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Research Article

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Effects of Acetyl Glyceryl Ether of Phosphorylcholine (Platelet Activating Factor) on Ventricular Preload, Afterload, and Contractility in Dogs

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Abstract. Acetyl glyceryl ether of phosphorylcholine (AGEPC), platelet activating factor, is a potent hypotensive agent that may mediate changes in blood pressure during anaphylaxis and may be involved in blood pressure variations of renal origin. This study was designed to characterize the hemodynamic mechanisms responsible for hypotension induced by this recently identified phospholipid. Intravenous administration of AGEPC to anesthetized open-chest dogs ($n = 5$) produced hemodynamic alterations which, for the purpose of analysis, were divided into three phases based on changes in the mean systemic blood pressure. During phase I (5–30 s) mean systemic blood pressure decreased to levels 5 to 10% below baseline values in association with a rise in cardiac output and a decrease in systemic vascular resistance. Phase II (30–90 s) consisted of a substantial reduction in systemic blood pressure to its nadir, 50% of baseline values, together with a decrease of similar magnitude in cardiac output and a rise in systemic vascular resistance. Phase III (90 s–60 min) exhibited a gradual recovery of mean systemic blood pressure toward normal with a several-fold rise in systemic vascular resistance and a continued low cardiac output. On the right side of the circulation, the predominant effect of AGEPC was a marked transient increase in pulmonary artery pressure in phase I, associated with an elevation of pulmonary resistance during phase II. Diethylcarbamazine blocked virtually all of these hemodynamic changes induced by AGEPC; FPL 55712 substantially blocked the rise in systemic vascular resistance in phase III. These results suggest that leukotrienes may mediate at least some of the hemodynamic effects induced by AGEPC, but further studies will be required when more specific leukotriene blocking agents become available. As assessed during phase III with the end-systolic pressure-dimension relation, myocardial performance itself was diminished. The occurrence of an AGEPC-induced negative inotropic effect was further confirmed in isolated Krebs-perfused guinea pig hearts and isolated blood-perfused rabbit hearts. The results indicate that the mechanism of AGEPC-induced hypotension is complex, affecting both vascular tone and the inotropic state of the myocardium.

Introduction

In 1970, while investigating the phenomenon of leukocyte-dependent histamine release from platelets, Henson reported the isolation of a soluble factor from sensitized leukocytes that induced histamine release from platelets (1). Subsequent investigators termed the substance platelet activating factor (2, 3), and it has been isolated from numerous cells and tissues (4). More recently, this factor was identified as an acetyl glyceryl ether of phosphorylcholine (AGEPC),[1] and, concomitantly, it was suggested that antihypertensive polar renomedullary lipid, a hypotensive agent (5) isolated from kidney, is also AGEPC (6). It exhibits varied biological actions in vivo and in vitro, including platelet and neutrophil activation, initiation of wheal and flare reaction, increases in vascular permeability (7), and constriction of guinea pig ileum. Infusion of AGEPC in the rabbit or baboon induces dose-dependent profound cardiovascular and pulmonary changes similar to those documented in IgE-induced systemic anaphylaxis in the rabbit. Although the deleterious effects on pulmonary

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1. *Abbreviation used in this paper:* AGEPC, acetyl glyceryl ether of phosphorylcholine.

mechanics depend upon the presence of platelets, the bases for the hemodynamic effects have remained substantially unexamined (4).

Early hemodynamic studies emphasized the potent hypotensive effect of AGEPC, and measurements of changes in systemic blood pressure were made at various times after administration of this phospholipid. The interrelations, both temporal and functional, between the hemodynamic determinants of blood pressure, including systemic vascular resistance and cardiac output, were not well characterized. Therefore, in view of the potential involvement of this phospholipid in hypotension associated with anaphylaxis and its putative role in some instances of renally modulated hypertension (8), the present study was designed to characterize the hemodynamic effects of intravenously administered AGEPC, with particular attention to the time course of the alterations as well as the determinants of cardiac output. The results indicate that the mechanism of AGEPC-induced hypotension is complex, affecting both vascular tone and left ventricular performance itself.

Methods

AGEPC. AGEPC was synthesized from beef heart plasmalogens to give a product that was virtually identical over time, as assessed by mass spectroscopy and thin-layer chromatography of the synthesized compound in several solvent systems to show that deacylation had not occurred (6). Fast atom bombardment mass spectroscopy (9–11) of AGEPC in a glycerol matrix demonstrated primary peaks of 524 and 552 *m/e* (mass-to-charge ratio), representing the hexadecyl and octadecyl parent ions, respectively, with the former predominating (Fig. 1).

AGEPC was stored in $CHCl_3/CH_3OH$, 2:1, at −20°C at a concentration of 3.8 μmol/ml. Immediately before use, an aliquot of AGEPC was placed in a test tube, the solvent was evaporated under nitrogen gas, and the AGEPC was redissolved in 2 ml of 0.9% NaCl containing 2 mg bovine serum albumin (BSA)/ml by sonication for 3 s in a water bath.

In vivo dog preparation. Mongrel dogs (n = 14) weighing 19–30 kg were anesthetized with intravenously administered sodium thiopental (25 mg/kg) followed by α-chloralose (80 mg/kg) dissolved in polyethylene glycol and 0.9% NaCl (40:60 vol/vol). Positive pressure ventilation with room air supplemented by oxygen was used. A thoracotomy was performed through the left fifth intercostal space, the pericardium was opened, the heart was suspended in a pericardial cradle, and electromagnetic flow probes (Narcomatic; Narco Bio-Systems, Inc., Houston, TX) were placed around the ascending aorta and the pulmonary artery and connected to flow meters (model RT-500 Narcomatic; Narco Scientific, Inc.). Catheters connected to pressure transducers (P23Db; Statham Instruments, Inc., Oxnard, CA) were inserted into the ascending aorta, left ventricular cavity, and pulmonary artery for the recording of systemic arterial blood pressure, left ventricular pressure, and pulmonary artery pressure, respectively. Transducer signals were am-

