Relationship between Regional Myocardial Procainamide Concentration and Regional Myocardial Blood Flow during Ischemia in the Dog

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SUMMARY We measured myocardial blood flow and myocardial procainamide concentration in 24 sections of left ventricle following occlusion of the left circumflex coronary artery in 11 dogs. 14C-labeled procainamide was infused at a constant rate (40 µg/kg per min) following completion of the coronary artery occlusion. At the end of 4 hours, the infusion was terminated and the dogs were killed. Regional myocardial blood flow was determined at 30 minutes and at 4 hours, using 125I- and 51Cr-labeled microspheres. Regional myocardial procainamide concentration was determined by analysis of 14C content in the samples, using a combustion technique with entrainment of liberated 14CO2. The amount of 14C activity in each sample was calculated following corrections made for 51Cr and 125I activity, using the channels ratio method. Twenty-four regional sections of left ventricle were divided into 12 anteroseptal control sections and 12 posterolateral "ischemic" sections. Myocardial blood flow to the control region was 1.17 ± 0.17 (mean ± SEM) ml/min per g and myocardial procainamide concentration was 4.62 ± 0.36 µg/g. Plasma procainamide concentration was 2.2 ± 0.1 µg/ml. Flow to ischemic sections, when measured at 30 minutes and 4 hours, did not change. Myocardial procainamide concentrations in the ischemic sections differed significantly from procainamide in the control sections only when flow fell to 31-40% of control values (P < 0.01). At flows less than or equal to 10% of control flow, myocardial procainamide concentration was reduced to only 42 ± 4% of control drug levels. We conclude that in our experiments there is a substantial concentration of procainamide in severely hypoperfused and probably densely infarcted ventricular myocardium.

PROCAINAMIDE, like other antiarrhythmic drugs, lacks predictable effectiveness.1 The ability to determine plasma concentrations of many drugs has enabled investigators to define a "therapeutic range" for plasma drug levels and thus provide the clinician with a useful method for establishing an adequate dosage regimen.2 However, a "therapeutic" plasma drug concentration does not always result in a therapeutic concentration at the effector site under either transient or steady state conditions.3, 4 In patients with ischemic heart disease, lack of response or toxicity may result in part from a different concentration of drug in ischemic as opposed to normal myocardium. In fact, Bella et al.5 reported a localized decrease in digoxin concentration in ventricular myocardium in the presence of ischemia which they suggested might parallel reductions in flow.

We developed a method that allows simultaneous measurement of procainamide concentration and blood flow within small samples of myocardium. Results of our previous work demonstrate that, when drug is administered by constant infusion over 4 hours to anesthetized, open chest, nonischemic dogs, myocardial procainamide concentration ([PA]M) is independent of myocardial blood flow (MBF) over a normal range of flows.6 The aim of the present investigation was to determine the extent to which [PA]M is correlated with MBF in the setting of decreased flow caused by coronary artery occlusion in dogs.

Methods

Eleven mongrel dogs weighing 14–20 kg were anesthetized with morphine sulfate (2 mg/kg, im) and α-chloralose (85 mg/kg, iv), intubated with a cuffed endotracheal tube and ventilated with a Harvard Apparatus respiration pump. A solution of 0.9% NaCl was infused (10 ml/kg per hr) into a superficial brachial vein throughout the experiment. Anesthesia was maintained with additional doses of α-chloralose. The right femoral artery and vein were exposed and catheterized. A polyvinyl chloride catheter with an outside diameter of 3 mm was introduced into the femoral artery, advanced to the ascending aorta, and connected to a Statham P23Db pressure transducer. Phasic and mean aortic blood pressure and the ECG (lead II) were recorded continuously on an Electronics for Medicine DR-8 recorder. Procainamide was infused through the femoral vein catheter. A catheter was passed into the bladder and secured to prevent leakage and provide continuous drainage. A left thoracotomy then was performed, and a catheter was inserted into the left atrium and secured by a purse string suture.

