is not clear without evidence that allergen-induced C activation, which is usually mild and
Table 3. Inhibition of large multilamellar liposome-induced cardiac changes in pigs by blockers of complement activation and action

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CAS</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCR1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 mg/kg</td>
<td>28, 71 (2)</td>
<td>4, 4 (2)</td>
<td>2, 1 (2)</td>
</tr>
<tr>
<td>2.0 mg/kg</td>
<td>40, 99 (2)</td>
<td>4, 3 (2)</td>
<td>2, 1 (2)</td>
</tr>
<tr>
<td>GS1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.6 mg/kg</td>
<td>41.3 (SD 12.5) (4)</td>
<td>3.8 (SD 0.5) (4)</td>
<td>2.6 (SD 0.6) (4)</td>
</tr>
</tbody>
</table>

Individual values with n = 2 or means (SD) for n = 4 animals (in parentheses). These data were reported earlier (47). Pulmonary hypertensive responses to 5 mg MLV (0.10 – 0.16 mg lipid/kg) were measured in pigs before and after treatment of animals intravenously with specified doses of soluble complement receptor type 1 (sCR1) and GS1, a porcine anti-C5a antibody (see MATERIALS AND METHODS and Ref. 43). Inhibition of reaction, posttreatment rise of PAP was related to pretreatment rise to quantify inhibitory effect of drugs on pulmonary hypertension in terms of percentage. Entries are % inhibition obtained by the formula: 100 – (PAPpost/PAPpre), where PAPpre and PAPpost are MLV-induced rise of PAP before and after drug treatment, respectively. CAS values for reactions before and after treatment (before, after). The “after” values are significantly lower than the “before” values for GS1, and posttreatment values are also consistently lower for the 2 doses of sCR1 with 2 animals in each group.

occurs at the site of allergen exposure (52), leads to reactogenic levels of C5a in the heart despite the extremely short (seconds to minutes) half lives of anaphylatoxins (15, 20). This lack of crucial information regarding the “C amplification of cardiac anaphylaxis” theory, together with the unexplained adverse cardiac effects of C-activating liposomes and other drugs (see Clinical Relevance of C-Mediated Cardiac Anaphylaxis) prompted us in the present study to examine whether peripheral C activation can explain cardiac anaphylaxis in vivo in a large animal model.

Pathophysiology of C Activation-Related ECG Changes

As previously outlined in detail, liposome-induced and C-mediated hemodynamic changes in pigs result from multiple, interdependent, and adverse processes, including eicosanoid (mainly thromboxane A2)-mediated pulmonary and coronary vasoconstriction (43, 47), possibly combined with microthrombus formation and microembolization of capillaries by neutrophil-platelet aggregates (25, 35, 37, 47). The resultant falls in left cardiac preload and coronary flow lead to myocardial ischemia, decreased contractility, and reduced cardiac output and hypotension, all feeding a vicious cycle that either resolves spontaneously or leads to death via circulatory collapse. The present study further refines this scheme inasmuch as we suggest that ischemic adenosine release from the heart causes bradycardia with arrhythmias, and, hence, it represents an additional factor aggravating cardiac dysfunction. Accordingly, the ECG changes, catalogued and analyzed for the first time in the present study, can most easily be rationalized with ischemia-related membrane dysfunction plus adenosine-induced and A1-mediated electric conduction problems, as discussed below in more detail.

Although cardiac Purkinje fibers and the working cardiomyocytes are not known to express G protein-linked receptors that mediate ion channel conduction changes in response to anaphylatoxins (16, 31), the late (after 10–15 min) ECG alterations, particularly the protracted and biphasic T-wave changes, could reflect direct membrane damage in the cells caused by the terminal C complex (C5b-9) (13, 37).

Adenosine Release as Underlying Cause of Paradoxical Bradycardia

One of the original observations in this study was the occurrence of a special physiological phenomenon referred to as relative or paradoxical bradycardia (9, 29). Our focus on adenosine as an underlying cause of this event was based on the facts that even moderate myocardial ischemia can lead to substantial local adenosine production and release from the heart and that extracellular adenosine is known to cause bradycardia via hyperpolarization of Purkinje fibers in the sinus and AV nodes and throughout the entire cardiac conduction system (4, 26, 53). Also, Del Balzo et al. (8) described that the negative dromotropic effect of intracoronary injection of rhuC5a in isolated guinea pig hearts was mediated by adenosine.

Our findings that adenosine and CPA mimicked, whereas CPX inhibited the bradycardic effect of intravenous C activators, corroborate the above data of Del Balzo et al. (8) and extend their observations inasmuch as we provide pharmacological evidence for a critical role of A1 receptors in the phenomenon. In addition, our experiments with CPX provide arguments against another possible explanation of paradoxical bradycardia: operation of the classical vagus-mediated Bezold-
Jarisch reflex that may underlie vasovagal syncope and sudden death (3). The phenomenon involves nonspecific irritation of cardiac chemosensitive mechanoreceptors leading to arterial hypotension, bradycardia, and apnea. There are two major reasons why our experiments argue against the involvement of the Bezold-Jarisch reflex in C activation-related paradoxical bradycardia. First, CPX is not known to inhibit cholinergic neurotransmission (11), and, second, CPX led to pharmacological uncoupling of bradycardia from arterial hypotension, which is not easy to reconcile with the joint, central inhibition of sympathetic vasoconstrictor and cardioaccelerator and respiratory centers in the medulla oblongata during the Bezold-Jarisch reflex (3). Nevertheless, in the absence of specific experimental evidence or literature data, it cannot be excluded a priori that adenosine, via A1 receptors, may trigger a selectively heart-slowing cardiopulmonary chemoreflex or that it selectively interferes with the cardiac cholinergic system in pigs (in fact, CPX itself caused tachycardia, Table 4). Exploration of these possible mechanisms was beyond the scope of this investigation and may require further studies.

