Effects of p-Chlorophenoxyisobutyrate on Myocardial Free Fatty Acid Extraction, Ventricular Blood Flow, and Epicardial ST-segment Elevation During Coronary Occlusion in Dogs

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SUMMARY The effect of p-chlorophenoxyisobutyrate (CPIB) on ST-segment elevation in epicardial electrocardiographic recordings was studied during coronary artery occlusion in dogs. Occlusion alone raised the sum of ST-segment elevations (ΣST) to 26 ± 6 mV (mean ± SEM). Intravenous (i.v.) administration of CPIB 30 min before re-occlusion reduced ΣST to 14 ± 3 mV (P < 0.03). A continuous i.v. infusion of isoproterenol increased ΣST to 74 ± 11 mV. Pretreatment with CPIB reduced ΣST during isoproterenol infusion to 40 ± 7 mV (P < 0.005). CPIB had no effect on mean aortic blood pressure, heart rate, or regional myocardial blood flow, as measured by radioactive microspheres. Arterial free fatty acid (FFA) concentration was reduced by CPIB from 466 ± 41 to 221 ± 44 μEq/L (P < 0.001) in the basal state, and from 1966 ± 183 to 1429 ± 209 μEq/L (P < 0.001) during isoproterenol infusion. The reduction in arterial FFA concentration was associated with a proportionate decrease in the myocardial extraction of FFA. Similar changes were observed when CPIB was administered during an occlusion which had been established 10 min earlier. These observations support other evidence that the severity of acute myocardial ischemic injury in dogs is positively correlated with the myocardial extraction of FFA, and that the severity of the ischemic injury can be reduced by effective antilipolytic therapy.

ACUTE MYOCARDIAL INFARCTION (AMI) in man is accompanied within one hour by an increase in the plasma concentration of free fatty acids (FFA), probably due to increased adipose tissue lipolysis as a result of enhanced sympatho-adrenal activity.7,8 Those patients with the highest plasma FFA concentrations have been reported to be at the greatest risk of developing serious ventricular arrhythmias and death,9,10 although not all investigations have confirmed this association.9 The hypothesis that this might reflect a toxic effect of FFA on the ischemic myocardium,11 rather than a direct consequence of increased catecholamine activity, has been supported by the recent demonstration that the incidence of ventricular arrhythmias during the early phase of AMI in man can be reduced by effective antilipolytic therapy in the absence of changes in plasma catecholamine concentration.11 A toxic effect of FFA on the heart could be mediated through an alteration of membrane potentials or an increase in the severity of the ischemic injury to the myocardium. Increases in plasma FFA concentration similar to those observed in clinical studies have been reported to increase the frequency of ventricular arrhythmias12 and the severity of myocardial ischemic injury13 during experimental coronary occlusion in dogs. In healthy dogs, elevated plasma FFA enhance myocardial oxygen consumption (MVO2) without improving the mechanical activity of the heart,13,14 suggesting that the deleterious effect of FFA during coronary occlusion may reflect an increase in the oxygen requirement of the ischemic tissue.

Such observations have raised the possibility that the survival of the ischemic cells following acute coronary occlusion may be enhanced by measures which decrease the delivery of FFA to the ischemic cells. Substantial reductions in plasma FFA concentrations have been reported in animals treated with p-chlorophenoxyisobutyrate (CPIB),15,16 although it is not known whether this is accompanied by a reduction in the utilization of FFA by the myocardium,17 as has been demonstrated with β-pyridyl-carbinol and its active metabolite, nicotinic acid.12,18

We have studied the effect of CPIB on the extraction of FFA by the heart and the extent and magnitude of epicardial ST-segment elevation during experimental coronary artery occlusion in dogs. The effect of antilipolytic therapy on regional myocardial blood flow during coronary occlusion was also examined, with the aid of radioactive microspheres.

