Cardiac dysfunction caused by purified human C3a anaphylatoxin

(complement/histamine/leukotrienes/thromboxane/isolated heart)

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ABSTRACT The purpose of this investigation was to define the cardiac effects of complement-derived C3a anaphylatoxin, in view of the possibility that cardiac dysfunction may occur as a result of complement activation. Purified human C3a was administered by intracoronary bolus injections into isolated guinea pig hearts. As a function of dose, C3a caused tachycardia, impairment of atrioventricular conduction, left ventricular contractile failure, coronary vasoconstriction, and histamine release. These effects were abolished by cleavage of the COOH-terminal arginine by carboxypeptidase B. The magnitude of C3a-induced tachycardia correlated with the amount of endogenous cardiac histamine released into the coronary effluent. Whereas the tachycardia was markedly reduced by the histamine H₂ antagonist cimetidine, the contractile failure and the coronary vasoconstriction caused by C3a were antagonized by the leukotriene antagonist FPL 55712 and by the cyclooxygenase inhibitor indomethacin, respectively. This suggests that histamine, leukotrienes, and vasoactive prostanoates may mediate the various cardiac effects of C3a. Our findings indicate that C3a anaphylatoxin has marked cardiac effects at concentrations that are likely to be attained with a degree of C3 activation commonly seen in various disease states. Thus, our data are compatible with the hypothesis that generation of anaphylatoxins may induce cardiac dysfunction in clinical conditions.

The complement system plays a primary role in normal host defense mechanisms, and its activation is commonly associated with human disease (1). A consequence of complement activation by the classical or alternative pathway is the production of C3a and C5a anaphylatoxins. These low molecular weight biologically active peptides are specifically cleaved from the parent molecules C3 and C5, respectively, during the activation process. These factors exhibit a variety of activities, such as smooth muscle contraction, chemotaxis, enhanced vascular permeability, and release of a number of mediators, such as histamine, leukotrienes, prostaglandins, thromboxane, and platelet-activating factor (2). High C3a and/or C5a plasma levels are known to occur during immediate hypersensitivity reactions (3–5, 36). Previous studies from our laboratory have demonstrated that mediators of immediate hypersensitivity cause severe cardiac dysfunction (6–9). Earlier reports from other investigators have attributed some degree of cardioactivity to crude anaphylatoxin preparations (10, 11). Thus, it is conceivable that when complement is activated and anaphylatoxins are generated, cardiac dysfunction may ensue. The purpose of our investigation was to define the cardiac pharmacology of purified human C3a anaphylatoxin and to assess the role of various hypersensitivity mediators in the cardiac effects of C3a. We report that C3a has potent effects in the isolated guinea pig heart and that these consist of tachycardia, arrhythmias, left

ventricular contractile failure, and coronary vasoconstriction.

MATERIALS AND METHODS

Isolated Heart Perfusion. Male Hartley guinea pigs (250–300 g) were killed by cervical dislocation. The hearts were excised and perfused in a Langerdoff apparatus at a constant pressure of 40 cm of water with oxygenated Ringer's solution at 37°C as described (12). The composition of the Ringer's solution was as follows: 160 mM Na⁺/5.6 mM K⁺/2.2 mM Ca²⁺/164 mM Cl[−]/5.9 mM HCO₃[−]/5.5 mM glucose. Isometric left ventricular contraction and bipolar surface electrogram were continuously recorded from the right atrium and left ventricle. Sinus rate and atrioventricular conduction time were calculated from the R–R (or P–P) and P–R intervals, respectively. Coronary flow rate was continuously monitored by measuring the volume of coronary effluent collected during 2-min periods.

Preparation of C3a and C3a des-Arg. C3a anaphylatoxin was prepared from purified human third complement component (C3) (13) by treatment with trypsin (14) followed by molecular sieve chromatography as modified by Polley and Nachman (15). COOH-terminal arginine was removed by incubating 2.36 nmol of C3a at 37°C (pH 7.6) for 30 min with 0.165 nmol of carboxypeptidase B in a total vol of 112 μ l. As a control, C3a was incubated at 37°C (pH 7.6) for 30 min with saline (15).