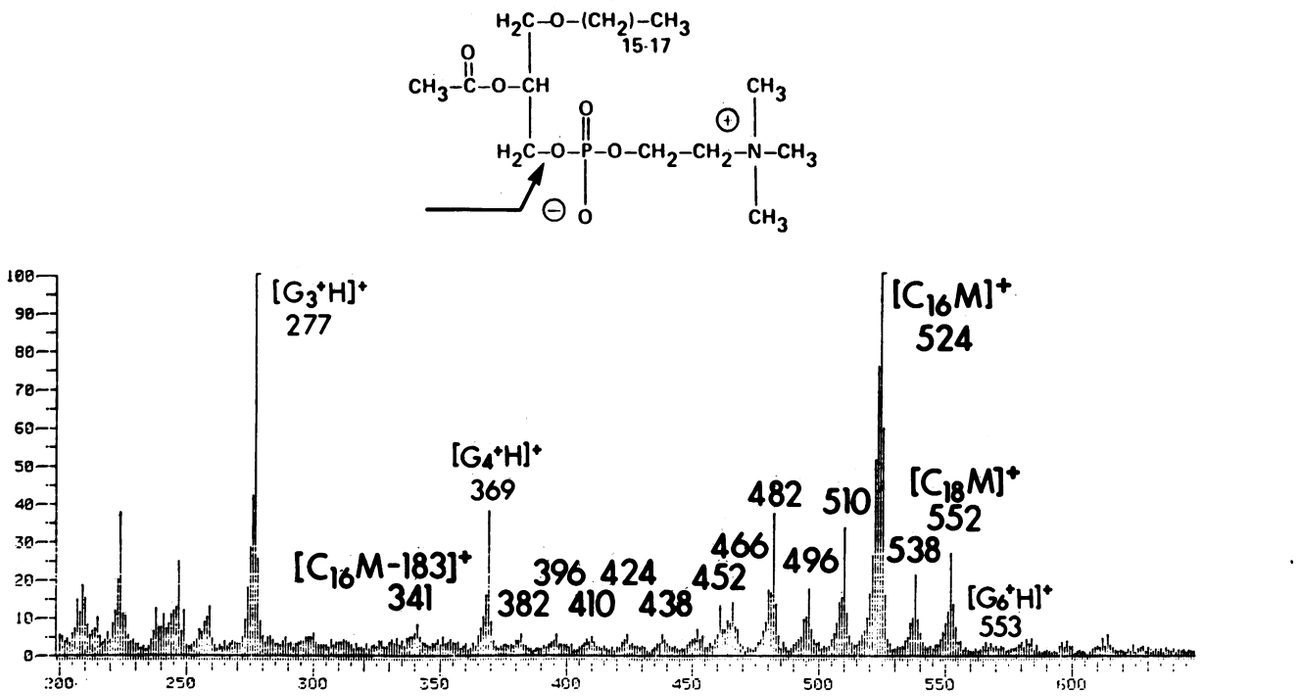

Figure 1. Fast atom bombardment mass spectrum of AGEPC. The relative percentage of each species (ordinate) is plotted against the mass/charge ratio (abscissa). This spectrograph represents a 50-μg sample of AGEPC, and the primary peaks at 524 and 552 *m/e* represent the hexadecyl and octadecyl parent ions, respectively. The peak at 341 is consistent with the loss of the phosphocholine moiety from the hexadecyl parent ion. Peaks at 14 m/e intervals represent extrusion of methylene groups. Glycerol (G) was used as a matrix.

plified and differentiated with Honeywell amplifiers (model 143) and differentiators (model 132), and recorded with a Honeywell Visicorder (model 1858; Honeywell, Inc., Test Instruments Div., Denver, CO).

After surgical manipulation, the preparation was allowed to equilibrate for 30 min. Continuous measurements of left ventricular pressure, left ventricular dP/dt (change in pressure over time), mean systemic arterial blood pressure, mean systemic arterial blood flow, mean pulmonary arterial blood pressure, and mean pulmonary arterial blood flow were made in each dog. After completion of each experiment the data were analyzed as follows: for each hemodynamic parameter listed above the percentage change from the baseline value was determined at 5-s intervals from the continuous recordings. The mean±SEM of the percentage change was calculated for each hemodynamic parameter in the five dogs and plotted over time, generating a series of curves which were almost continuous on a compressed scale and which are depicted as continuous in (Figs. 2, 3, and 4). Standard error bars are expressed only at points where there are maximum changes in the hemodynamic parameters, to avoid cluttering of the figures.

End-systole pressure-diameter relation. To assess myocardial inotropy in vivo, end-systolic pressure-diameter relations (12, 13) were evaluated before and after injection of AGEPC into five dogs. Real time two-dimensional echocardiographic images of the left ventricle were obtained with an ultrasonography system (Honeywell, Inc., Test Instruments Div.) that employs a 3.5-MHz transducer applied directly to the epicardial surface of the left ventricle. The mitral valve was imaged, and the transducer was angulated caudally to visualize the papillary muscles, which were used as landmarks to ensure that rotation of the transducer was avoided throughout the study. Only images that displayed a circular left ventricular geometry were used. The aortic blood pressure tracings were displayed simultaneously in real time on the ultrasonography system, superimposed over the two-dimensional echocardiographic image. Two-dimensional echocardiography was chosen rather than M-mode to ensure a true rather than a tangential measurement of the minor axis of the left ventricle, to obtain a perpendicular cross section of the left ventricular wall, and to employ lateral resolution (not available with M-mode) to provide adequate assessment of the overall contraction pattern in the beating heart (14, 15). To alter loading conditions of the left ventricle, one snare was placed around the inferior vena cava ~2 cm proximal to the right atrium, and a second snare was placed around the descending aorta proximal to the diaphragm. Each dog underwent simultaneous baseline recordings of right- and left-sided pressures and flows and an echocardiogram. Preload was then lowered by gradual tightening of the inferior vena cava snare until the peak systolic blood pressure was reduced at least 5 to 15 mmHg, where it was maintained for 2 min before pressure and echocardiographic data were recorded. The snare was released, and, after all values had returned to baseline, afterload was elevated by constriction of the snare around the descending aorta to obtain a peak systolic blood pressure elevation of at least 5 to 15 mmHg and maintained at this level for 2 min. After pressure and echocardiographic data had been recorded, the snare was released.

Analysis of the data, displayed via a Betamax ½ inch videocassette recorder (Sony Corp. of America, Long Island City, NY), was performed off-line with a microprocessor-based microcomputer (E-Z view II; Microsonics, Inc., Indianapolis, IN). End-systole was assumed to occur at the dicrotic notch of the aortic pressure tracing (12, 16), and end-systolic pressure was measured at this point. The echocardiographic left ventricular end-systolic diameter of the same beat was measured simultaneously at the dicrotic notch of the aortic pressure tracing. Three beats were used to measure diameter and end-systolic pressure at baseline and after preload and afterload interventions. Data points (Fig. 6) represent mean±SEM.