Following control observations, the left circumflex coronary artery was ligated within 1.5 cm of its origin using the two-stage method of Harris.7 Procainamide then was infused at a rate of 40 µg/kg per min for 4 hours. Procainamide solution for infusion was prepared in the...
following manner: 100 μCi of 14C-procainamide* was added to the amount of procainamide hydrochloride required for the 4 hours of infusion and diluted with normal saline to a final volume of 53 ml. The rate of infusion of procainamide solution was kept constant with a Harvard Apparatus infusion pump. Blood samples for analysis of plasma procainamide concentration were obtained through the aortic catheter prior to and at hourly intervals during the infusion period. Five minutes prior to the end of the infusion period, approximately 3 × 10^6 125I-labeled microspheres (3M Co.), diameter 7-10 μm, were injected into the left atrium as previously described. Simultaneously, blood was withdrawn continuously at 15 ml/min over a 2-minute period through the aortic catheter, using a Harvard Apparatus peristaltic pump to provide reference blood samples. The heart then was excised and the right atrium, left atrium, and right ventricle (free wall) were rapidly dissected away from the left ventricle. The left ventricle immediately was frozen in a dry ice-ethanol mixture (−37°C). All samples then were stored at −5°C. In five of the 11 dogs, additional injections into the left atrium of 3 × 10^6 125I-labeled microspheres were performed 30 minutes after left circumflex coronary artery ligation to evaluate changes in myocardial blood flow during the period of drug infusion.

Left ventricular tissue samples for analysis of 51Cr, 125I, and 14C content were obtained as follows: the left ventricular apex and base were discarded, leaving one central ring. The ring was divided into six transmural segments (anterior free wall, septum, posterior free wall, posterior papillary muscle area, lateral wall, and anterior papillary muscle area). Each segment was subdivided into four layers of equal thickness from epicardium to endocardium (labeled 1 through 4, respectively), resulting in 24 regional sections. Each layer was sectioned further into two triangular subsections by a diagonal cut, so that each triangle would have equal representation from the inner and outer surface of the section. Two samples were obtained from the long side of each triangle and used for analysis of 14C. The remainder of both triangles was used for analysis of 51Cr and 125I (Fig. 1).

The analysis of 14C in the duplicate samples was performed as previously described. In short, 200- to 300-mg samples of myocardium (wet weight) were combusted in oxygen and the liberated 14CO₂ was trapped in a solution of ethanolamine ethylene glycol monomethyl ether. Radioactivity of the samples was determined in a Beckman liquid scintillation spectrometer. Contamination by counts in the β range caused by 125I and 131J was subtracted from the total counts per minute of each sample using the channels ratio method. Procainamide (PA) concentration (μg/g sample weight) in each sample was determined using the formula:

\[
[\text{PA}] \text{μg/g} = \frac{\text{unlabeled PA (μg) in infusate}}{\text{labeled PA (dpm) in infusate}} \times \frac{14\text{C (dpm) in sample}}{\text{weight of sample (g)}}
\]

The values of procainamide concentration from the two samples of each section were used to calculate a mean value for procainamide concentration in each of the 24 sections. Samples of left ventricle used for analysis of 51Cr and 125I consisted of the portions of triangular subsections not used in the analysis of 14C. There were 24 such samples from each dog; average sample weight was 1.539 g. Blood reference and myocardial samples were placed in vials and counted in a gamma spectrometer at window settings selected to correspond to the peak energy of each nuclide. The counts per minute were corrected for background.

Blood flow (ml/min) of each sample was determined using the formula: \( F_0 = F_T \times C_M + C_R \), where \( F_T = \) tissue sample blood flow (ml/min), \( F_R = \) reference blood flow (ml/min), \( C_M = \) tissue sample cpm, and \( C_R = \) reference cpm. Sample blood flow was divided by sample weight to express flow as ml/min per g of tissue.

### Data Analysis

Sections of left ventricle were grouped into a control region and an "ischemic" region. The control region consisted of 12 sections from the anterior papillary, anterior, and septal transmural segments. The ischemic region consisted of 12 sections from the posterior, posterior papillary, and lateral transmural segments. The \([PA]_M\) obtained from each sample in the ischemic region was divided by the mean \([PA]_M\) from that dog's control region; the resultant fraction was multiplied by 100 to allow \([PA]_M\) in ischemic regions to be expressed as a percent of control drug concentration. Similarly, MBF in each sample of the ischemic region was divided by the appropriate mean control MBF and multiplied by 100 to express flow as a percent of control.