Experimental Procedure. For the determination of dose-response relationships for the cardiac effects of C3a, C3a was injected intra-aortically in a constant volume (0.4 ml) of warm oxygenated Ringer's solution. To avoid tachyphylaxis, only one dose of C3a was administered to each heart. In some experiments, C3a was administered to the hearts in the presence of cimetidine (donated by Smith Kline & French), FPL 55712 (donated by Fisons, Loughborough, U.K.), or indomethacin (purchased from Sigma). In these studies, cimetidine (3 μ M), FPL 55712 (0.48 μ M), or indomethacin (10 μ M) were continuously perfused through the heart from 20 min before C3a administration until the end of the experiment. Histamine was assayed in the coronary perfusate by a radioenzymatic method (16).

Statistical Analysis. Differences between mean responses were considered significant at $P < 0.05$, using a two-tailed Student's t test.

RESULTS

The administration of C3a to the isolated guinea pig heart caused marked changes in several parameters of cardiac function. As shown in Fig. 1, with increasing doses (0.5–24 μ g) C3a enhanced the sinus rate (by 6–63 beats/min, equivalent to a 3%–30% increase), prolonged the P–R interval (by 3%–50%), decreased left ventricular contractile force (by 3%–32%), and diminished coronary flow rate (by 7%–30%). As shown in Table 1, no such effects were obtained with the administration of C3a des-Arg. The time course of the changes in sinus rate, atrioventricular conduction, contrac-

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FIG. 1. Dose-response relationships for the effects of purified human C3a anaphylatoxin on sinus rate (A), atrioventricular conduction time (i.e., P-R interval) (B), left ventricular contractile force (C), and coronary flow rate (D) in isolated guinea pig hearts. Each point (mean, 4–6 hearts; $\pm$ SEM) represents the maximum change from control occurring within 4 min of the administration of each C3a dose. Control values (mean $\pm$ SEM) were as follows: sinus rate, 219 ± 5 beats/min; P-R interval, 65 ± 2 msec; contraction, 6.4 ± 0.3 g; coronary flow, 4.5 ± 0.2 ml/min ($n = 30$).

tility, and coronary flow caused by 3 doses of C3a is shown in Fig. 2. The sinus rate (Fig. 2A) began to increase within 15 sec from the administration of C3a, reached a peak between 1 and 4 min, depending on the dose, and returned to control levels within 30 min after the administration of C3a. The duration of the P-R interval (Fig. 2B) also increased quite rapidly after the administration of C3a, with greater increments occurring with larger doses, an indication of a progressive impairment in atrioventricular conduction. Indeed, with the 24- μg dose of C3a, second degree atrioventricular conduction block occurred in 3 of 5 experiments and lasted ≈ 2 min. With increasing doses of C3a, the return of the P-R interval to control values was progressively slower. Left ventricular

contractile force (Fig. 2C) decreased rapidly and reached a peak 1 min after the administration of C3a. With the 24- μg dose, however, the decrease in contractility was preceded, in 3 of 5 experiments, by a brief increase in contractile force. Contractility returned to control levels by 30 min after the administration of C3a. Coronary flow rate (Fig. 2D) decreased with increasing doses of C3a, reached a maximum by 2 min and rapidly returned to near control values.

After the administration of C3a, endogenous cardiac histamine was released into the coronary effluent (Fig. 3); the larger the dose of C3a, the greater the total amount of histamine released; also, with increasing doses of C3a, a progressively greater proportion of histamine was released within

FIG. 2. Time course of the effects of purified human C3a anaphylatoxin ($\circ$, 2.5 μg ; Δ , 10 μg ; $\bullet$, 24 μg) on sinus rate (A), atrioventricular conduction (i.e., P-R interval) (B), left ventricular contractile force (C), and coronary flow rate (D) in isolated guinea pig hearts. Each point is the mean change ($n = 4-6$; $\pm$ SEM where indicated) from control at each time. Control values are the same as in Fig. 1. (B) Time action curve for the 24- μg dose of C3a is interrupted to indicate occurrence of atrioventricular conduction block (arrow).

Table 1. Relative cardiac effects of C3a and C3a des-Arg

	C3a	C3a des-Arg
Sinus rate	+25 ± 3	+7 ± 5
P-R interval	+18.0 ± 5.1	+1.0 ± 1.0
Contractility	-24.1 ± 3.6	-3.1 ± 1.1
Coronary flow	-19.2 ± 4.3	-4.7 ± 1.0

Numbers indicate mean percentage changes in atrioventricular conduction time, left ventricular contractility, and coronary flow, or changes in beats/min for sinus rate ($\pm$ SEM; $n = 4$), in isolated guinea pig hearts, after administration of a 6- μ g dose of C3a or C3a des-Arg. C3a was incubated for 30 min at 37°C (pH 7.6) with carboxypeptidase B or saline, respectively. Each change caused by C3a was significantly different ($P < 0.03$) from the corresponding C3a des-Arg value.