Summary of dog protocols. All dogs were surgically prepared as described above and all received AGEPC (0.4 μg/kg) intravenously through the right femoral vein. In five dogs, after baseline conditions were recorded, a control intravenous injection of the vehicle (2 ml of a 2 mg BSA/ml 0.9% NaCl solution) was administered. 5 min later AGEPC was administered, and the hemodynamic data, along with echocardiographic recordings of right and left ventricular end-diastolic diameters, were recorded for 1 h to characterize the hemodynamic alterations induced by this phospholipid. In two dogs, diethylcarbamazine (50 mg/kg), an inhibitor of leukotriene synthesis (17–19), was infused over 20 min, and AGEPC was injected 2 min later. In two additional dogs FPL 55712 (1 mg/kg), a leukotriene receptor blocker (20) obtained from Fisons plc, Loughborough, England, was infused 30 s before the administration of AGEPC. In five dogs the end-systolic pressure-diameter relation was evaluated, as previously described, before AGEPC. After all values returned to baseline, AGEPC was given, and, 3 to 30 min later, i.e., during the prolonged phase of lowered cardiac output (see text), recordings of pressures, flows, and echocardiograms were obtained. Preload and afterload were altered to define end-systolic pressure-diameter relations after AGEPC and repeat recordings were made.

Isolated perfused rabbit hearts. To assess further the effect of AGEPC on myocardial performance, in a preparation relatively independent of loading conditions, AGEPC was perfused through isolated rabbit hearts. Nonfasted New Zealand White rabbits (n = 4), weighing 1.5–2.0 kg, were stunned with a blow to the head, the hearts were quickly removed through a sternotomy incision and perfused retrogradely through the aorta with a roller pump (Ismatec MP-4; Gilford Instrument Laboratories, Inc., Corning Glass Works, Oberlin, OH) at 20 ml/min at 37°C with nonrecirculating modified Krebs-Henseleit (21) solution containing 0.4 mM BSA equilibrated with 95:5% O_2/CO_2. A fluid-filled latex balloon was placed in the left ventricle through the left atrium and mitral valve, creating an isovolumically beating heart, and connected to a pressure transducer for continuous recording of left ventricular pressure. Coronary artery perfusion pressure was recorded via the pressure transducer. By alteration of the volume of the balloon, the left ventricular end-diastolic pressure was maintained at 9–10 mmHg. A waterjacket surrounding the isolated heart maintained monitored myocardial temperature at 37°C. Pacemaker electrodes were sutured onto the right atrium and the heart rate was paced at 180 beats/min.

After the preparation had equilibrated, 0.5 ml of vehicle (2 mg BSA/ml 0.9% NaCl) was injected into the perfusing Krebs-Henseleit solution 2 cm proximal to the coronary ostia. When 5 min had elapsed, AGEPC (10^{-9}, 10^{-8}, and 10^{-7} M, in 0.5 ml of vehicle) was injected in a similar manner at 5-min intervals. Left ventricular pressure and coronary perfusion pressure were recorded simultaneously.

Isolated cross-perfused rabbit hearts. Since AGEPC had no effect in isolated rabbit hearts perfused with Krebs-Henseleit buffer alone (see below), and since this agent was known to activate certain cellular elements in the blood, AGEPC was further evaluated in blood-perfused isolated rabbit hearts. Nonfasted New Zealand white rabbits (n = 4) weighing 2.0–2.5 kg were anesthetized with sodium thiopental (25 mg/kg) and α-chloralose (80 mg/kg). The rabbits were intubated with a polyethylene tube via a tracheostomy site and ventilated under positive pressure with room air supplemented by oxygen. Catheters were inserted into the right femoral artery and the left femoral vein; another catheter connected to a pressure transducer was placed in the left

carotid artery for measurement of systemic arterial blood pressure. Heparin (1,000 U) was administered intravenously. From a separate rabbit (n = 8), an isolated perfused heart was prepared as described above, and once both preparations were stable, the intact rabbit's circulation was diverted through the right femoral artery line to the isolated perfused heart at 6 ml/min (controlled by a roller pump) and returned from the isolated heart to the intact rabbit through the left femoral vein.

Vehicle and AGEPC were administered in the same manner as described above for the isolated perfused heart. The left ventricular pressure of the isolated heart and the systemic arterial blood pressure of the intact rabbit were recorded continuously. Once AGEPC was injected proximal to the isolated heart it became necessary to divert the effluent blood from the isolated heart to prevent hypotension in the intact rabbit. The blood lost was replaced with fresh rabbit blood.

Isolated perfused guinea pig hearts. To ascertain if AGEPC produced a similar response in the isolated heart of a species other than the rabbit, it was tested in the isolated guinea pig heart. Five nonfasted white guinea pigs weighing 400 g each were stunned with a blow to the head, and the hearts were removed and perfused as in the isolated rabbit heart, at a rate of 14 ml/min with equilibrated Krebs-Henseleit buffer. In one heart, left ventricular pressure was measured with a fluid-filled catheter and in the other four with a fluid-filled latex balloon; coronary perfusion pressure was measured also. All pressures were recorded via pressure transducers. After equilibration of the isolated guinea pig heart, AGEPC (10^{-9}, 10^{-8}, and 10^{-7} M) was injected at 5-min intervals as in the isolated rabbit heart.

Results

AGEPC-induced changes in blood pressure in vivo. To ascertain the dosage of AGEPC to be used subsequently in intact dogs, doses of AGEPC (from 0.1 to 0.8 μg/kg) were administered in accordance with previously reported effects of varying doses of AGEPC in several animal species in the literature (4). When AGEPC was infused intravenously in dogs, there resulted a clear-cut depression in mean systemic arterial blood pressure that was dose dependent: at 0, 0.1, 0.2, 0.4, and 0.8 μg/kg AGEPC, mean systemic arterial blood pressure was reduced from a control value of 144 mmHg by 0, 9, 32, 39, and 41%, respectively. Since 0.8 μg/kg AGEPC resulted in the death of one dog and since a near maximal response was reproducibly obtained with 0.4 μg/kg, equivalent to $\sim 8 \times 10^{-9}$ M, this latter dose was selected for experiments where both pressures and flows were simultaneously recorded to ascertain the hemodynamic alterations induced by AGEPC.

AGEPC-induced changes in systemic and pulmonary hemodynamics. Five dogs received AGEPC (0.4 μg/kg) for characterization of the hemodynamic alterations induced by this agent. Before the injection of AGEPC the mean baseline hemodynamic values (±SE) were: mean systemic arterial blood pressure, 111±9 mmHg; mean systemic arterial blood flow, 2.15±0.36 liters/min; systemic vascular resistance, 4,700±1,400 dyn·s·cm^{-5}; mean pulmonary arterial blood pressure, 17±2 mmHg; mean pulmonary blood flow 2.20±0.35 liters/min; and pulmonary vascular resistance, 728±132 dyn·s·cm^{-5}. Baseline left ventricular dP/dt averaged 2,849±320 mmHg/s, and heart rate averaged 138±14/min.