Values for \([PA]_M\) in the sections from ischemic regions were compared to \([PA]_M\) in the sections from control regions for each decile decrement of percent flow and for each 0.1 ml/min per g decrement of absolute flow. To evaluate the influence of transmural location, \([PA]_M\) in layer 4 was compared to \([PA]_M\) in layer 3 of the ischemic regions. The above analyses were performed by a standard analysis of variance. Blood pressure and heart rate changes were analyzed by a multivariate analysis of variance. MBF at 30 minutes was compared to MBF at 4 hours by paired t-test. All results were expressed as mean ± SEM.

### Results

Under control conditions, mean arterial blood pressure was 114 ± 4 (mean ± SEM) mm Hg, and heart rate was 128 ± 11 beats/min. After coronary ligation, the mean arterial blood pressure was 105 ± 7 mm Hg and the heart rate was 139 ± 9 beats/min. These changes were not statistically significant. Mean arterial blood pressure and heart rate did not vary significantly during the period of drug infusion.

### Control Regions

Mean MBF in the control regions for all 11 dogs was 1.17 ± 0.17 ml/min per g (Table 1). Mean \([PA]_M\) in control regions of all dogs was 4.62 ± 0.36 μg/g. The ratio of \([PA]_M\) to plasma [PA] was 2.1 ± 0.1.
Ischemic Regions

Ligation of the left circumflex coronary artery produced reductions in MBF in the posterolateral region. The results from a typical experiment are illustrated in Figure 2. Reductions in flow were most pronounced in the “endocardial” layer (layer 4) of the posterior papillary muscle segment (Fig. 2 and Table 1).

$[PA]_m$ was compared to MBF in the ischemic regions (Figs. 2 and 3). A statistically significant decrease from control $[PA]_m$ did not occur until blood flow in ischemic regions fell to 31–40% of control. Drug concentration continued to decrease with flow until, at flows of ≤10% of control, the $[PA]_m$ equaled 42 ± 4% of control. Expressed as absolute flow, drug concentration began to fall at flows of 0.4 to 0.5 ml/min per g and reached a concentration of 39 ± 4% of control drug concentration at flows ≤0.1 ml/min per g.

It is surprising to find these relatively high concentrations of procainamide in areas of severe hypoperfusion. The presence of procainamide in areas with severely depressed MBF could be due to drug diffusion or could be a result of decreasing MBF occurring during the period of drug infusion.

Diffusion of drug from blood in the left ventricular chamber was evaluated as a source for procainamide in ischemic regions by comparing $[PA]_m$ in samples from layer 4 to $[PA]_m$ in samples from layer 3, in a similar flow range. No differences in $[PA]_m$ between layers 4 and 3 were noted.

Myocardial blood flow was determined at the end of the drug infusion period and may not have reflected flow earlier in the course of the infusion. To evaluate the hypothesis that the concentration of procainamide in areas with severely depressed MBF (determined at the end of the drug infusion period) could have resulted from high flows to those areas earlier in the course of the infusion, we measured MBF at 30 minutes as well as at 4 hours after onset of drug infusion in five dogs (Table 2). In control and moderately ischemic areas, the relationship of 30-minute to 4-hour flows was variable with no statistically significant change noted. In the samples for which MBF was <0.2 ml/min per g at 4 hours, the MBF at 30 minutes did not differ from the flow at 4 hours (Table 2).

Discussion

The most important finding of this study is that procainamide is present in areas of myocardium that are severely hypoperfused. We found that mean drug concentration was 42% of control concentration in myocardial sections receiving ≤10% of control MBF at 4 hours (Fig. 3). Irwin et al. have shown that in awake dogs such flows at 2 hours postocclusion result in almost total infarction of the

