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The anaphylatoxins C3a and C5a are vasodilators in the canine coronary vasculature *in vitro* and *in vivo*

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Abstract

The effect of complement fragments on coronary blood flow *in vivo* and the contraction of coronary arteries *in vitro* was determined. In pentobarbital anesthetized dogs, intraarterial bolus injection of C3a and C5a, zymosan-activated serum and methylcholine in the coronary vascular bed caused transient and dose-dependent increases in coronary blood flow. Similar increases were obtained with 25 μg of C3a ($104 \pm 13\%$, $n=5$) and 0.1 μg of methylcholine ($102 \pm 4\%$, $n=3$). Smaller increases in blood flow were elicited by 25 μg of C5a ($41 \pm 18\%$, $n=4$) and 0.2 ml of zymosan-activated serum ($48 \pm 5\%$, $n=4$). None of these responses were associated with significant changes in left ventricular contractile force measured with a strain gauge, arterial blood pressure, and heart rate. C3a dilated the coronary vascular bed in conscious dogs with an activity equal to or greater than that observed in anesthetized dogs. Isolated canine coronary arteries that were precontracted with serotonin relaxed in response to C3a, whether or not the endothelium was intact. Overall these data suggest that physiologically high doses of anaphylactic complement fragments vasodilate the canine coronary circulation.

Introduction

Activation of the complement cascade has been observed in the rat [1], dog [2] and monkey [3] heart during myocardial ischemia. It is not certain whether this process is a causative factor or an epiphenomenon of mycocardial infarction, however depletion of complement prior to ischemia has been found to be cardioprotective [4]. The anaphylactic complement fragments C3a and C5a possess potent vasospastic and chemotactic activities [5] which could contribute directly or indirectly to the impairment of cardiac function and coronary blood flow (CBF) that accompany reperfusion of ischemic myocardium. The observation by several laboratories that the intracoronary injection of C5a into pigs reduces coronary blood flow and causes cardiac dysfunction [6–8] suggests that the anaphylatoxins could contribute to the pathophysiology of myocardial ischemia. In the present study the effect of C3a, C5a and zymosan-activated plasma (ZAS) on the coronary vascular bed of dogs *in vivo* and on epicardial canine coronary arteries *in vitro* was determined.

Materials and methods

Vascular responses to anaphylatoxins in vivo

In the anesthetized preparation five male mongrel dogs (17 to 22 kg) were given sodium pentobarbital (30 mg/kg, i.v.) and ventilated with room air

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using a model 607 respirator pump (Harvard Apparatus, South Natick, MA). A carotid artery was catheterized to monitor arterial blood pressure (ABP) by use of a P23Db transducer (Gould Inc., Oxnard, CA). A left thoracotomy was performed at the 5th intercostal space and the left circumflex coronary artery was isolated proximal to the obtuse diagonal branch. A cannula (27-gauge, U-shaped hypodermic needle attached to 0.03 inch bore tygon tubing) was inserted into the LCCA for intracoronary injections, and distal to this an EP407 and EP408 electromagnetic flow probe (Carolina Medical Instruments, King, NC) was attached to the vessel. A Walton-Brodie strain gauge arch (Warren Research Products, Charleston, SC) was sutured to the left ventricle in the left circumflex coronary artery perfusion bed to measure isometric contractile force as described previously [9]. CBF, ABP, heart rate and developed contractile force were recorded continuously on a model 7 polygraph (Grass Medical Instruments, Charleston, SC).

In the conscious preparation the left circumflex coronary artery of six male dogs was instrumented with the intracoronary cannula and flow probe as described for the anesthetized preparation. Surgery was performed under aseptic conditions and a week was allowed for recovery. Experimental studies were performed in infection-free, conscious and unsedated dogs that were resting quietly in a nonrestrictive sling. CBF, ABP and heart rate were recorded as described previously.

Intracoronary injections (0.2 ml) of C3a (anesthetized and conscious dogs) and C5a (anesthetized dogs only) were given over 15 sec in order of increasing dose (3 to 25 μg) and spaced approximately 10 min apart for recovery of baseline CBF. In most experiments methylcholine chloride (MeCh, 0.1 μg) and ZAS (0.2 ml) were administered in a similar manner. The order of C3a and C5a injections was randomized. In three of the anesthetized dogs the 6 μg C3a dose was repeated after the 25 μg dose to determine if sensitivity to the anaphylatoxin had diminished. In two of the conscious dogs responses to 25 μg of C3a were repeated after 1 to 2 days to assess reproducibility.