Methods

Surgical Techniques

Experiments were carried out in 23 mongrel dogs of both sexes (15–20 kg body weight), fasted for 12–15 hours. The dogs were anesthetized with sodium pentobarbitral, 25 mg/kg body weight, followed by maintenance doses of 30–40 mg. Ventilation was maintained through a cuffed endotracheal tube with a positive pressure respirator (Harvard Apparatus Co. Inc., Mass.). Thoracotomy was performed through an incision in the left fifth intercostal space, and the heart suspended in a pericardial cradle. A branch of the left anterior descending (LAD) coronary artery was dissected free for a distance of 0.5 cm and a ligature placed loosely around it. Subsequent occlusions of the artery were performed with a releasable metal clip. The left femoral artery was cannulated for measurement of mean aortic blood pressure (AP), and the left femoral vein as a route of infusion. AP was monitored with a Statham P23Db transducer and recorded on a 4-channel recorder (Devices Ltd.,
Herts, U.K.). Arterial blood was sampled through a catheter inserted into the right femoral artery. In seven experiments coronary sinus blood was withdrawn simultaneously through a catheter introduced via the left jugular vein under fluoroscopic guidance. Arterial oxygen saturation was monitored with a Kipp type M01 Hemoreflexor, and pH and pCO₂ with a Type AME 1c Astrup Micro Equipment (Radiometer, Copenhagen).

**Epicardial ST-segment Mapping**

Epicardial electrocardiographic (ECG) measurements were performed with a mobile cotton-wick electrode, as described by Maroko et al., from 10–15 anatomically recognizable sites supplied by the dissected coronary artery and from the surrounding left ventricular tissue. Measurements were recorded, together with the standard limb leads, on a Devices 4-channel recorder at a paper speed of 25 mm/sec. The sensitivity of the epicardial recordings was set at 1mV/mm deflection. Those sites at which ST-segment elevation exceeded 2 mV were considered to be ischemic. Results were expressed as the sum of ST-segment elevations recorded from all sites (2ST), and as the number of sites at which ST elevation exceeded 2 mV². It should be noted that the values obtained with this technique are related not only to the severity of the ischemic injury, but also to the number and location of the recording sites, and cannot therefore be used to compare the results of different experiments. For such a purpose it would have been necessary to use a standardized grid system, which has disadvantages because of anatomical variations from dog to dog. In the present study, however, such a system was not required since each animal served as its own control, the electrode sites remaining constant throughout a given study, and the effect of CPIB being assessed by paired analysis.

**Regional Myocardial Blood Flow**

Regional myocardial blood flow was measured by means of radioactive microspheres labelled with 125I, 141Ce, or 85Sr (3M Riker Laboratories, Loughborough, U.K.: nominal diameter, 15μm). Prior to injection 2.0–2.5 × 10⁶ microspheres were subjected to ultrasound in 10% dextrose for 10 or more min to disperse aggregates (Dawe Sonicleaner, type 6441A), and drawn into a syringe containing 0.1 ml 5% Tween 80 to prevent reaggregation. The suspension was diluted with 10% dextrose to give a final concentration of Tween 80 of less than 0.5% and then continuously agitated until injected. Ten to 13 min after each coronary occlusion blood for the estimation of reference blood flow (RBF) was withdrawn from a femoral artery to a weighed heparinized syringe at a constant rate (approx. 9 ml/min) for a period of 2 min using a Harvard infusion/withdrawal pump. Immediately after the commencement of blood withdrawal, the microspheres were injected through a left atrial cannula over a period of about 10 sec. Between 5 and 30 min after the last injection the dog was sacrificed with sodium pentobarbital. The heart was excised, the free wall of the left ventricle dissected out, and visual fat and large vessels removed. Between 10 and 14 full thickness biopsies were made and divided into epicardial and endocardial layers of wet weight 1.5–4.0 g. The method was designed to give a minimum of 1000 microspheres/g wet weight of ischemic myocardium. Each layer was then divided into 10–20 blocks to produce relatively constant geometrical factors for the measurement of radioactivity in a three channel gamma counter (Gamma-Guard, Tracerlab Ltd.). Approximately 27% and 9% of 85Sr radioactivity was counted in the 141Ce and 125I channels respectively, while 3% of 141Ce radioactivity was counted in the 125I channel. These factors were determined during each counting procedure using the reference blood samples (containing only one isotope) as standards in a similar geometrical setting. Regional blood flow was estimated by weighing, using an assumed blood specific gravity of 1.05 g/ml. Regional myocardial blood flow (MBF) was calculated for each biopsy using the formula:

\[
\text{MBF} = \frac{C M \times \text{RBF}}{\text{CR}}
\]

where MBF is expressed in ml g⁻¹ min⁻¹, CM is the biopsy radioactivity in cpm/g wet weight, CR is the total cpm in the reference blood sample, and RBF is expressed in ml/min.¹⁸,¹⁹

**Laboratory Procedures**

Plasma CPIB concentrations were measured by a spectrophotometric method. Plasma FFA concentrations were assayed in duplicate by the method of Dole as modified by Trout et al. and were corrected for the presence of CPIB as described by Barrett and Thorp. Plasma free glycerol was estimated in duplicate according to Chernick. Blood glucose was measured in duplicate by an automated method.