<table>
<thead>
<tr>
<th>Layer no.</th>
<th>Ant Pap segment</th>
<th>Anterior segment</th>
<th>Septal segment</th>
<th>Posterior segment</th>
<th>Post Pap segment</th>
<th>Lateral segment</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>1.30 ± 0.17</td>
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<td>1.16 ± 0.18</td>
<td>0.64 ± 0.08</td>
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<td>2</td>
<td>1.16 ± 0.23</td>
<td>1.10 ± 0.18</td>
<td>1.15 ± 0.19</td>
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<td>0.53 ± 0.11</td>
</tr>
<tr>
<td>3</td>
<td>1.09 ± 0.15</td>
<td>1.11 ± 0.16</td>
<td>1.25 ± 0.18</td>
<td>0.49 ± 0.12</td>
<td>0.24 ± 0.10</td>
<td>0.36 ± 0.10</td>
</tr>
<tr>
<td>4</td>
<td>1.29 ± 0.18</td>
<td>1.09 ± 0.18</td>
<td>1.11 ± 0.19</td>
<td>0.43 ± 0.15</td>
<td>0.16 ± 0.07</td>
<td>0.33 ± 0.08</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. Abbreviations: Post Pap = posterior papillary muscle segment; Ant Pap = anterior papillary muscle segment.
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FIGURE 2 Regional myocardial blood flow (ml/min per g) and procainamide concentration ([PA]μg/g) in a dog following left circumflex coronary artery occlusion. The average regional myocardial blood flow to the control region (anterior, septal, and anterior papillary muscle segments) was 1.67 ml/min per g and the average [PA]μg to the control region was 4.12 μg/g. Plasma [PA] was 1.7 μg/ml. Note that the reduction of flow and drug were most marked in the subendocardial layer (layer 4) of the posterior papillary muscle segment. Abbreviations used: POST PAP = posterior papillary muscle segment; ANT PAP = anterior papillary muscle segment.

section. Since flows did not change in ischemic areas between 30 minutes and 4 hours after occlusion, it is reasonable to assume that in this study sections receiving ≤10% of control flow at 4 hours also were almost completely infarcted. We conclude therefore that following prolonged infusion a substantial concentration of procainamide exists in densely infarcted myocardium.

Studies elucidating the mechanisms of ventricular arrhythmias following coronary occlusion emphasize the importance of reentry in areas of nonhomogeneous infarction or at the border between infarcted and normal myocardium. The results of Irvin et al. suggest that nonhomogeneous infarct or border zone areas occur at flows >20% of control. As can be seen in Figure 3, flows >20% of control in our experiments resulted in mean drug concentrations that exceeded 66% of control levels. Thus, following coronary artery occlusion, myocardial areas thought to be arrhythmogenic have procainamide concentrations near to concentrations found in normally perfused areas.

How procainamide gets to the severely ischemic myocardium is not clear. One possibility is that plasma "skimming" occurs in collateral channels so that microspheres fail to measure plasma perfusion in ischemic regions. Tripp et al. induced canine infarction by coronary ligation and then compared calculations of regional MBF, using microspheres and tritiated water. Flows measured by these two techniques were similar in both normal and ischemic myocardium, ruling out the possibility of significant "skimming."

One cannot predict how much diffusion of procainamide...
occurred in the myocardium. Similar values of $[PA_M]$ at similar flows in layers 3 and 4 support the contention that diffusion of procainamide from the left ventricular cavity over a 4-hour period is an insignificant source of procainamide in ischemic "endocardial" samples. These data do not rule out the possibility of intracavitary diffusion of drug to subendocardial Purkinje fibers.

The presence and direction of blood flow changes over time following experimental infarction are uncertain and probably vary with the experimental model. The presence of procainamide in ischemic "endocardial" samples. These data do not rule out the possibility of intracavitary diffusion of drug to subendocardial Purkinje fibers.

The presence and direction of blood flow changes over time following experimental infarction are uncertain and probably vary with the experimental model.8, 15, 16 The present study demonstrates no decline of MBF to ischemic regions from 30 minutes to 4 hours after occlusion (Table 2) and is in agreement with the data of Rivas et al.8 and Bishop et al.16 It is unlikely therefore that changes in regional MBF during the course of drug infusion explain the presence of procainamide in myocardium that is markedly hypoperfused 4 hours post coronary occlusion.