Vascular responses to complement in vitro

Male mongrel dogs were anesthetized as described previously and the left anterior descending coronary artery was excised. The artery was placed in a physiological salt solution at 4 °C, which had the following composition (mmol/l): NaCl 130; KCl 4.7; KH_2PO_4 1.18; $MgSO_4 \cdot 7H_2O$ 1.17; $CaCl_2 \cdot H_2O$ 1.6; $NaHCO_3$ 14.9; dextrose 5.5; $CaNa_2$ EDTA 0.03. Arteries were then cut into helical strips (1.5 × 10 mm) and mounted vertically in a tissue bath containing physiological salt solution aerated with 95% O_2-5% CO_2 at 37 °C to a pH 7.4. Arterial strips were attached to FT.03 force transducers (Grass Medical Instruments) and placed under 1.0 g of tension. After 2 h of equilibration responses to C3a (0.1 to 1.0 μg/ml) were determined either directly or after eliciting contraction with serotonin (1 μmol/l). Additional experiments were performed on arteries that were rubbed prior to mounting to disrupt the endothelium, which was verified by loss of acetylcholine-induced relaxation.

Materials and statistical analysis

ZAS was prepared by incubating canine serum with 1 mg/ml of zymosan particles (Sigma Chemical Co., St. Louis, MO) at 37 °C for 30 min and then separating the serum by centrifugation. Human C5a and C3a were isolated by an enzyme-linked immunoabsorbent assay from serum which was activated with zymosan in the presence of a carboxypeptidase inhibitor to prevent formation of des arg metabolites [10], and they were suspended in saline. Complement fragments and ZAS were stored at −70 °C and thawed immediately before use.

All data in the text and figures are presented as mean ± SEM. Statistical analyses were performed by computer-assisted *t*-test for two-way comparisons, or analysis of variance with repeated measures and contrast for multiple comparisons (Systat, Evanston, IL). A $p < 0.05$ was considered significant.

Results

Vascular responses to complement in vivo

Anesthetized dogs reacted to intracoronary injection of C3a and C5a with increases in CBF. No reductions in CBF were detected in any animal tested. The dose-dependent and reversible nature of these vasodilator responses is demonstrated by

the physiological recordings obtained from one dog during administration of C3a (Fig. 1). Qualitatively similar results were obtained with C5a, ZAS and MeCh in this and other experiments, although there was considerable variability among animals in the magnitude of the CBF increases. Overall, baseline CBF (27 ± 2 ml/min) was increased 29 ± 5 ml/min by C3a (25 µg; $n=5$), 11 ± 6 ml/min by C5a (25 µg; $n=4$), 15 ± 3 ml/min by ZAS (0.2 ml; $n=4$), and 29 ± 3 ml/min by MeCh (0.1 µg; $n=3$) (Fig. 2). C3a was more active than C5a in both this analysis, and also when coronary vascular responses were quantitated as volume (ml) increases determined as area under the CBF trace by planimetry (Fig. 2). CBF was not affected by intracoronary injections of C3a and C5a which had been heat-inactivated (56 °C for 1 h), or canine serum which was not activated by zymosan particles (not shown). Baseline mean ABP (123 ± 4 mm Hg) heart rate (177 ± 4 beats/min) and developed left ventricular contractile force (92 ± 5 g) were not significantly altered during peak increases in CBF induced by 25 µg of C3a (124 ± 5 mm Hg, 179 ± 4 beats/min and 100 ± 6 g, respectively; $n=5$). Injections of C5a, ZAS, and lower doses of C3a likewise had no significant effect on ABP or left ventricular contractility (not shown).

The effect of C3a on CBF in conscious dogs was studied in separate experiments. Coronary vascular responses to C3a in the conscious dog were characterized by dose-dependent increases in CBF that were qualitatively similar to those observed in anesthetized dogs (Fig. 3). The increase in CBF (50 ± 4 ml/min, $n=5$) above baseline levels (21 ± 1 ml/min) elicited by 25 µg of C3a was greater ($p<0.01$) than the corresponding response observed in anesthetized dogs. However, the total volume of this flow response did not differ significantly between anesthetized and conscious preparations (22 ± 6 and 25 ± 2 ml, respectively). When dogs were exposed to a single 25 µg injection of C3a after 1 or 2 days the magnitude of the flow increase was within 20% of the initial response.