**Design of Experiments**

**Effects of Pretreatment with CPIB on Subsequent Coronary Occlusion.** The effects of pretreatment with CPIB on the response to subsequent coronary occlusion were studied in ten dogs. In seven animals the following experimental design was employed. Epicardial electrocardiograms were recorded before and 5, 10, and 15 min after an initial control occlusion. The clip was then removed and a recovery period of 30 min allowed. The arterial FFA concentration was then raised by a continuous intravenous (i.v.) infusion of isoproterenol (0.2–0.3 µg/kg/min). Coronary occlusion alone does not alter plasma FFA concentrations in anesthetized dogs. This was confirmed in five of the present experiments, in which arterial FFA concentrations 15 minutes after occlusion (414 ± 16 µEq/L, mean ± SEM) were not significantly different from those immediately before occlusion (406 ± 23 µEq/L). Five minutes after the start of the infusion (by which time a stable hemodynamic response had been attained) the artery was reclosed. Epicardial ECG recordings were again made at 5 min intervals for 15 min, after which time the clip was released and the isoproterenol infusion discontinued. After another recovery period of 30 min CPIB was administered as the sodium salt by slow i.v. injection in 0.9% saline at the dose of 20 mg/kg body weight. Thirty minutes later the foregoing sequence was repeated, identical occlusions of the artery being induced at first in the absence and then in the presence of isoproterenol, and ST-segment elevation being mapped immediately before and 5, 10, and 15 min after each occlusion. In the remaining three dogs (307, 309, 313)
studies were carried out either in the absence or presence of isoproterenol only.

The reproducibility of the ST-segment mapping procedure was checked in the same experiments by performing repeated occlusions under identical conditions. In ten comparisons, values for \( \Sigma ST \) measured 15 min after coronary occlusion agreed consistently within \( \pm 7\% \) (first occlusion: \( \Sigma ST = 46 \pm 12 \) mV, mean \( \pm \) SEM; second occlusion: \( 47 \pm 12 \) mV [paired t-test: \( P > 0.30 \)].

Measurements of \( \Delta P \) and HR were made simultaneously with each ECG recording. Arterial blood was collected 15 min after each occlusion into a heparinized tube, and plasma for FFA and CPIB estimations separated immediately by centrifugation at 4°C. In nine dogs arterial blood was also collected for blood glucose determination.

In five additional dogs the effect of CPIB on regional myocardial blood flow during coronary occlusion was assessed by means of radioactive microspheres. Measurements were made during an initial control occlusion (three dogs), during second occlusion performed in the presence of isoproterenol (five dogs), and during a third occlusion performed in the presence of isoproterenol 30 min after i.v. CPIB (five dogs), the order of injection of the differently labeled microspheres being randomized in order to avoid systematic errors due to possible differences in their behavior. (The effect of CPIB on myocardial blood flow was assessed in the presence of isoproterenol, rather than under basal conditions, since it had previously been found that the reduction of epicardial ST-segment elevation by CPIB was more marked in the former situation.)

**Effects of CPIB Administration during Established Coronary Occlusion.** In six dogs an i.v. infusion of isoproterenol (0.2–0.3 \( \mu \)g/kg/min) was maintained for the duration of the study. Five minutes after its commencement a branch of the LAD coronary artery was permanently occluded. Five and ten minutes later recordings were made of epicardial ECG, \( \Delta P \), and HR. CPIB was then administered as previously described, and the recordings repeated after further intervals of 5 and 10 minutes. Simultaneously with each recording, arterial blood was sampled for the measurement of plasma FFA, CPIB, and free glycerol concentrations. In five of these dogs, and in an additional two animals, coronary sinus blood was also sampled immediately before and ten minutes after CPIB administration for the determination of the arterial-coronary sinus concentration differences of FFA and CPIB.