Causal Role of C5a in Liposome Reactions

The correlation between 1) liposome-induced C5a formation in pig serum in vitro and the degree of adverse reactions in vivo, 2) the reproduction of cardiac anaphylaxis with rhuC5a, and 3) the inhibition of ECG abnormalities with inhibitors of C5a formation or action provides strong support for a key causal role of C5a in liposome-induced cardiac changes in our model. The experiments using rhuC5a also allowed for some calculations with regard to the extent of C5a rise during mild and severe cardiac reactions to exogenous C5a. Thus, with the consideration that the normal plasma levels of C5 and C5a in pigs are ~175 and 20 ng/ml, respectively, and that the plasma volume in pigs is ~33 ml/kg, the barely reactiveogenic 330 ng/kg rhuC5a dose led to about 30–40% rise of plasma C5a, whereas the 440 μg/kg rhuC5a dose, which mimicked severe cardiac anaphylaxis, caused approximately a 600–700-fold rise of C5a. Thus it is possible that severe liposome reactions may involve a several hundredfold rise of plasma C5a in pigs. It should be added though that in the absence of intermediary test doses and information on the clearance rate of rhuC5a in pig blood, it is difficult to apply these figures to humans. Also, our study did not rule out a significant role for C3a in the most severe or lethal reactions.

Clinical Relevance of Mediated Cardiac Anaphylaxis

The clinical relevance of our study lies in highlighting the possible mechanism by which Taxol, Doxil, and some other C-activating liposomal or micellar drugs and radiocontrast media (39, 40) can cause acute cardiac adverse events. In the case of Taxol, cardiac arrests were a major obstacle in the development of this drug (32, 33), and fatalities can still occur despite mandatory antiallergic premedication of patients (5, 19, 21, 50). With Doxil, the box insert warns of the presence of “acute infusion-related reactions in up to 10% of patients” (49) with “adverse cardiac events possibly or probably related to Doxil” (i.e., not to doxorubicin) in 4.3% of patients (2). Among the symptoms listed are tachycardia, bundle branch block, ventricular arrhythmias, and heart arrest (49). It should be emphasized with Doxil, however, that a major thrust of its clinical application, vis-à-vis free doxorubicin, is the reduction of long-term cardiotoxicity, which in fact is achieved with liposome encapsulation (49). With the consideration that the above anticancer drugs are used in the treatment of some half-million new cases of lung, breast, and ovarian cancer each year in the United States, the occurrence of C-mediated cardiac events can be roughly estimated in the order of hundreds to thousands per year, indicating a serious but preventable public health issue.

In conclusion, with the extension of our previous reports (43, 44, 46, 47) on the unique cardiopulmonary response of pigs to intravenous liposomes, the present study quantified the cardiac changes and showed that these changes can be caused, at least in part, by C5a. Complement activation could represent an independent pathogenic pathway, a yet unclassified subgroup of cardiac anaphylaxis, whose occurrence and, hence, clinical significance may far outweigh classical cardiac anaphylaxis. Inhibitors of C activation or C5a or adenosine action might be useful in preventing or ameliorating C activation-related cardiac anaphylaxis. As a further spin-off, our model may allow noninvasive and nonpharmacological triggering of transient myocardial ischemias in anesthetized closed-chest pigs, which may provide a potentially useful new tool in studying the mechanism and prevention of acute ischemic events in the heart.

Table 4. Predominant role of adenosine and A1 receptors in mediating complement activation-related paradoxical bradycardia

<table>
<thead>
<tr>
<th>Reaction Trigger/Inoculum</th>
<th>Heart Rate, % of Baseline</th>
<th>MAP, % of Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>CPX</td>
</tr>
<tr>
<td>PBS</td>
<td>100</td>
<td>128±12 (6)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>52±3 (9)</td>
<td>123±12 (5)</td>
</tr>
<tr>
<td>CPA</td>
<td>46±6 (3)</td>
<td>93±4 (4)</td>
</tr>
<tr>
<td>MLV</td>
<td>73±4 (13)</td>
<td>111±4 (15)</td>
</tr>
<tr>
<td>Dexecil</td>
<td>86±2 (4)</td>
<td>118±11 (6)</td>
</tr>
<tr>
<td>Zymosan</td>
<td>58±8 (6)</td>
<td>169±10 (5)</td>
</tr>
</tbody>
</table>

Endpoint values are means ± SE (no value indicates "not tested"); n, number of bolus intravenous injections (in parentheses) in up to 9 pigs. Entries are heart rate and mean arterial pressure (MAP) readings at the peak of reactions, expressed as percentage of preinjection baseline. No significant change implied at 100%.

Doses of reaction inducers and inhibitors were the following treatments: adenosine, 0.6 mg/kg; N’-cyclopentyl-adenosine (CPA), 0.3 mg/kg; 8-cyclopentyl-1,3-dipropyl-3,7-dihydro-1-purine-2,6-dione (CPX), 0.25 mg/kg; 4-[2-{7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-a][1,3,5]triazin-5-ylamino}ethyl]phenol (ZM-241385), 1.5 mg/kg; MLV and zymosan, 0.2 mg/kg; and Doxil (doxorubicin), 0.01 mg/kg. In the case of Doxil, because of tachyphylactic responses, only first injections were considered.
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GRANTS
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DISCLOSURE
Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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