Vascular responses to complement in vitro

Canine coronary arteries that were contracted with serotonin relaxed upon addition of 0.5 µg/ml of C3a (Fig. 4), whether or not the endothelium was intact. Higher C3a concentrations (1 µg/ml) did

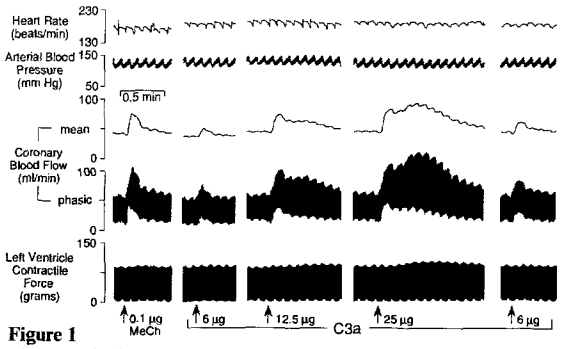

Figure 1
Physiological responses of a single pentobarbital-anesthetized dog were determined during sequential intraarterial injections of methylcholine (MeCh) and C3a into the coronary circulation. In this and in two other experiments the response to 6 µg of the anaphylatoxin was similar before and after the 25 µg dose.

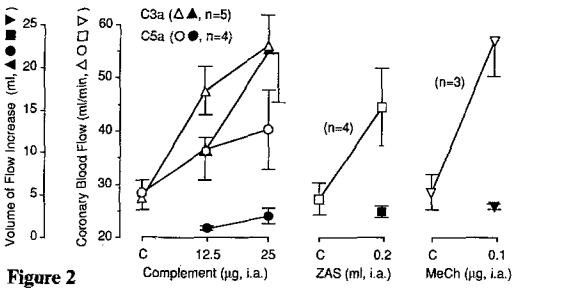

Figure 2
Intraarterial (i.a.) injections of C3a, C5a, zymosan-activated serum (ZAS) and methylcholine (MeCh) were given to pentobarbital-anesthetized dogs. Coronary vascular responses were measured as mean above control baseline flow (C) and as total increase in blood volume. C3a responses were greater than C5a responses ($p<0.05$ at 12.5 and 25 µg).

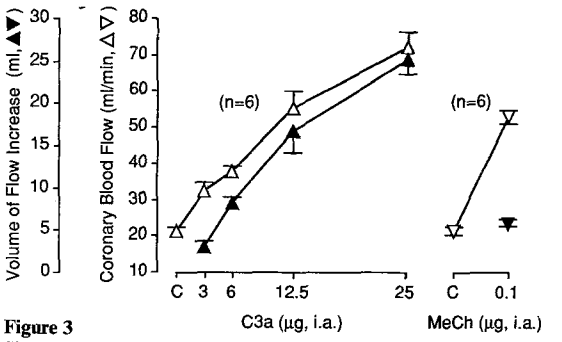

Figure 3
Single intraarterial injections (i.a.) of C3a and methylcholine (MeCh) were given to conscious dogs. Coronary vascular responses were measured as increase above control baseline flow (C) and as total increase in blood volume, which was measured as area under the CBF trace by planimetry.

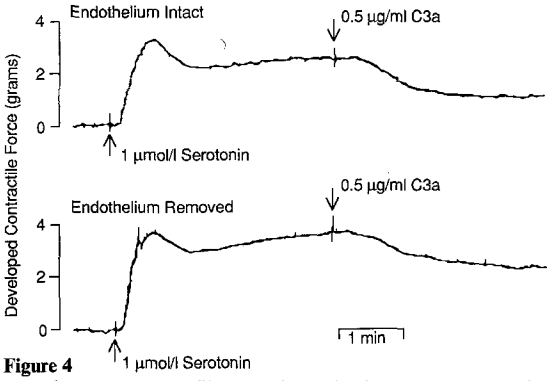

Figure 4
Vascular responses to C3a were determined *in vitro* in two canine coronary artery strips that were first contracted with serotonin. The endothelium was removed from one the arteries by rubbing. Data are representative of 3 similar experiments.

not cause greater relaxation and similar responses were observed in arteries exposed to C3a (0.5 µg/ml) without serotonin-induced contraction (not shown). The maximum reduction in contractile force induced by C3a was greater in pharmacologically contracted (−150 mg) compared to unstimulated arteries (−48 mg).