**Statistics**

Each dog served as its own control. Student’s t-test for paired data was used to calculate probability values. \( P > 0.05 \) was regarded as not statistically significant.

**Results**

**Effects of Pretreatment with CPIB**

The effects of CPIB on the response to subsequent coronary occlusion are summarized in tables 1 and 2. Occlusion of a branch of the LAD coronary artery was followed by marked changes in epicardial ST segments. After 15 minutes of occlusion \( \Sigma ST \) averaged 26 \( \pm \) 6 mV (mean \( \pm \) SEM). After CPIB administration re-occlusion of the artery resulted in a much smaller \( \Sigma ST \), averaging 14 \( \pm \) 3 mV (\( P < 0.03 \)). CPIB also reduced the number of sites with evidence of ischemic injury (ST > 2 mV) from an average of 4.9 \( \pm \) 1.1 to 2.9 \( \pm \) 1.0 (\( P < 0.05 \)). These effects of CPIB occurred in the absence of any changes in \( \Delta P \), HR, or blood glucose concentration (table 1). However, arterial FFA concentrations were reduced from 466 \( \pm \) 41 \( \mu \)Eq/L to 221 \( \pm \) 44 \( \mu \)Eq/L (\( P < 0.001 \)) (table 1).

Prior to CPIB the infusion of isoproterenol increased occlusion-induced \( \Sigma ST \) from 26 \( \pm \) 6 mV to 74 \( \pm \) 11 mV (\( P < 0.001 \)), and the number of sites with ischemic injury from 4.9 \( \pm \) 1.1 to 9.0 \( \pm \) 0.9 (\( P = 0.02 \)) (table 1 and 2). After CPIB \( \Sigma ST \) associated with subsequent coronary occlusion and isoproterenol infusion was markedly reduced to 40 \( \pm \) 7 mV (\( P < 0.005 \)), while the number of sites with ischemic injury was reduced to 7.6 \( \pm \) 1.3 (\( P < 0.05 \)). Intravenous infusion of isoproterenol before CPIB reduced \( \Delta P \) from 97 \( \pm \) 5 to 91 \( \pm \) 3 mm Hg (\( P < 0.05 \)), and increased HR from 134 \( \pm \) 7 to 176 \( \pm \) 6 beats/min (\( P < 0.001 \)), blood

**Table 1. Effects of Pretreatment with CPIB on Epicardial ST-segment Elevation, Arterial FFA Concentration (FFA), \( \Delta P \), HR, and Blood Glucose Concentration of 15 Minutes after Coronary Occlusion**

<table>
<thead>
<tr>
<th>Dog</th>
<th>( \Sigma ST^* ) (mV)</th>
<th>( \Delta P ) (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Bd glucose (mg/100 ml)</th>
<th>Plasma CPIB concentration (( \mu )Eq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>303</td>
<td>8</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>139</td>
</tr>
<tr>
<td>304</td>
<td>3</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>129</td>
</tr>
<tr>
<td>305</td>
<td>17</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>148</td>
</tr>
<tr>
<td>306</td>
<td>5</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>125</td>
</tr>
<tr>
<td>307</td>
<td>20</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>159</td>
</tr>
<tr>
<td>308</td>
<td>26</td>
<td>17</td>
<td>-</td>
<td>-</td>
<td>166</td>
</tr>
<tr>
<td>309</td>
<td>31</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>166</td>
</tr>
<tr>
<td>312</td>
<td>62</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>208</td>
</tr>
<tr>
<td>315</td>
<td>34</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>159</td>
</tr>
</tbody>
</table>

*Sum of ST-segment elevations at 10–15 sites.

†Number of sites at which ST-segment elevation exceeded 2 mV.

NS = \( P > 0.05 \).