Although no changes occurred after the administration of vehicle, AGEPC induced marked hemodynamic changes (Figs. 2 and 3). Within several minutes after the intravenous injection of AGEPC profound systemic hypotension ensued. This hypotension, which lasted as long as an hour, changed characteristically and reproducibly over time. Purely for the purposes of analysis, the temporally complex hemodynamic alterations of both the systemic and pulmonary components of the circulation were divided into three phases based on changes noted on the left side of the circulation and depicted in Fig. 2. Such nomenclature is convenient though continuous recordings of hemodynamics were made, and it is recognized to be arbitrary. Thus, the phases will be described in detail below, but for the purposes of definition, phase I lasts from ~5–30 s after injection and it is characterized by mild reductions in systemic blood pressure and increases in aortic blood flow. Phase II is notable for a sudden collapse of systemic blood pressure to ~50% of the control values, a reduction that occurs ~30 s after injection of AGEPC and lasts for a total of ~60 s. After an elapsed time of ~90 s, mean systemic blood pressure gradually returns toward baseline values, and thus phase III is defined by the presence of this return toward near-normal pressure but severely depressed aortic blood flow

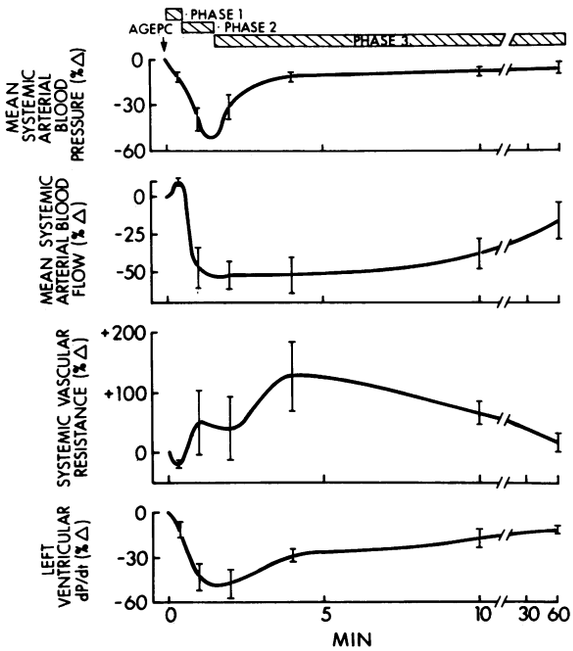

Figure 2. Hemodynamic alterations in the systemic circulation induced by AGEPC. Percentage change in the hemodynamic parameters is plotted against time after intravenous administration of AGEPC (0.4 μg/kg) to dogs as described in Methods. The bars represent the mean±SE, and the baseline absolute values for these hemodynamic parameters are provided in Results.

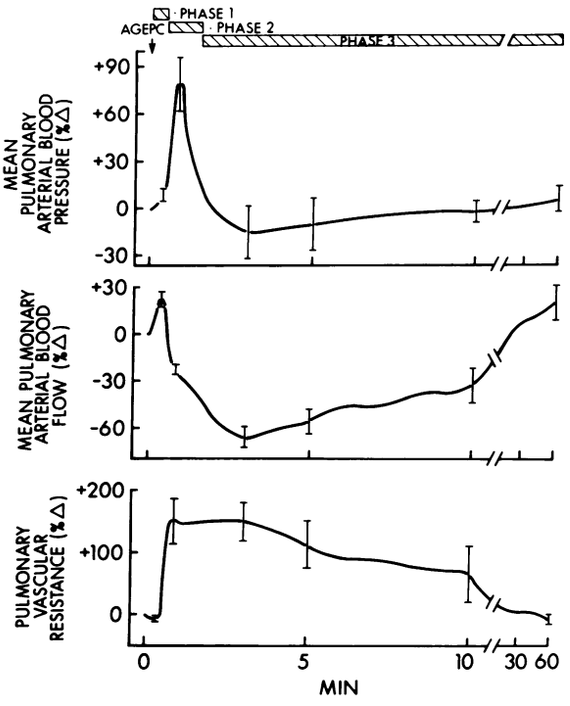

Figure 3. Hemodynamic alterations in the pulmonary circulation induced by intravenous administration of AGEPC (0.4 μg/kg). The percentage change in the hemodynamic parameters is plotted against time. The bars represent the mean±SE.

values. These changes were observed every time AGEPC was infused (n = 5 dogs) and can be appreciated from a pattern analysis of Fig. 2. Although the three phases were designated based on these changes in mean systemic blood pressure, the nomenclature is used in the description of Fig. 3 and, subsequently, merely to indicate the temporal relationship to changes noted in Fig. 2 and to serve as a basis for discussion. Fully quantitative descriptions of changes in the hemodynamic parameters can be derived from the figures presented and the absolute units for those presented above; hence, the textual description will not repeat these in the interests of clarity.

In detail, on the left side of the circulation (Fig. 2), phase I (from ~5 to 30 s after injection) consisted of a mild reduction in systemic blood pressure from 111 to 98 mmHg and a reduction in systemic vascular resistance with an increase in aortic flow. Left ventricular dP/dt also fell. During phase II (from ~30 to 90 s) marked systemic hypotension occurred (systemic pressure dropped from 98 to 52 mmHg), associated with declines in aortic blood flow and left ventricular dP/dt and a rise in systemic vascular resistance. Phase III (from ~90 s to 60 min) was characterized by a gradual return of systemic blood pressure to near normal levels with continued depression of dP/dt, marked reduction of aortic flow, and elevation of systemic vascular resistance (+125% of baseline values). There was no significant change in heart rate during any of the phases.

On the right side of the circulation during phase I (Fig. 3) there was a slight rise in mean pulmonary artery pressure and a decrease in pulmonary vascular resistance. In phase II mean pulmonary artery pressure rose markedly, mean pulmonary blood flow decreased, and pulmonary vascular resistance rose. During phase III pulmonary artery pressure returned toward normal, and mean pulmonary vascular resistance initially remained high but later returned toward normal.

Since AGEPC has been thought to produce α-adrenergic-blockade (22), the effect of prazosin pretreatment on these typical left- and right-sided hemodynamic changes was characterized (23). First, phenylephrine (0.2 mg) was administered intravenously to document reactivity to this alpha-adrenergic agent: there was a prompt and resultant rise in mean systemic arterial blood pressure from 97 to 115 mmHg which lasted 10 min. After 15 min, prazosin (0.4 mg/kg) was administered intravenously over 30 min, with a mild reduction of mean systemic blood pressure from 97 to 78 mmHg. The adequacy of α-adrenergic blockade was demonstrated by rechallenge with phenylephrine (0.2 mg); there was complete abolition of the effects in systemic and pulmonary artery blood pressure previously observed with phenylephrine administration. After 15 min of further equilibration, AGEPC, 0.4 μg/kg, was administered intravenously. All three phases of hemodynamic changes on both sides of the circulation ensued as described in Figs. 2 and 3, including marked and sustained hypotension. After 30 min, when both systemic blood pressure and flow had returned to baseline, phenylephrine (0.2 mg) was readministered with no effect, demonstrating that the pressor response to phenylephrine remained blocked. Thus, α-adrenergic receptor function does not seem to be necessary for the hemodynamic actions of AGEPC.

