Regional Cardiac Prostaglandin Release during Myocardial Ischemia in Anesthetized Dogs

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SUMMARY Cardiac prostaglandin release was studied in closed-chest dogs during acute coronary occlusion. Aortic and coronary sinus blood was obtained before, and at intervals after, balloon occlusion of the left anterior descending artery in seven dogs. Samples were assayed for prostaglandins F, E, and A by radioimmunoassay. All dogs demonstrated prostaglandin F release. Mean ± SE postocclusion aortic levels were 0.26 ± 0.01 ng/ml; coronary sinus levels were 0.67 ± 0.01 ng/ml (P < 0.001). In six dogs, prostaglandin E also was released. Mean postocclusion aortic levels were 0.24 ± 0.01 ng/ml; coronary sinus, 0.44 ± 0.01 ng/ml (P < 0.001). There was no release of prostaglandin A. To examine the site of prostaglandin release, simultaneous samples from the aorta, the coronary sinus, and the great cardiac vein were obtained before and after left circumflex artery occlusion in six dogs. Mean postocclusion aortic prostaglandin F was 0.32 ± 0.01 ng/ml. Coronary sinus prostaglandin F was 1.69 ± 0.03 ng/ml (P < 0.001), whereas the great cardiac vein level remained at 0.34 ± 0.01 ng/ml (P > 0.05). Prostaglandin E was released from both ischemic and nonischemic regions. Mean aortic prostaglandin E was 0.21 ± 0.01 ng/ml; great cardiac vein, 0.55 ± 0.02 ng/ml (P < 0.001); and coronary sinus, 1.07 ± 0.04 ng/ml (P < 0.001). These results have led us to conclude that the different local availability of prostaglandins E and F may influence the cardiac response to ischemia.

THE RELEASE of prostaglandins from tissues subjected to acute ischemia was first demonstrated by McGiff et al.1 in the canine kidney. Prostaglandin (PG) biosynthesis has been documented in the isolated perfused rabbit heart exposed to hypoxia, mechanical massage, elevated preload, vagal stimulation, and adenosine triphosphate or acetylcholine administration.2,4 Ischemia and anoxia have manifested variable effects on PG biosynthesis.3,5,7,8 whereas acidosis, hyperthermia, hypothermia, hyperosmolality, and hyperkalemia all were without effect.9 Studies utilizing the open-chest dog have shown an increase in prostaglandins in coronary venous blood during postocclusive reactive hyperemia.10 The same finding is reported following coronary occlusion in the canine heart-lung preparation.11 These data are significant because of the known vascular actions of these compounds. It generally is agreed that intracoronary administration of prostaglandin E (PGE) produces increased inotropy, chronotropy, and coronary blood flow, whereas intravenous administration produces a marked fall in systemic vascular resistance and arterial pressure.12–14 In contrast, intracoronary administration of prostaglandin F (PGF) has little effect on coronary hemodynamics or ventricular function, but this agent does increase systemic arterial pressure when infused intravenously in large doses.15

It has been suggested that prostaglandins may play a role in the cardiac response to ischemia. In the present study, the cardiac release of PGF, PGE, and PGA was studied in closed-chest dogs during acute coronary occlusion. The identification and quantification of PG release by radioimmunoassay, the time course, and the regional distribution of release were determined.

Methods

Initial experiments (group I) were designed to determine whether prostaglandins are released from the heart following coronary occlusion; and if so, which are released and the time course of release. Subsequent experiments (group II) investigated the site of PG release by selective sampling of venous drainage from ischemic and nonischemic myocardial regions.

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GROUP I

Seven healthy mongrel dogs, weighing 20–31 kg, were anesthetized with sodium pentobarbital (35 mg/kg, iv) and ventilated mechanically with room air using a Harvard respirator. All dogs received 3,000 U of heparin iv. Blood volume removed for sampling was replaced simultaneously by normal saline.

A No. 7 French Sones catheter was positioned fluoroscopically approximately 2 cm beyond the orifice of the coronary sinus (CS). Aortic (AO) pressure was measured by a Statham P23Db transducer connected through a fluid-filled system to a catheter placed in the descending AO. The lead V6 electrocardiograph (ECG) was monitored continuously. Pressure and ECG were recorded on a DR-12 Electronics for Medicine multichannel recorder.

Baseline AO and CS blood samples (7 ml each) were drawn simultaneously. Under fluoroscopic control, a balloon-tipped catheter then was positioned in the proximal left anterior descending coronary artery. This placement was confirmed by injection of sodium meglumine and diatrizoate. The balloon then was inflated, completely occluding the left anterior descending coronary artery. S-T segment elevation consistent with ischemia was noted immediately in all dogs. Following occlusion, and at 5- to 10-minute intervals thereafter, blood samples were drawn from AO and CS (7 ml each) into plastic syringes. Sampling times following occlusion differed in individual dogs because of variations in hemodynamic and electrical responses to ischemia. Experiments were continued until death occurred from ventricular arrhythmias, or for 3 hours in surviving dogs.