Abbreviations: \( Ooc \) = occlusion; \( IP \) = isoproterenol; FFA = free fatty acid; \( \Delta P \) = mean arterial pressure; HR = heart rate; Bd gluc = blood glucose.
TABLE 2. Effects of Pretreatment with CPIB on Epicardial ST-segment Elevation, Arterial FFA Concentration (FFA), AP, HR and Blood Glucose Concentration 15 Minutes after Coronary Occlusion Performed during a Continuous i.v. Infusion of Isoproterenol

<table>
<thead>
<tr>
<th>Dog</th>
<th>ΣST* (mV)</th>
<th>ST &gt; 2 mV† (number of sites)</th>
<th>FFA (μEq/L)</th>
<th>AP (min Hg)</th>
<th>HR (beats/min)</th>
<th>Bd glue (mg/100 ml)</th>
<th>Plasma CPIB concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Occ IP</td>
<td>Occ IP</td>
<td>Occ IP</td>
<td>Occ IP</td>
<td>Occ IP</td>
<td>Occ IP</td>
<td>Occ IP</td>
</tr>
<tr>
<td>303</td>
<td>19 5</td>
<td>4 1</td>
<td>1200 975</td>
<td>82 83</td>
<td>185 180</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>304</td>
<td>71 43</td>
<td>11 11</td>
<td>1930 1275</td>
<td>90 92</td>
<td>185 185</td>
<td>81 60</td>
<td>—</td>
</tr>
<tr>
<td>305</td>
<td>92 65</td>
<td>9 6</td>
<td>1560 945</td>
<td>93 97</td>
<td>132 185</td>
<td>80 70</td>
<td>—</td>
</tr>
<tr>
<td>306</td>
<td>58 40</td>
<td>10 9</td>
<td>2820 2315</td>
<td>82 82</td>
<td>175 176</td>
<td>95 82</td>
<td>—</td>
</tr>
<tr>
<td>308</td>
<td>64 17</td>
<td>7 4</td>
<td>1880 1160</td>
<td>98 90</td>
<td>175 170</td>
<td>77 75</td>
<td>—</td>
</tr>
<tr>
<td>312</td>
<td>124 54</td>
<td>9 9</td>
<td>1880 1380</td>
<td>83 80</td>
<td>154 149</td>
<td>136 141</td>
<td>—</td>
</tr>
<tr>
<td>313</td>
<td>70 34</td>
<td>12 11</td>
<td>2640 2400</td>
<td>102 105</td>
<td>200 198</td>
<td>52 60</td>
<td>—</td>
</tr>
<tr>
<td>315</td>
<td>87 58</td>
<td>10 10</td>
<td>1720 1000</td>
<td>95 90</td>
<td>155 148</td>
<td>42 54</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td>74 40</td>
<td>9.0 7.6</td>
<td>1966 1429</td>
<td>91 90</td>
<td>176 174</td>
<td>80 77</td>
<td>—</td>
</tr>
<tr>
<td>SEM</td>
<td>11 7</td>
<td>0.9 1.3</td>
<td>183 209</td>
<td>3 3</td>
<td>6 6</td>
<td>12 11</td>
<td>—</td>
</tr>
</tbody>
</table>

*Sum of ST-segment elevations at 10–15 sites.
†Number of sites at which ST-segment elevation exceeded 2 mV.

For abbreviations see table 1.

TABLE 3. Effects of Isoproterenol and CPIB on Regional Myocardial Blood Flow in Nonischemic and Ischemic Free Ventricular Wall 10–18 Minutes after Coronary Occlusion

<table>
<thead>
<tr>
<th></th>
<th>Occ MBF (ml/g-1 min-1)</th>
<th>IP MBF (ml/g-1 min-1)</th>
<th>P</th>
<th>IP occ MBF (ml/g-1 min-1)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonischemic myocardium*</td>
<td>1.30 ± 0.09 (N = 21)</td>
<td>1.88 ± 0.09 (N = 34)</td>
<td>&lt;0.001</td>
<td>1.85 ± 0.07 (N = 34)</td>
<td>NS</td>
</tr>
<tr>
<td>Epi</td>
<td>1.36 ± 0.10 (N = 21)</td>
<td>1.62 ± 0.07 (N = 34)</td>
<td>&lt;0.001</td>
<td>1.61 ± 0.05 (N = 34)</td>
<td>NS</td>
</tr>
<tr>
<td>Endo</td>
<td>0.97 ± 0.04 (N = 21)</td>
<td>1.17 ± 0.04 (N = 34)</td>
<td>&lt;0.001</td>
<td>1.16 ± 0.03 (N = 34)</td>
<td>NS</td>
</tr>
<tr>
<td>Ischemic myocardium†</td>
<td>0.86 ± 0.16 (N = 6)</td>
<td>0.89 ± 0.08 (N = 11)</td>
<td>NS</td>
<td>0.94 ± 0.10 (N = 11)</td>
<td>NS</td>
</tr>
<tr>
<td>Epi</td>
<td>0.71 ± 0.14 (N = 6)</td>
<td>0.71 ± 0.11 (N = 11)</td>
<td>NS</td>
<td>0.76 ± 0.12 (N = 11)</td>
<td>NS</td>
</tr>
<tr>
<td>Endo</td>
<td>1.32 ± 0.12 (N = 6)</td>
<td>1.45 ± 0.16 (N = 11)</td>
<td>NS</td>
<td>1.50 ± 0.12 (N = 11)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (number of biopsies).