Effect of leukotriene antagonists on AGEPC-induced hemodynamic alterations. Since α- and β-adrenergic antagonists, histaminergic blockers, acetylcholine, indomethacin, and antiplatelet antibodies have been ineffective in blocking the reported hemodynamic effects of AGEPC (4, 22, 24), and since this agent is thought to activate the lipooxygenase pathway in isolated organ preparations (25, 26), we examined the effects of pretreatment with diethylcarbamazine or FPL 55712, leukotriene blocking agents, on AGEPC-induced hemodynamic alterations.

First, two dogs received diethylcarbamazine (50 mg/kg) without significant alteration of their baseline hemodynamic values, which were, respectively: mean systemic arterial blood pressure, 86 and 115 mmHg; mean systemic aortic blood flow, 3.58 and 2.00 liters/min; mean systemic vascular resistance 1,900 and 4,600 dyn·s·cm^{-5}; mean pulmonary arterial blood pressure, 20 and 11 mmHg; mean pulmonary arterial blood flow, 3.30 and 2.12 liter/min; and pulmonary vascular resistance, 480 and 415 dyn·s·cm^{-5}). Within 2 min, AGEPC (0.4 μg/kg) was administered but, contrary to the findings depicted

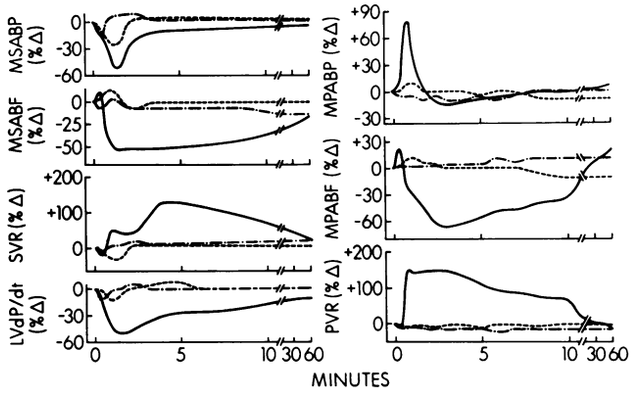

Figure 4. Effect of diethylcarbamazine pretreatment on hemodynamic alterations in the systemic and pulmonary circulations induced by AGEPC. Percentage change in the hemodynamic parameters (mean systemic arterial blood pressure, MSABP; mean systemic arterial blood flow, MSABF; systemic vascular resistance, SVR; left ventricular d*P*/d*t*, LVd*p*/d*t*; mean pulmonary arterial blood pressure, MPABP; mean pulmonary arterial blood flow, MPABF; pulmonary vascular resistance, PVR) is plotted against time after intravenous administration of AGEPC (0.4 μg/kg) to five dogs (solid curves). The plots represent the mean values as shown in Figs. 2 and 3. Two separate dogs—see Methods and Results—were pretreated with diethylcarbamazine (50 mg/kg) followed by AGEPC (0.4 μg/kg). There is a marked diminution of the effects induced by AGEPC (dashed curves).

in Figs. 2 and 3, there was little, if any, significant change in the hemodynamic parameters recorded (Fig. 4). Thus, diethylcarbamazine pretreatment prevents essentially all of the hemodynamic effects induced by AGEPC, suggesting the involvement of leukotrienes in such effects and demonstrating that this leukotriene antagonist is the first effective pharmacologic agent in preventing AGEPC-induced hemodynamic perturbations.

Because diethylcarbamazine probably also has nonspecific blocking effects, further implication of leukotrienes in the hemodynamic mechanism of action of AGEPC was sought by examining the effect of FPL 55712 pretreatment (1 mg/kg) in two dogs subsequently infused with AGEPC (0.4 mg/kg). In both cases, phase I and II hemodynamic changes remained essentially unmodified, except for a slight blunting of the increase in pulmonary vascular resistance. However, during phase III, systemic vascular resistance did not rise to 125% of baseline values as shown in Fig. 2. Instead this elevation was almost completely blocked by FPL 55712, with increases in systemic vascular resistance of only 20%. The half-life of FPL 55712 in vivo is short, but the abolition of the rise in systemic vascular resistance in the presence of this agent, as well as an attenuation of this during diethylcarbamazine pretreatment, suggests that leukotrienes may mediate the marked rise in systemic vascular resistance induced in phase III by AGEPC. Leukotrienes may mediate other hemodynamic alterations but their involvement cannot currently be inferred since FPL 55712 did not prevent them.

Right and left end-diastolic diameters after AGEPC. After AGEPC injection, left ventricular end-diastolic diameter and right ventricular end-diastolic diameter decreased simultaneously in parallel (Fig. 5). The reduction of end-diastolic diameters began 30 s after AGEPC, became maximal at 210 s, and was reversed 7 to 8 min later, at a time that left ventricular d*P*/d*t* remained depressed. Since these results suggested the occurrence during phase III of a negative inotropic effect induced by AGEPC, we assessed additional criteria for implicating such an effect which would be less influenced by changes in preload and afterload.

End-systolic pressure-diameter relations. End-systolic pressure end-systolic diameter relationships were determined in five dogs before AGEPC and during phase III after infusion of the agent, since left ventricular end-diastolic diameter returned to normal during this phase. The data points were obtained as described in Methods. The set of three data points gathered before AGEPC, representing a control value, a preload inter-

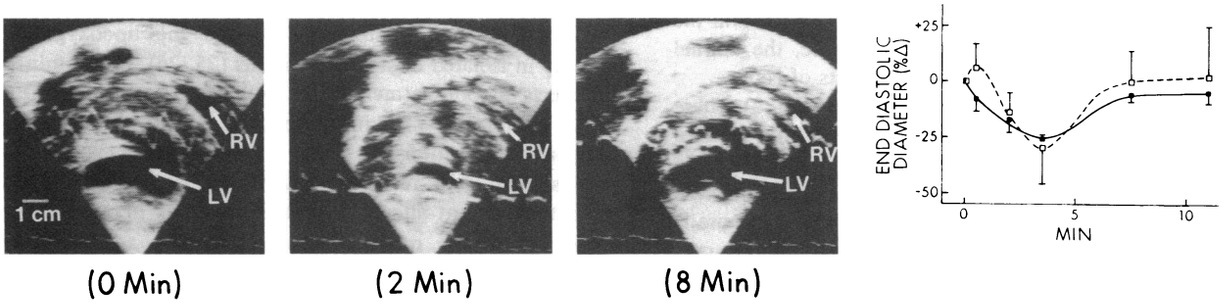

Figure 5. Time course of AGEPC-induced changes in left and right ventricular end diastolic diameter. AGEPC, 0.4 μg/kg, was infused into dogs (n = 5) and the right and left ventricular (RV and LV, respectively) cavity size was assessed by continuous recording by two-dimensional echocardiography. End-diastolic diameter for each was then determined as described in Methods and plotted at the intervals shown. There was no overall change in geometry of either ventricle except for size. Left ventricular end-diastolic diameter is represented by ■——■, the right ventricular end-diastolic diameter by □- - -□. The vertical bars represent the mean value of the end-diastolic diameter±SE.