Following occlusion, arterial pressure decreased by about 20% in every dog. None went into shock unless ventricular tachycardia or fibrillation occurred.

In two control dogs, the identical protocol was carried out without inducing coronary occlusion. Samples were obtained from similar sites at 15-minute intervals for a total of 90 minutes.

GROUP II

Six dogs (22–29 kg), anesthetized with sodium pentobarbital (35 mg/kg, iv) were studied. A No. 7 French Sones catheter was positioned fluoroscopically in the proximal CS 2 cm beyond the orifice; another was passed more distally into the great cardiac vein (GCV) as far anteriorly as 2 cm beyond the orifice; the latter was then inflated, completely occluding the left circumflex coronary artery. Its position was confirmed by injection of contrast under fluoroscopic control into the left circumflex coronary artery. S-T segment elevation consistent with ischemia was noted immediately in all dogs. Following occlusion, and at 5- to 10-minute intervals thereafter, blood samples were drawn from AO and CS (7 ml each) into plastic syringes. Sampling times following occlusion differed in individual dogs because of variations in hemodynamic and electrical responses to ischemia. Experiments were continued until death occurred from ventricular arrhythmias, or for 3 hours in surviving dogs.

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PROSTAGLANDIN ANALYSES

Immediately after withdrawal into syringes, blood samples were transferred to heparinized tubes which were capped and stored on ice. All tubes were centrifuged promptly at 1,000 g for 10 minutes. The plasma was decanted into scintillation vials which were sealed tightly and stored in a freezer at −20°C. Hemolyzed samples were discarded. Extreme care was taken to remove all red blood cells and platelets from the supernatant fluid prior to freezing.

Plasma samples were assayed for PGF, PGE, and PGA by radioimmunoassay, as standardized in this laboratory. Plasma samples (2 ml) were acidified mildly and extracted with ethyl acetate. Silicic acid column chromatography was used to separate the three major groups of prostaglandins. Values are expressed as major groups, since the chromatography is unable to differentiate subgroups (i.e., PGE1, PGE2, and PGA). Varying concentrations of methanol in benzene and ethyl acetate were used for elution. Antibodies were generated in rabbits by immunization utilizing the respective prostaglandin bound to bovine serum albumin. The full characterization of the antibodies has been reported. Charcoal-coated dextran in buffer was used to separate bound and unbound PG. All assays had at least 65% recovery efficiency. The interassay and intrasay variation was less than 15%. All samples were run in duplicate, but in different sample sizes. Results are reported as corrected means of the two values. The lower limit of sensitivity of the assay is approximately 0.1 ng/ml.

DATA ANALYSIS

The first sample obtained immediately after occlusion never showed any arteriovenous PG difference. This sample, therefore, was not included in analysis in any experiment. All subsequent postocclusion samples were analyzed by several approaches. First, each dog was treated as a separate, independent experiment in which paired AO and CS, AO and GCV, or CS and GCV samples were compared by a paired t-test. In addition, for each of the three sampling sites, the mean ± SE postocclusion value was calculated for each dog. Second, individual dogs were grouped to assess the overall response. Because the number of samples obtained after occlusion differed in individual studies, the group means for each sampling site were determined from weighted means of each dog's prostaglandin level; the weighting factor used was the inverse of the individual variances. Paired, weighted AO and CS, AO and GCV, or CS and GCV samples were compared by a t-test. Analysis of PG levels at baseline before occlusion and at the time of maximal PG release after occlusion was also undertaken. At each of these sampling times, arterial and venous values were compared by a paired t-test. Mean ± SE arteriovenous differences at these times were also determined.

Probability (P) less than 0.05 was considered significant.
### Table 1 Prostaglandin (PG) Levels following Coronary Occlusion

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Plasma prostaglandin concentration (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td>AO</td>
</tr>
<tr>
<td>1 (2) P</td>
<td>0.75 ± 0.03</td>
</tr>
<tr>
<td>2 (7) P</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>3 (12) P</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>4 (5) P</td>
<td>0.30 ± 0.02</td>
</tr>
<tr>
<td>5 (9) P</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>6 (4) P</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>7 (6) P</td>
<td>0.29 ± 0.01</td>
</tr>
</tbody>
</table>

Mean ± se* P | 0.30 ± 0.01 | 0.33 ± 0.02 | 0.24 ± 0.01 | 0.44 ± 0.01 | 0.26 ± 0.01 | 0.67 ± 0.01 |

AO = aorta; CS = coronary sinus; NS = not significant.