Discussion

In anesthetized dogs intracoronary injections of C3a, C5a and ZAS transiently increased but never decreased CBF, and this occurred without concurrent changes in ABP, heart rate or regional myocardial contractility. Intravenous administration of C5a has been found by others to reduce ABP in dogs [11], and therefore the absence of a ABP response in our preparation demonstrates that anaphylatoxin exposure was effectively limited to the coronary circulation. While it is possible that the use of human C3a and C5a could have influenced the vascular response, the fact that canine ZAS produced similar although smaller responses argues against this. Of the two anaphylatoxins C5a had less vascular activity since reductions in coronary vascular resistance were more than two-fold greater with C3a. Although we did not address the issue of tachyphylaxis to repeated high injections of anaphylatoxins in these experiments, the response to 6 µg of C3a was not diminished after exposure to the 12.5 and 25 µ doses (Fig. 1).
Vascular responses to C3a, C5a and ZAS were qualitatively similar and therefore only C3a was studied in conscious dogs. These experiments were performed to eliminate interfering factors related to anesthetic or acute surgical trauma. Responses in conscious and anesthetized dogs proved to be qualitatively similar, although maximum CBF increases (ml/min) to C3a were greater in conscious animals. In the conscious canine 3 µg of C3a elicited a transient 11 ± 3 ml/min ($n = 5$) increase in CBF, which assuming a 15 sec injection into the respective baseline CBF (~ 20 ml/min) corresponds to a transient blood level of ~ 0.6 µg/ml. This estimate compares well with the reported C3a plasma concentration (0.6 ± 0.09 µg/ml) detected in patients undergoing thrombolytic therapy for acute myocardial infarction [12]. When the reactivity of the canine coronary vasculature to anaphylatoxin was compared to a cholinergic stimulus, the C3a dose producing an equivalent response was 50-fold greater than the MeCh dose. However the molecular weight (g/mole) of C3a (3×10^3) greatly exceeds that of MeCh (196), and if vasodilation resulted from receptor occupation this difference should be corrected for in any comparison. We did not study >25 µg injections of C3a and C5a due to the limited supply and concerns regarding the physiological relevance of higher doses. Thus it is not known if the vascular responses were maximal. C3a produced only vasorelaxation of coronary artery strips *in vitro*. The epicardial artery response was thus similar to the small vessel response observed *in vivo*, and it did not appear to involve EDRF since it persisted in the absence of endothelium.
Other investigators have observed reductions in both CBF and myocardial contractility in response to the intracoronary injection of C5a into anesthetized pigs [6–8]. The C5a dose (0.5 µg) that was active in the pig [7, 8] was 25-fold lower than the threshold dose of C5a which increased CBF in our anesthetized canine preparation. One hypothesis which addresses the disparity between these results and ours raises the issue of species differences in the production or reactivity to secondary mediators, especially TxA_2. There is good evidence that autocoids and prostaglandins are involved in the action of activated complement on smooth muscle [5, 13, 14]. TxA_2-receptors participate in CBF reductions produced by C5a in the pig as evidenced by the near complete antagonism obtained with TxA_2-receptor antagonists [7, 8], although peptide leukotrienes may also contribute to the response

[7]. The coronary vasculature of the pig is very reactive to Tx-mimetics, such as U-46,619, and increased production of TxA_2 (measured as TxB_2) has been found to accompany C5a-induced vasoconstriction [7, 8]. In contrast, the coronary vasculature of the adult dog has been reported to be relatively unreactive to endogenous and pharmacologic activators of TxA_2-receptors [15, 16]. Other canine vascular beds including the pulmonary, mesenteric and renal circulations respond more consistently to local injection of U-46,619 with dose-dependent reductions in vascular resistance [16, 17].

Our results emphasize the importance of species variability in the vascular reactivity to anaphylatoxins and demonstrate that complement fragments are relatively weak vasodilators in the canine coronary circulation. Since other pharmacologic interventions were not examined, we do not know if the action of anaphylatoxins on the canine coronary circulation are direct or indirect. We also do not know if acute exposure of the canine vasculature to activated complement causes neutrophil sequestration [6] or neutrophil-dependent plasma exudation [18] as reported with other species. In comparison to our canine studies, activation of the complement cascade in human pathophysiology may be both more sustained and accompanied by reperfusion of ischemic tissue. These additional factors could in turn lead to a qualitatively different myocardial vascular response.

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