We speculate that the presence of procainamide in areas of low MBF results from a high percentage extraction of drug from plasma by the hypoperfused myocardium. We calculated, by using a regional MBF of 0.01 ml/min per g and the hourly plasma procainamide concentrations obtained in this study, that even in such severely hypoperfused samples sufficient procainamide was delivered to explain the quantities of drug found in these samples. Whether the percentage extraction of drug by ischemic tissue differs from that of normal myocardium is unclear. Intracellular acidosis can theoretically increase uptake of procainamide across an intact cell membrane;17 however, the sarcolemma is known to become defective in infarcted cells,18 leaving doubt as to whether pH changes could effect uptake in our model.

A major limitation of the experimental model in this study is the lack of precise knowledge of the pattern of localization of both MBF and $[PA_M]$ within each tissue sample. Those samples to which flow was very low must have been fairly homogeneously hypoperfused. Similarly, samples in which $[PA_M]$ was equal to control $[PA_M]$ most likely had homogeneous drug concentrations. However, one cannot differentiate samples with homogeneously distributed moderate reductions of flow or drug from samples with nonhomogeneous mixtures of flow or drug resulting in the same average measurements. Thus, any tissue sample with an apparently moderate reduction in flow or drug concentration could have resulted from a combination of severely ischemic plus more normal myocardium within the single sample. For example, Figure 3 shows that a sample in the ischemic region receiving 35% of control MBF would achieve 75% of control drug concentration. These values could result from a homogeneous decrease in flow and drug in the sample. Alternatively, the MBF and $[PA_M]$ measured could result from 50% of control MBF and 100% of control $[PA_M]$ in one half of the sample plus 10% of control MBF and 50% of control $[PA_M]$ in the other half of the sample. If heterogeneity of flow and drug distribution is assumed to be present in moderately ischemic samples, then the flow reduction at which $[PA_M]$ falls below control concentrations must actually be less than the 31-40% of control flow value observed in this study.

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References

LUNG WATER AND VASCULAR PERMEABILITY IN SHEEP/Brigham et al. 851

SUMMARY. To compare vascular permeability and water content in the lungs of newborn lambs with those in adult sheep, we measured extravascular water and permeability surface area products (PS) for "C-urea and "C-sucrose in nine 3- to 5-day-old lambs and 13 yearling sheep. In normal, unanesthetized animals, we injected a mixture of "Cr-erythrocytes, "H-albumin, "H-water, and either "C-sucrose or "C-urea as a bolus into the right atrium and sampled blood from the thoracic aorta, calculating extravascular water and PS for urea and sucrose from the time-concentration curves of the tracers. We also measured extravascular lung water and dry bloodless lung weight postmortem. Normalized to dry lung weight, extravascular lung water by both techniques was similar in newborns and adults (indicator dilution values = 3.2 ± 0.03 (SEM) ml/g for lambs and 3.2 ± 0.03 for sheep; postmortem values = 4.07 ± 0.26 g/g for lambs and 4.03 ± 0.17 for sheep). In newborn lambs, PS calculated by integral extraction was 0.28 ± 0.04 (SEM) g/g dry lung for "C-urea and 0.18 ± 0.04 for "C-sucrose. These values were not significantly different from those for adult sheep ("C-urea PS = 0.26 ± 0.03; "C-sucrose PS = 0.08 ± 0.03). We conclude that, when normalized to dry lung weight, newborn and adult sheep have similar lung water and vascular permeability to small hydrophilic molecules and that indicator methods for measuring PS and lung water are feasible in newborns.

PULMONARY edema is a prominent pathological finding in hyaline membrane disease. Since the edema fluid is rich in large proteins, at least part of the lesion may involve increased microvascular permeability. Techniques for measuring permeability in newborns could help to clarify the pathogenesis of this disease. There have been only a few studies of lung water and vascular permeability in newborn animals. We and others have used multiple indicator methods to measure permeability and water content in adult living animals and humans, but these methods seldom have been used in newborns. We wanted to see whether indicator dilution measurements were feasible in newborn lambs and to compare lung water and vascular permeability in normal newborn lambs with those in normal adult sheep. We used "C-urea and "C-sucrose as lung vascular permeability indicators and measured lung water content by both indicator dilution and postmortem methods. Newborn and adult lungs had similar amounts of extravascular water when normalized to dry lung weight. Permeability surface area products for "C-urea and "C-sucrose were similar in newborn and adult sheep when normalized to dry lung weight.

Lung Water and Vascular Permeability in Sheep
Newborns Compared with Adults
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