Failure of High Doses of Propranolol to Reduce Experimental Myocardial Ischemic Damage

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SUMMARY Myocardial creatine phosphokinase (CPK) activity and myocardial blood flow (MBF, 15 ± 5 μm/spheres) were measured at 24 hours after ligation of the left anterior descending coronary artery in nine untreated anesthetized dogs, in eight dogs pretreated with intravenous propranolol 5 mg/kg and in eight which had both pretreatment as well as infusion of propranolol (1.25 mg/kg/hour) after occlusion. Loss of CPK activity from the border and center zones of the myocardial infarct was similar in extent in dogs which had pretreatment but no infusion of propranolol as it was in the control group. Loss of CPK from the center zone was greater (P < 0.005) in dogs receiving pretreatment followed by constant infusion of the drug. Propranolol had no significant effect on collateral blood flow to the border or center zone of the infarct. In separate experiments, there was no important difference in hemodynamic measurements, except a slower heart rate (P < 0.01), when pretreated dogs were compared with control dogs up to 2 hours after coronary ligation. We conclude that propranolol given in this dose does not influence myocardial damage, on the basis of regional myocardial blood flow or tissue CPK depletion values at 24 hr after coronary occlusion.

REDUCTION OF MYOCARDIAL OXYGEN CONSUMPTION with beta-adrenergic blocking drugs is of proven clinical value for the treatment of angina pectoris and might also be beneficial in the treatment of the acute imbalance between oxygen supply and demand which occurs during the early stages of myocardial infarction. In spite of an initially encouraging report, however, oral administration of propranolol in early randomized trials was not found to reduce mortality from myocardial infarction in patients.

More recently, Maroko et al. suggested that propranolol could reduce the intensity of experimental myocardial ischemia, as judged by electrocardiographic as well as other criteria. The findings have been consistent with the results of histological studies of the posterior papillary muscle after circumflex coronary artery ligation in the dog and with the reports that propranolol used experimentally preserved high energy stores in infarcting myocardium and reduced gross anatomic infarct size. Moreover, a recent clinical study has shown that propranolol may improve myocardial oxygenation in patients with uncomplicated infarction.

The present study was designed to re-examine the effect of high doses of propranolol, using our previously described model for the measurement of myocardial blood flow, both at 15 min and 24 hours after coronary occlusion, and measurement of the intensity of creatine phosphokinase (CPK) depletion at 24 hours after onset of infarction. The effects of propranolol on these parameters were correlated with the hemodynamic alterations produced by the drug in relation to its plasma concentrations.

Methods

Forty-eight mongrel dogs, weighing 18–32 kg (mean 24 kg) were divided into two groups.

Group I (25 dogs), designated the survival group, was divided into three subgroups; coronary ligation without propranolol (N = 9); treatment with propranolol 5 mg/kg 10 min before coronary ligation (N = 8); and treatment with propranolol 5 mg/kg 10 min before coronary ligation followed by continuous infusion with propranolol 1.25 mg/kg/hr from 4 until 24 hr after coronary ligation (N = 8). These dogs survived for 24 hours after the onset of infarction, and myocardial CPK depletion was measured at this time.

Group II, designated the nonsurvival group, was composed of 14 control dogs and nine treated dogs surviving for two hours after coronary ligation. We studied the effects of propranolol (5 mg/kg given 10 min before coronary ligation) on hemodynamics, myocardial blood flow, and ST-segment elevation in these animals.

Group I

The animals were prepared after overnight fasting. Anesthesia was induced with thiopentone sodium (10 mg/kg) and maintained with sodium pentobarbital (2–3/mg/kg/hr) intravenously. Positive pressure respiration was established through a cuffed endotracheal tube with 20–30% oxygen and 40–60% nitrous oxide; initial adjustments were made to keep the arterial blood gases and body temperature within physiological limits.

Under sterile conditions, the femoral artery and vein were cannulated, the heart was exposed through a left thoracotomy, and a catheter was placed in the left atrium through a purse-string suture. Pressures in the left atrium and femoral artery were recorded with Statham P23Db transducers and a Sanborn 350 or a Gould Brush 480 recorder. Heparin (1500-3000 units) was given intravenously.

Epicardial ECGs were recorded with a steel ball electrode at 14 positions over the anterior surface of the left ventricle (LV) which were determined by their relationship to the...
epicardial blood vessels (fig. 1). Four of these positions were above the level of the ligature (normal zone), four were at the level of the ligature (border zone) and six were over the center of the infarct below the level of the ligature (center zone). ST-segment elevation was measured 0.06 sec after the end of the S wave, using the TP interval as the isoelectric line and was calculated at each site by averaging results from at least five complexes. Areas with QRS width greater than 0.06 sec were excluded from analysis. Myocardial blood flow (MBF) was measured according to our previously described method by injecting approximately 10⁶ microspheres 15 ± 5 μ in diameter, labeled either with ⁴¹Ce or ⁸⁵Sr. Blood propranolol levels were measured by a fluorometric method.