the first 2 min (i.e., 40%, 50%, and 80% of total at 6, 10, and 24 μ g, respectively). The amounts of endogenous histamine released by the hearts correlated with the increase in sinus rate: the higher the amount of histamine released, the greater the tachycardia (Fig. 4). This correlation was highly significant ($P < 0.001$), suggesting that the C3a-induced tachycardia might be mediated by the release of endogenous histamine. Since the positive chronotropic effect of histamine is mediated by H_2 receptors (17), if C3a-induced tachycardia were histamine-mediated it ought to be antagonized by an H_2 blocker. As shown in Fig. 5, cimetidine reduced by $\approx 60\%$ the tachycardia caused by a 24- μ g dose of C3a. An attenuation of C3a-induced tachycardia was also observed in the presence of the leukotriene end-organ antagonist FPL 55712; however, this reduction was less than one-half that obtained with cimetidine and did not attain a level of statistical significance. In addition, FPL 55712 failed to significantly antagonize the decrease in coronary flow rate caused by the 24- μ g dose of C3a. On the other hand, FPL 55712 significantly reduced by $\approx 40\%$ the negative inotropic effect of the 24- μ g

FIG. 3. Release of endogenous histamine from isolated guinea pig hearts by administration of purified human C3a. Each bar represents the mean amount of histamine released ($\pm$ SEM) by C3a into the coronary effluent in the time period indicated ($n = 4-5$). Mean basal histamine release prior to C3a administration was 0.84 ± 0.4 ng/g ($n = 30$). For each preparation, basal histamine release was subtracted from total histamine release to yield C3a-induced histamine release.

FIG. 4. Relationship between histamine release and sinus tachycardia caused by administration of purified human C3a into isolated guinea pig hearts. Histamine was measured over 2-min intervals for 10 min after administration of 6–24 μ g of C3a. Data shown are derived from seven experiments. In five experiments, histamine was released within 4 min after C3a administration (10 points); in the remaining two experiments, histamine was released only in the first 2 min after C3a (2 points). Curve was calculated by regression analysis.

dose of C3a (Fig. 5). The cyclooxygenase inhibitor indomethacin modified neither the positive chronotropic nor the negative inotropic effect of C3a; however, indomethacin completely abolished, and actually reversed, the decrease in coronary flow caused by C3a. Cimetidine did not modify the negative inotropic effect of C3a and only moderately, but not significantly, attenuated the C3a-induced coronary constriction (Fig. 5).

DISCUSSION

Our findings demonstrate that the administration of purified human C3a anaphylatoxin causes marked dysfunction in the isolated guinea pig heart. C3a-induced cardiac dysfunction is characterized by tachycardia, arrhythmias, contractile failure, and coronary vasoconstriction. The COOH-terminal ar-

FIG. 5. Effects of cimetidine, FPL 55712, and indomethacin on changes in cardiac function induced by administration of purified human C3a anaphylatoxin into isolated guinea pig hearts. Each bar represents the change from control (mean $\pm$ SEM; $n = 4$) caused by a 24- μ g dose of C3a, either in the absence or in the presence of each pharmacologic antagonist. *, Significance at $P < 0.05$. Cimetidine, FPL 55712, and indomethacin did not by themselves modify sinus rate, contractility, and coronary flow.

ginine appears to be essential for the cardiac effects of C3a: no significant changes in sinus rate, ventricular contractility, atrioventricular conduction, and coronary flow rate occurred when carboxypeptidase B-treated C3a was administered. Thus, with the notable exception of the platelet-stimulating activity of C3a, which is unaffected by the removal of the COOH-terminal arginine (15), C3a des-Arg appears to be devoid of biological activity in most tissues, including the heart (see Table 1) and the lung (18).