Each value represents the mean of all samples drawn in each dog after occlusion. Number of samples (in parentheses) was determined by the course of the experiment. The sample immediately following occlusion was disregarded (see text). Statistical significance was determined by a paired t-test.

* Weighted mean determined by weighing means of individual animals by inverse of their variances.

### Results

#### GROUP I

All seven dogs demonstrated release of PGF after coronary occlusion (Table 1). Mean postocclusion AO levels were 0.26 ± 0.01 ng/ml, whereas CS levels were 0.67 ± 0.01 ng/ml (P < 0.001). Three of these dogs showed a PGF release greater than 1 ng/ml. In six of seven experiments, CS levels of PGE were significantly greater than AO levels after occlusion. After occlusion, AO PGE averaged 0.24 ± 0.01 ng/ml, while CS was 0.44 ± 0.01 ng/ml (P < 0.001). There were no significant postocclusion differences between AO and CS PGA in any individual dog or for the group of dogs (P > 0.05).

Before coronary occlusion (baseline), there were no significant AO-CS differences (Fig. 1). After occlusion, maximal release of PGE averaged 0.76 ± 0.23 ng/ml. Maximal PGF averaged 0.91 ± 0.33 ng/ml.

The two control experiments tested the effects of the experimental manipulations and blood withdrawal. There was no detectable PG release.

Release of PGE or PGF occurred within 10 minutes after occlusion in all dogs. Once present, PG release was maintained until the dog died or until the experiment was terminated. PG release was greater in magnitude, and appeared earlier, in those dogs surviving for less than 20 minutes.

#### GROUP II

Plasma PGE and PGF levels, measured for a representative experiment, are shown in Figures 2 and 3. AO PGE remained constant after occlusion, whereas both CS (ischemic drainage) and GCV (nonischemic drainage) PGE concentrations were elevated significantly. AO PGF also remained constant throughout the experiment. Proximal CS drainage, predominantly representing the ischemic region, contained elevated PGF levels immediately after occlusion.
CARDIAC PROSTAGLANDIN RELEASE/Berger et al.

and throughout the study. However, there was no release of PGF from the normal zone drained by the distal GCV. There was no PGA release from either site. Five of six dogs demonstrated significant release of PGE from the GCV drainage, and all six showed release from the CS (Table 2). Mean AO PGE was 0.21 ± 0.01 ng/ml, Mean GCV PGE was 0.55 ± 0.02 ng/ml (P < 0.001), and mean CS PGE, 1.07 ± 0.04 ng/ml (P < 0.001, compared with AO levels). All six dogs demonstrated release of PGF from the ischemic region (proximal CS), while only one showed release from the nonischemic region (distal GCV). Mean AO PGF was 0.32 ± 0.01 ng/ml. CS PGF was significantly elevated at 1.69 ± 0.03 ng/ml (P < 0.001). There was no significant release of PGA in any of the individual dogs following coronary occlusion. However, there was a small, but statistically significant increase in mean GCV PGA. Relative to the sensitivity of the assay, this difference (0.03 ng/ml) is too small to be meaningful.

When arteriovenous differences for PG were analyzed, no significant difference was noted prior to occlusion. The arteriovenous differences at the time of maximal PG release after occlusion are shown in Figure 4. PGE was released only from the ischemic zone, with a maximal arteriovenous difference of -1.39 ± 0.15 ng/ml. In contrast, PGE was released from both ischemic and nonischemic regions, -1.09 ± 0.09 and -0.52 ± 0.04 ng/ml, respectively.

Discussion

The present study demonstrates the release of PGE and PGF from the heart during myocardial ischemia. This finding in the intact anesthetized dog is in agreement with earlier studies using the perfused rabbit heart and with the work by Alexander et al. using the canine heart-lung preparation. In contrast to the data of Kraemer and Folts, release of PGA was not shown at any time. The validity of PGA values in the dog can be questioned because of the presence in canine plasma of a PGA isomerase, which converts PGA to PGB.

The release of both PGE and PGF from the heart during ischemia resembles the findings of McGiff et al. in the kidney. During canine renal ischemia, PGE and PGF were identified in renal vein blood by bioassay and thin layer chromatography. Recent studies from that group have demonstrated the enzymatic conversion of PGE to PGF in the rabbit kidney, suggesting that some of the PGF measured may have been released as PGE. Similar conversion also might occur in the heart.

It is unclear where cardiac PG biosynthesis and release originate. Local tissue action is characteristic of the prostaglandins because of their rapid metabolism by the lung and liver. Both myocardial PG synthetase and PG dehydrogenase have been isolated and characterized from the canine left ventricle. PG synthesis and breakdown, therefore, could occur locally in the myocardium. As recently suggested, however, PG synthesis may occur in vascular smooth muscle as well.