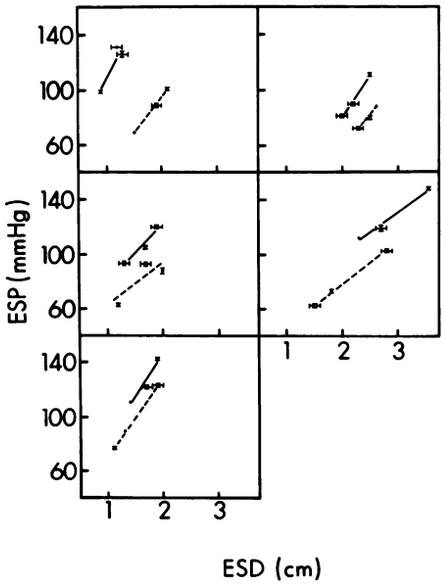

Figure 6. AGEPC-induced changes in end-systolic pressure-diameter relationships in the dog during phase III. The end-systolic pressure (ESP) vs. the end-systolic diameter (ESD) was determined as described in detail in the Methods. Each box represents one dog. The end-systolic pressure-diameter relationship for each dog before the injection of AGEPC (0.4 μg/kg) is represented by the solid line (●——●), always above the line representing the relationship after administration of AGEPC. After the administration of AGEPC the end-systolic pressure-diameter relationship for each dog is, in each case, shifted down and to the right, represented by the dashed line (●- - -●). The horizontal bars represent the mean values of the end-systolic diameters±SE, and the vertical bars represent the mean values of the end-systolic pressures±SE (e.g., ⊢I⊣). Where bars are absent (·), SE = 0.

vention, and an afterload intervention, was used to determine a correlation coefficient for the end-systolic pressure-diameter relation for each of the five dogs during the control evaluation; a second similar set of data points obtained after the administration of AGEPC was used to determine a correlation coefficient for the end-systolic pressure-diameter relation during the post-AGEPC period. The end-systolic pressure-diameter relation so determined was linear for each of the five dogs both during the control evaluation and after the administration of AGEPC (r = 0.88 to 0.99; Fig. 6). In addition, after the administration of AGEPC, there was a consistent shift in the end-systolic pressure-diameter relation down and to the right, such that at any given end-systolic pressure, the end-systolic diameter was increased (Fig. 6), a finding consistent with a decrease in the inotropic state of the left ventricle (27–29). These results further substantiate the occurrence of a decrease in contractility in phase III.

AGEPC-induced changes in left ventricular pressure in isolated hearts. Additional experiments to characterize the negative inotropic effect induced by AGEPC were carried out in isolated, perfused hearts, a preparation independent of systemically induced changes in preload and afterload. When isolated rabbit hearts (n = 4), perfused in a retrograde fashion with Krebs-Henseleit buffer, were exposed to vehicle (2 mg BSA/ml NaCl) or increasing concentrations of AGEPC (10^{-9}, 10^{-8}, or 10^{-7} M), similar to those given dogs in vivo, no changes were noted in left ventricular pressure (Fig. 7 *A*). In contrast, when isolated rabbit hearts were perfused with blood from a donor rabbit and then given AGEPC (10^{-9} M) there was a marked reduction in developed left ventricular pressure to 53±12% of baseline within 3 min (Fig. 7 *A*); within 20 to 25 min, left ventricular pressure returned to baseline values (Fig. 7 *B*). No changes occurred with vehicle in this preparation, which was stable over the time course of the experiment.

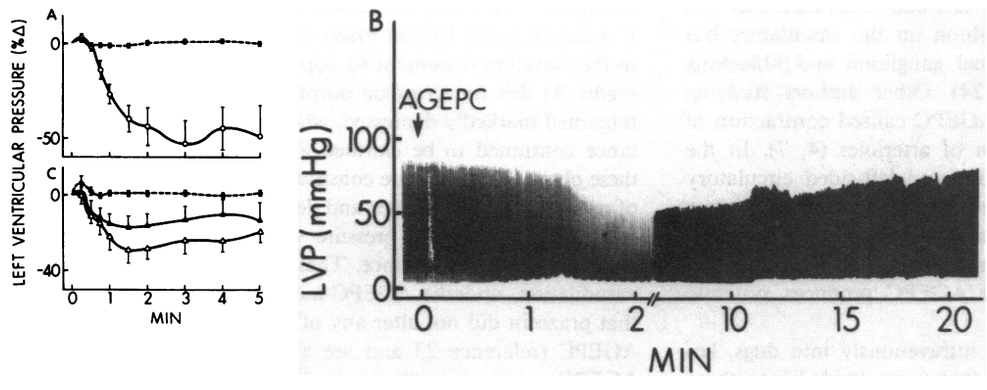

Figure 7. AGEPC-induced depression of left ventricular function in isolated perfused rabbit and guinea pig hearts. The percentage change in peak systolic left ventricular pressure (LVP) is plotted against time. Fig. 7 *A* shows the effects of AGEPC (from 10^{-9} to 10^{-7} M) on isolated rabbit hearts (n = 4) perfused with Krebs-Henseleit buffer (●- - -●) and of AGEPC (10^{-9} M) on isolated rabbit hearts (n = 8) cross-perfused with blood from a donor rabbit (○- - -○). Fig. 7 *B* shows the actual pressure tracings from an isolated blood-perfused rabbit heart to indicate the recovery of left ventricular pressure after administration of AGEPC as described above. Fig. 7 *C* shows the effects of AGEPC on the peak systolic left ventricular pressure in isolated guinea pig hearts (n = 5) perfused with Krebs-Henseleit buffer at two concentrations: 10^{-9} (▲——▲) and 10^{-8} M (△——△). There is a dose-dependent decrease in peak systolic left ventricular pressure which does not require the presence of blood as in the isolated rabbit hearts. AGEPC, 10^{-7} M, had no increased effect on the changes in peak systolic left ventricular pressure as compared with AGEPC, 10^{-8} M. Control tracings (♦- - -♦) after infusion of vehicle were stable.