NS = P > 0.05.

*Myocardium distant from the occluded artery.
†Myocardium within the area of distribution of the occluded artery.

Abbreviations: MBF = myocardial blood flow; epi = epicardial; endo = endocardial.

Effects of CPIB Administration During Established Coronary Occlusion

The acute effects of CPIB when given during an established coronary occlusion and isoproterenol infusion are summarized in table 4. Five and ten minutes after giving CPIB (i.e., 15 and 20 minutes following coronary occlusion) values for ΣST and arterial FFA concentration were both significantly reduced relative to those recorded immediately before treatment, while AP and HR remained unchanged. In contrast, ΣST has previously been found to increase or remain unchanged between 10 and 20 min following coronary occlusion during isoproterenol infusion in the absence of CPIB administration (10 min: 61 ± 8 mV; 15 min 67 ± 8 mV: N = 12, P < 0.005, 10 min: 94 ± 19 mV: 20 min: 98 ± 16 mV: N = 5, P = NS). The reduction in arterial FFA concentration was associated with a proportionate decrease in the arterial-coronary sinus FFA concentration difference (table 5). No significant uptake of CPIB by the myocardium could be detected: mean arterial and coronary sinus CPIB concentrations were 225 ± 18 and 218 ± 21.
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TABLE 4. Acute Effects of CPIB on ST, AP, HR and the Arterial Concentrations of FFA and Free Glycerol when Administered during an Established Coronary Occlusion and Isoproterenol Infusion

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol infusion (0.2 – 0.3 μg/kg/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPIB (20 mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (number of dogs).
Statistical comparisons were performed by paired t-test analysis against those results (3rd column) obtained immediately before the administration of CPIB.

Discussion

The present observations demonstrate that pretreatment with CPIB substantially reduced the extent and magnitude of epicardial ST-segment elevation after experimental coronary artery occlusion in dogs, and reduces the augmentation of ST-segment elevation induced by isoproterenol infusion. The absolute reduction in ST elevation during isoproterenol infusion was greater than that recorded during occlusion in the absence of an isoproterenol infusion. This difference was observed both when the administration of CPIB preceded a subsequent re-occlusion of the artery, and when the drug was given during an occlusion established 10 minutes earlier.

Epicardial ST-segment elevation has been reported to reflect the severity of experimentally induced myocardial ischemia, and to correlate with both the local reduction in myocardial oxygen tension produced by both acute coronary occlusion and the subsequent depletion of myocardial creatine kinase activity during sustained occlusion. The latter relationship has been shown to persist under a variety of conditions, including antilipolytic therapy. Since irreversible myocardial injury does not occur during the first 20 minutes of ischemia, and reproducible results for ΣST were obtained in the absence of intervention, the reduction in ST-segment elevation achieved with CPIB probably indicates limitation of the acute myocardial ischemic injury.

The severity of acute myocardial ischemic injury has been shown to be influenced by factors which alter the oxygen requirements of the heart relative to oxygen supply. The major determinants of myocardial oxygen requirements include the mechanical factors of contractility, heart rate, and wall tension. However, the lack of any effect of CPIB on AP or HR in the present investigation renders it unlikely that there was reduction in ischemic injury due to altered mechanical activity.

An effect of CPIB on the coronary collateral circulation can be excluded from the results of the microsphere studies, which demonstrated that blood flow to both the ischemic and non-ischemic zones of the left ventricular wall was unaltered by treatment. Furthermore, the failure of CPIB to alter regional myocardial blood flow suggests that the reduction in ST-segment elevation was also unrelated to its known effects on platelet aggregation, blood clotting mechanisms, and blood viscosity.