In this study, PGE was found in the venous effluent from both ischemic and nonischemic regions, whereas PGF release was limited to the ischemic region. These findings suggest that the roles of PGE and PGF may be different. Several earlier studies have demonstrated different physiological effects for these two prostaglandins in the cardiovascular system. PGE (but not PGF) increases myocardial adenosyl cyclase, increases vascular Na⁺-K⁺-ATPase, and inhibits adrenergic transmission by postjunctional depression. On the other hand, PGF in low doses (but not PGE) act at the cardioregulatory and vasomotor centers of the hindbrain to regulate vagal tone to the heart.

The physiological importance of cardiac release of prostaglandins during ischemia remains unclear. They may be involved in local regulation of coronary blood flow, having been implicated in vasomotor autoregulation in the kidney, brain, and uteroplacental bed. PGE increases adenosine levels in myocardial tissue and perfusate. This suggests an interrelationship between PG and adenosine, which has been proposed as a mediator of metabolic regulation of coronary flow. In one study of experimental myocardial infarction, PGE increased coronary blood flow to the ischemic area in comparison with the preinfarction state. However, indo-
TABLE 2  Regional Prostaglandin (PG) Levels following Coronary Occlusion

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Plasma prostaglandin concentration (ng/ml)</th>
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<tbody>
<tr>
<td></td>
<td>PGA</td>
</tr>
<tr>
<td></td>
<td>GCV AO CS GCV AO CS GCV AO CS</td>
</tr>
<tr>
<td>1 (4) P</td>
<td>0.39±0.01 0.37±0.01 0.38±0.01 0.60±0.04 0.20±0.01 1.03±0.11</td>
</tr>
<tr>
<td>2 (4) P</td>
<td>0.35±0.02 0.35±0.01 0.35±0.01 0.55±0.07 0.24±0.01 1.00±0.17</td>
</tr>
<tr>
<td>3 (4) P</td>
<td>0.36±0.01 0.36±0.01 0.35±0.02 0.46±0.03 0.20±0.01 0.65±0.08</td>
</tr>
<tr>
<td>4 (2) P</td>
<td>0.51±0.01 0.48±0.02 0.50±0.02 0.57±0.03 0.23±0.01 1.18±0.15</td>
</tr>
<tr>
<td>5 (2) P</td>
<td>0.39±0.01 0.37±0.01 0.38±0.03 0.81±0.05 0.19±0.01 1.38±0.06</td>
</tr>
<tr>
<td>6 (5) P</td>
<td>0.44±0.01 0.44±0.01 0.45±0.02 0.54±0.07 0.22±0.02 0.99±0.07</td>
</tr>
<tr>
<td>Mean ± se*</td>
<td>0.41±0.01 0.38±0.01 0.38±0.01 0.55±0.02 0.21±0.01 1.07±0.04</td>
</tr>
</tbody>
</table>

AO = aorta; CS = coronary sinus (proximal); GCV = great cardiac vein (distal); NS = not significant.

Each value represents the mean of all samples drawn in each dog after occlusion. Number of samples (in parentheses) was determined by the course of the experiment. The sample immediately following occlusion was disregarded (see text). Statistical significance was determined by a paired t-test.

* Weighted mean determined by weighting means of individual animals by the inverse of their variances.

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methacin, which blocks PG synthesis and release from the heart in various preparations,4-6, 7, 8, 30 has not demonstrated a consistent effect on cardiac reactive hyperemia.7, 8, 11, 30-33

After acute coronary occlusion in the cat, administration of PGF increased survival time, decreased plasma creatine phosphokinase, and decreased myocardial lysosomal activity in comparison to controls.24, 35 Recent reports have suggested that release of cardiac lysosomal hydrolases may signal irreversible cell death. They also have been implicated in the development of collateral blood flow.24 Stabilization of these enzymes in vivo by a locally synthesized agent, such as PGF, may maintain cellular viability and local perfusion. However, these findings are contrasted by studies in vitro of human leukocytes which showed that PGF did not inhibit lysosomal enzyme release.37

PG restored normal sinus rhythm and decreased the incidence of arrhythmias following coronary occlusion in cats.38 In a similar study in monkeys, a derivative of PGB enhanced recovery from ventricular arrhythmias due to coronary occlusion.39 This has suggested a favorable effect on electrical stability of the ischemic myocardium.

The known physiological and pharmacological actions of PGE and PGF, therefore, would suggest that their local availability following a decrease in coronary perfusion may affect the cardiac response to ischemia.

Acknowledgments

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