Isolated guinea pig hearts were also used, in view of possible species differences. When these hearts were perfused with Krebs-Henseleit buffer (n = 5) and given vehicle (2 mg BSA/ml NaCl), there was no change in left ventricular pressure. However, when the same hearts were exposed to increasing doses of AGEPC (10^{-9}, 10^{-8}, and 10^{-7} M) there was a marked reduction in developed left ventricular pressure to 17±7% below baseline with the 10^{-9} M dose (Fig. 7 *C*). With the subsequent dose (10^{-8} M) there was a further decrease in peak systolic left ventricular pressure to 29±7% below baseline, but there was no further decrease in the left ventricular pressure with the final dose (10^{-7} M). These results demonstrated the occurrence of a myocardial depressant effect of AGEPC, independent of autonomic innervation, preload, afterload, and heart rate, which reduces developed left ventricular pressure. Furthermore, these results support the interpretation of the data obtained in vivo, indicating the occurrence of an AGEPC-induced negative inotropic effect.

Discussion

AGEPC, an acetyl glyceryl ether of phosphorylcholine, elicits inflammatory and anaphylactic-like responses as well as circulatory alterations in several species (4). The circulatory alterations include systemic hypotension, lasting from several minutes to an hour, increased pulmonary vascular resistance, and decreased cardiac output. Several investigations have attempted to clarify the mechanism of action by which AGEPC induces these hemodynamic alterations. Smith et al. found that AGEPC increased the diameter of both venules and arterioles of the exposed cremasteric muscle of the rat. Since it also prevented the pressor response to norepinephrine in vivo, the authors concluded that AGEPC was an α-adrenergic antagonist that caused vasodilation (22). Subsequently, Sybertz et al. studied the hypotensive effect of AGEPC in conscious normotensive rats and in pithed rats and concluded that this agent acted in a nonspecific fashion on the vasculature but was "devoid of adrenergic neuronal, ganglionic and β-blocking activity at hypotensive doses"(24). Other authors studying various tissues concluded that AGEPC caused contraction of smooth muscle and constriction of arterioles (4, 7). In the present study, recordings of right- and left-sided circulatory changes were monitored in a systematic and continuous manner to define the hemodynamic alterations induced by AGEPC, characterize their temporal relationships, and elucidate the mechanism of action by which AGEPC produces systemic hypotension.

When AGEPC was injected intravenously into dogs, hemodynamic alterations resulted that were divided into three phases for the purpose of analysis based upon changes that occurred in the mean systemic arterial blood pressure (Fig. 2). In phase I, on the left side of the circulation there was a decrease in mean systemic arterial blood pressure accompanied by a rise in mean systemic arterial blood flow with a fall of larger magnitude in systemic vascular resistance, alterations consistent with dilation of the systemic vascular bed. Concomitantly, there was a decrease in pulmonary vascular resistance consistent with vasodilation as seen in the arterial circuit, but a rise of greater magnitude in mean pulmonary arterial blood flow resulted in a rise in mean pulmonary pressure. During the same period (phase I) left ventricular dP/dt decreased without a significant change in left ventricular end-diastolic diameter, suggesting the occurrence of a depression of myocardial performance in view of the decrease in afterload.

In phase II, mean systemic arterial blood pressure became maximally depressed along with mean systemic arterial blood flow, whereas systemic vascular resistance rose. Mean pulmonary artery pressure increased markedly and reached a maximum before falling, whereas mean pulmonary artery blood flow declined and pulmonary vascular resistance rose steeply and remained elevated. Left ventricular end-diastolic diameter continued to fall, and left ventricular dP/dt decreased to its lowest point. These alterations in phase II are consistent with a marked depression of cardiac pump function and a resultant decrease in cardiac output and mean systemic arterial blood pressure. The increase in mean pulmonary artery pressure and mean pulmonary vascular resistance despite a fall in cardiac output are not inconsistent in view of the recent findings that suggest that AGEPC induces the release of leukotrienes in isolated lungs which in turn cause a rise in pulmonary artery pressure and pulmonary vascular resistance (26).

The decrease in right ventricular end-diastolic diameter despite a rise in pulmonary vascular resistance reflects decreased right-sided filling. The decrease in left ventricular end-diastolic diameter together with a depressed left ventricular dP/dt may occur secondary to a depletion of intravascular volume as well as to a decrease in blood flow from the right to the left heart, reflecting the marked rise in pulmonary vascular resistance.

In phase III, mean systemic arterial blood pressure gradually returned toward normal after administration of AGEPC but remained ~10% below baseline values for as long as 60 min. It is during phase III that many of the measurements reported in the literature documenting hypotension have probably been made. At this time cardiac output and left ventricular dP/dt remained markedly depressed, whereas systemic vascular resistance continued to be elevated above baseline values. All of these phase III changes are consistent with continued depression of myocardial performance and decreased cardiac output while systemic arterial blood pressure was maintained by a rise in systemic vascular resistance. These abnormalities, rather than vasodilation, underlie AGEPC-induced hypotension. The fact that prazosin did not alter any of the hemodynamic effects of AGEPC (reference 23 and see above) strongly suggests that AGEPC is not primarily an α-adrenergic antagonist and supports the concept that the rise in systemic vascular resistance is not a compensatory α-adrenergic mediated response.

Previous work by Voelkel and co-workers had shown that when AGEPC is infused into isolated lungs at a constant flow rate, pulmonary artery pressure and, therefore, pulmonary vascular resistance rose. Lung effluent contained leukotrienes,

suggesting that these agents might be responsible for the increase in pulmonary vascular resistance (26). Because similar changes in right- and left-sided pressures were seen in dogs after injection of AGEPC in the present study, we postulated that a phenomenon similar to that noted by Voelkel et al. might be occurring. To test this hypothesis, diethylcarbamazine was administered before AGEPC. Although this agent is nonspecific for antagonism of leukotriene-induced effects, its use has frequently allowed implication of these arachidonate metabolites in complex biological phenomenon. Thus, pretreatment with diethylcarbamazine (Fig. 4) essentially prevented all of the AGEPC-induced changes in the hemodynamic parameters noted in Figs. 2 and 3. Further support for this view arises from the FPL 55712-induced blunting of the vascular responses of AGEPC. FPL 55712, a purported leukotriene receptor blocker, was administered before AGEPC. Whereas partial attenuation of the phase II rise in pulmonary artery pressure as well as the rise in pulmonary vascular resistance occurred, the rise in systemic vascular resistance in phase III was completely blocked. Although FPL 55712 may have other effects than blockade of leukotriene receptors (30), these results, together with those obtained with diethylcarbamazine, suggest that elevation of systemic vascular resistance during phase III may be mediated by leukotrienes since this was blocked by both antagonists. Just why diethylcarbamazine is more effective in blunting the earlier phases is currently unclear. It is also unclear why the effects of FPL 55712, which has a short serum half-life, were not present until phases II and III. This finding may relate to the pharmacologic dynamics of FPL 55712 in vivo, to the specificity of this agent for specific leukotriene type receptors in various tissues, and/or to the temporal appearance of leukotriene effects. Determination of actual leukotriene concentrations in blood in vivo, of course, before and after AGEPC, with and without appropriate pretreatment, would allow a more definitive implication of leukotrienes in the hemodynamic mechanism of action of AGEPC. However, in vivo such measurements are not so easily performed as they are in vitro, and there is no assurance that blood, rather than vascular tissue, would be the appropriate compartment to assay. In another sense, some specificity is suggested by the inability of commonly used agents, such as α- and β-adrenergic blocking agents, acetylcholine, antihistamines, cyclo-oxygenase pathway blockers, antiplatelet antibodies, and angiotensin II (22), to blunt the hemodynamic actions of AGEPC. Diethylcarbamazine and FPL 55712 are the only two agents available that have been effective in this regard; future studies with more specific agents with longer in vivo half-lives are necessary for confirmation of these findings, as such agents become available.