The cardiac effects of C3a resemble those observed in sensitized guinea pig hearts upon challenge with the specific antigen; from a quantitative standpoint, however, the changes caused by C3a are of lesser intensity and shorter duration than those provoked by cardiac anaphylaxis (7, 19, 20). In the latter, sinus tachycardia is caused by the release of endogenous cardiac histamine, which has marked H₂-mediated positive chronotropic effects (17, 21). Since we found that (i) histamine is released into the coronary effluent after the administration of C3a; (ii) the quantities of histamine released are proportional to the doses of C3a injected; (iii) the increments in sinus rate directly correlate with the amounts of histamine released; and (iv) C3a-induced tachycardia is antagonized by the H₂ blocker cimetidine, it is most probable that the positive chronotropic effect of C3a is mediated by the release of endogenous cardiac histamine. Furthermore, we found that cimetidine, which is known to antagonize the positive inotropic effect of histamine (17), prevents the positive inotropic effect of C3a. Thus, it is likely that the initial brief increase in contractility caused by the 24- μ g dose of C3a is also mediated by the release of endogenous cardiac histamine. On the contrary, the negative inotropic effect of C3a was antagonized by the sulfidopeptide leukotriene antagonist FPL 55712 (Fig. 5). Since FPL 55712 specifically inhibits the decrease in contractility, which is characteristic of cardiac anaphylaxis (7), as well as antagonizes the negative inotropic effect of leukotrienes C₄ and D₄ (22), it is reasonable to assume that the myocardial contractile failure induced by C3a is leukotriene-mediated. Indeed, C5a is known to elicit the release of leukotrienes from guinea pig lung tissue (23), and FPL 55712 has been found to antagonize C5a-induced contraction of lung parenchymal strip in the presence or the absence of pyrilamine (23, 24). On the other hand, C3a-induced contraction of lung parenchyma appears to be mediated primarily by cyclooxygenase metabolites of arachidonic acid (25). Similarly, we found that C3a-induced coronary vasoconstriction is completely abolished, and even reversed, by the cyclooxygenase inhibitor indomethacin (Fig. 5). We had previously found that indomethacin, or 1-(2-isopropylphenyl)imidazole, blocks the synthesis of thromboxane in the guinea pig heart and completely abolishes the coronary constriction of cardiac anaphylaxis (26). Thus, the prevention of C3a-induced coronary vasoconstriction by indomethacin, coupled with our preliminary finding that immunoreactive thromboxane is released from the guinea pig heart after the administration of C3a (unpublished observations), favor the hypothesis that thromboxane mediates the coronary-constricting effect of C3a. On the other hand, the increase in coronary flow caused by C3a in the presence of indomethacin resembles the reversal of anaphylactic coronary vasoconstriction by cyclooxygenase inhibitors and prostaglandin end-organ antagonists (26, 27), and probably reflects the coronary-dilating effects of histamine (17).

It could be argued that the decrease in contractility induced by C3a may have been caused by myocardial ischemia secondary to coronary vasoconstriction. However, it is most probable that the negative inotropic effect of C3a is a primary event. In fact, coronary flow rate recovered much earlier than left ventricular contractility (compare C and D in Fig. 2); moreover, when indomethacin completely abolished C3a-induced coronary vasoconstriction, the negative inotropic

effect of C3a was unaffected (Fig. 5).

Collectively, our findings suggest that various hypersensitivity mediators, some preformed, such as histamine, and others synthesized *de novo*, such as lipooxygenase and cyclooxygenase metabolites of arachidonic acid, mediate the various cardiac effects of C3a. Whether C3a may also exert direct effects on the heart, and if so, the extent of these effects, remains to be determined.

Earlier reports that crude anaphylatoxin, obtained by incubation of dextran with rat serum, has positive inotropic and chronotropic effects in guinea pig atria, and that a less crude rat anaphylatoxin preparation increases the force of contraction of the isolated guinea pig papillary muscle have appeared in the literature (10, 11). Since our initial presentation of cardiac dysfunction induced by purified human C3a anaphylatoxin (28), Huey *et al.* (29) have reported that purified human C3a and C5a produce a positive inotropic effect in spontaneously beating guinea pig atria. The interest in the cardiac effects of anaphylatoxins appears to be well justified. Complement activation is associated with numerous pathological states (1), including anaphylaxis (30, 31), and high plasma levels of C3a and/or C5a have been reported in patients undergoing hypersensitivity reactions (3–5, 36). We have found that the cardiac effects of C3a approach a maximum at a 10- μ g dose. Notably, a C3a concentration of 10 μ g/ml approximates the value that would be attained with a 10%–20% activation of C3. This degree of activation is commonly seen in various disease states (32). Although one could argue that C3a anaphylatoxin would be inactivated in the systemic circulation, there is no reason to believe that this process would be sufficiently rapid to prevent its cardiac effects. Thus, given the many clinical conditions in which complement can be activated (33), including myocardial ischemia (34) and cardiopulmonary bypass (35), our data support the premise that in certain circumstances cardiac dysfunction may be due at least in part to the generation of complement-derived anaphylatoxins.

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