CPIB reduced the arterial concentration of FFA by a mean of 40%, and this was associated with a proportionate decrease in the arterial-coronary sinus difference in FFA concentration. We have shown in other studies that CPIB also reduces the myocardial uptake of radioactive FFA from plasma during a continuous infusion of albumin-bound radiolabelled palmitate (Miller, N.E., Mjöls, O.D.: un-
published observations). The association of these changes with the failure of CPIB to alter myocardial blood flow indicates a reduction in the utilization of FFA by the heart as a consequence of the fall in arterial FFA concentration. Although proportionality between the arterial concentration and myocardial extraction of FFA has been well documented in other situations, it could not have been predicted that this would apply after CPIB administration in view of reports that CPIB may displace FFA to weaker binding sites on the albumin molecule. The reduction in arterial glycerol concentration suggests that the FFA-lowering effect of CPIB derived, at least in part, from an inhibition of lipolysis, in accordance with in vitro studies of adipose tissue metabolism.

In 1965 Challoner and Steinberg reported that high concentrations of FFA raised the oxygen requirements of the isolated perfused rat heart. This finding was extended by Mjös et al., who reported that raised arterial FFA concentrations increased MVO₂ in healthy dogs without influencing the mechanical activity of the heart. Furthermore, by inhibiting catecholamine-induced lipolysis with β-pyridyl-carbinol, it was shown that as much as 30% of the rise in MVO₂ induced by catecholamines was attributable to FFA, and was independent of their inotropic and chronotropic actions. β-pyridyl-carbinol was subsequently shown to reduce the severity of myocardial ischemic injury, as assessed by myocardial creatine kinase depletion, during experimental coronary occlusion in dogs, and it was suggested that this reflected a decrease in myocardial oxygen demand as a result of the fall in arterial FFA. The present demonstration that the reduction of myocardial FFA extraction by CPIB is associated with a decrease in epicardial ST-segment elevation in the absence of changes in hemodynamics or regional myocardial blood flow is consistent with this proposal.

In addition to their calorigenic activity, FFA have other metabolic actions which might also be expected to increase the severity of acute myocardial ischemic injury. Thus, the inhibition of glycolysis by FFA in the normally perfused heart raises the possibility that they may impair carbohydrate utilization during coronary occlusion. More recently it has been proposed that FFA may augment myocardial ischemic injury by increasing the intracellular concentration of long-chain acyl CoA esters, which have been shown in vitro to inhibit the translocation of adenine nucleotides across the mitochondrial membrane, and which are known to accumulate within the heart during experimental coronary occlusion.

Acute coronary occlusion has been shown to stimulate the release of catecholamine stores within the myocardium, and this would be expected to enhance the hydrolysis of intramyocardial triglyceride with a local release of FFA. Studies in this laboratory have indicated that CPIB inhibits catecholamine induced lipolysis in the isolated rat heart (de Deckere, E.A.M., Mjös, O.D., Miller, N.E.: unpublished observations). Thus, any limitation of myocardial ischemic injury achieved with CPIB could have been related not only to the fall in arterial FFA concentration but also to an inhibition of intramyocardial lipolysis within the ischemic zone.

Although it seems likely that the reduction in epicardial ST-segment elevation achieved with CPIB reflected a limitation of myocardial ischemic injury, there is also the possibility that it may have reflected, at least in part, a direct effect of FFA on cellular membrane potentials. An alteration in cellular action potentials by sodium palmitate has been noted in the isolated guinea pig heart, an observation which may be relevant to the possible arrhythmogenic activity of FFA. It was not established in these studies, however, whether the effect of FFA on action potentials was direct, i.e., at membrane level, or indirect and mediated through a disturbance of cellular metabolism.

The antilipolytic activity of CPIB in most human subjects appears to be substantially less than that in animals, and the results of the present study cannot be interpreted as indicating that CPIB might similarly reduce the consequences of acute myocardial ischemia in man. However, they strengthen the proposal that effective antilipolytic therapy during the early phase of AMI in man, when FFA concentrations may be increased by three-fold or more, might be of value in aiding the survival of the ischemic cells.

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