The marked changes in left ventricular dP/dt after intravenous injection of AGEPC suggested the occurrence of a depression in myocardial performance which could contribute substantially to the systemic hypotension observed. Although α-chloralose has been reported to alter myocardial performance as well as other cardiovascular parameters after the induction of anesthesia, the most notable changes are immediate, whereas later effects, including a mild to moderate rise in heart rate, are variable and dependent upon the method of administration and the physiologic preparation. Studies have indicated that baseline hemodynamic alterations due to α-chloralose are less profound than those seen with other types of anesthesia, and α-chloralose, therefore, seems preferable for studies that may involve the neural regulation of the cardiovascular system (31–33). This study, in which each dog served as its own control, showed no significant alteration in heart rate by which myocardial contractility might be affected. In the conscious closed-chest dog it has been shown that changes in left ventricular dP/dt accurately reflect acute changes in myocardial contractility, independent of alterations in afterload with a moderate sensitivity to major alterations in preload. However, in the open-chest dog there appears to be some dependence of left ventricular dP/dt on preload (34). To assess more accurately changes in myocardial performance after AGEPC, an independent measurement of contractility in vivo, the end-systolic pressure-diameter relation was used. This measurement is independent of loading conditions in both in vitro and in vivo settings. After AGEPC the end-systolic pressure-diameter curve was shifted downward and to the right so that at any given end-systolic pressure, the end-systolic diameter was larger; these findings are consistent with a decrease in the contractile state of the left ventricle (Fig. 6) (13, 27, 29).

To assess further the effect of AGEPC on myocardial performance when alterations in loading conditions are kept at a minimum, a Langendorf-perfused isolated rabbit heart preparation was used. Perfusion with Krebs-Henseleit solution, followed by injection of vehicle or AGEPC, produced no changes in developed left ventricular pressure (Fig. 7 *A*). This absence of an AGEPC-induced depression in myocardial performance in this setting may be attributable to an obligatory role of formed elements in the blood, species variation, an obligatory role for extracardiac intermediary metabolism of AGEPC, or any combination of these factors.

To evaluate these possibilities systematically, we used isolated rabbit hearts (n = 8) cross-perfused with blood from an intact rabbit. Administration of AGEPC immediately proximal to the coronary arteries of the isolated heart resulted in profound depression of developed left ventricular pressure, consistent with a decrease in myocardial contractility (Fig. 7 *A*). This depression lasted for ~20 min. Subsequently, developed left ventricular pressure returned to control values with a time course similar to that observed in vivo in dogs during phase III. Since there was no alteration in left ventricular pressure before the administration of AGEPC or with the administration of vehicle, there is no indication that the experimental protocol alone resulted in activation of any formed elements of the blood and thus led to a depression of myocardial contractility. In addition, because the isolated hearts were perfused at a constant flow, any change in coronary vascular resistance would be reflected in coronary perfusion pressure without a change in global flow of the isolated heart.

For this reason an alteration in coronary perfusion pressure seems an unlikely cause of a reduction in contractility in this setting. Similarly, although oxygen extraction was not measured directly, oxygen delivery should have remained constant in the face of a constant flow and should not have been a factor in the decrease in contractility. Therefore, these results indicate that AGEPC causes a depression in left ventricular contractility in the isolated rabbit heart similar to that seen in the intact dog, and that in the isolated rabbit heart, blood is required for the effect to be manifested.

To characterize potential species variations, we determined the effect of AGEPC on left-ventricular pressure development in isolated, perfused guinea pig hearts as well. In contrast to the findings in the buffer-perfused isolated rabbit heart, our results showed that AGEPC infused into the coronary arteries of isolated guinea pig heart produced a profound negative inotropic effect within 30 to 60 s (Fig. 7 *C*), in the absence of blood. Similar results were recently reported in another study by Levi et al. (35) in which isolated guinea pig hearts perfused with Krebs-Henseleit buffer were given AGEPC. After the administration of AGEPC there was a prompt decline in left ventricular contractile force which was both marked and long lasting, and it was not blocked by FPL 55712. Diethylcarbamazine was not used.

The results from the in vitro experiments described in this study as well as the observations of Levi et al. support the view that AGEPC produces a negative inotropic effect in vivo. The difference between the effectiveness of diethylcarbamazine and FPL 55712 remains unresolved. There appear to be at least two mechanisms, an intrinsic and an extrinsic one, underlying the effect, with some species variation present. The extrinsic effect, observed in the rabbit heart, could be mediated by transient aggregation of formed elements in the blood induced by AGEPC, a known aggregator of both platelets and leukocytes (4). A similar mechanism has been demonstrated to produce hypoxia by affecting the pulmonary vascular bed during initial stages of hemodialysis. It has been termed leukostasis (36). Metabolic products derived from the interaction of AGEPC and blood may therefore play a role in producing a transient negative inotropic effect. The intrinsic mechanism, observed in the guinea pig heart, remains unexplained but presumably reflects metabolic events in myocardium induced by AGEPC since its onset is not immediate.

The recent identification of AGEPC in patients and the present description and characterization of its hemodynamic effects indicate that this agent may account for many of the sequential, deleterious responses seen with anaphylaxis, including alterations in cardiac output and systemic vascular resistance (37–39). Whether a negative inotropic effect, either intrinsic or extrinsic, also occurs in anaphylaxis is not clear, but cases of profound hypotension lasting up to 60 h have been reported (37). The present findings suggest that the negative inotropic effect may be important in human anaphylaxis and that it may be potentially amenable to modification pharmacologically. Further studies with agents such as leukotriene receptor and/or synthesis blockers may prove useful in elucidating the molecular bases of hypotension in such syndromes.

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