The left anterior descending coronary artery (LAD) was dissected about 2 cm from its origin, usually distal to its first diagonal branch. After recording the epicardial ST-segment map, propranolol 5 mg/kg was infused intravenously over 10 min. Ten minutes after the end of infusion the artery was occluded in two stages. Epicardial ST-segment mapping was repeated in all animals at 15 min after LAD occlusion, and myocardial blood flow was measured immediately afterward. The pericardium was then approximated and the thoracotomy was closed in layers over the re-expanded lungs. Constant sedation and analgesia were maintained with 4-6 hourly intramuscular injections of papaveretum (0.3-0.4 mg/kg). Venous blood for propranolol assay was taken in the treated animals at 10 min after giving the drug (immediately before first-stage coronary artery ligation), and at 0 and 15 min after completion of ligation. In the eight dogs that were infused with propranolol from 4 until 24 hr after ligation blood levels were again measured immediately before starting the infusion (4 hr after ligation) and at 10-12, 16-18 and 22-24 hr after ligation.

Twenty-four hours after coronary occlusion the dogs were reanesthetized and the heart exposed through the initial thoracotomy incision. The epicardial electrogram was obtained in identical positions as on the previous day, but was used only for the identification of pathological Q waves since fibrinous pericarditis may have obscured the ST-segment changes. After recording arterial and left atrial pressures, MBF was measured again using microspheres labeled with a different radionuclide.

Ventricular fibrillation was then induced and the heart removed. Seven full thickness pieces of myocardium, each approximately 2 cm × 1 cm × 1 cm and corresponding to two contiguous ECG recording sites, were taken from the anterior left ventricular surface (fig. 1). Two of these samples represented the normal zone of myocardium adjacent to the infarct, two pieces represented the border zone, and three the center of the infarct. The upper and lower edges of these pieces of myocardium corresponded with the diagonal arteries which were above and below the ligature (fig. 1); thus the normal zone was considered to end at the first diagonal artery, and the border zone was bounded by the first and second diagonals. One further sample was taken from the posterior LV wall, remote from the infarct. These eight pieces of myocardium were each divided into approximately equal halves for measurement of MBF and tissue CPK respectively. The mean weight of the pieces used for CPK analysis was 1.4 ± 0.4 (SD) g.

Tissue samples for MBF were divided into epicardial and endocardial halves (mean weight 1.0 ± 0.4 g), the radioactivity of the myocardial and reference blood samples being measured in a Packard Autogamma well-type scintillation counter using an integral counting technique. Myocardial CPK was estimated by the method of Shell et al. Whole-thickness pieces of LV wall were extracted in sucrose/EDTA medium, homogenised in an ultra-Turrax TP 18-10 (Janke and Kunkel KG), and diluted 1/100 into TRIS buffer containing 0.2% bovine serum albumin. CPK activity was assayed by the method of Rosalki at 340 nm using a Bausch and Lomb system 400. A commercial test kit (calbiochem) was used, and the reaction was carried out at 30°C. The protein content of the homogenate was estimated by the biuret method, and myocardial CPK level was expressed in I.U./mg of protein.

Group II

Methods were the same as for the survival experiments (group I) except that the pulmonary artery was cannulated for cardiac output measurements using 2.5 mg indocyanine green. Hemodynamic measurements were continued on this anesthetized open-chest preparation until 2 hr after completion of LAD ligation, after which ventricular fibrillation was induced.

Cardiac output, arterial, and left atrial pressures were recorded immediately before giving propranolol in the treated animals, immediately before two-stage ligation of the LAD in all animals (10 min after propranolol in the treated animals), and again in all animals at 15, 30, 60, 90

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**Figure 1.** Schematic illustration of the experimental preparation. The stippled area distal to the ligature (center of the diagram) represents the ischemic zone. Closed circles represent the ECG electrode positions. Inserts illustrate the method of tissue sampling for the measurement of MBF and CPK activity from the normal (above first diagonal artery), border (between first and second diagonals) and center (below second diagonal) zones. An additional tissue sample was also taken from the back of the LV, remote from the infarct.
and 120 min after completion of ligation. MBF was measured at 15 and 60 min after LAD ligation using approximately 10 µm microspheres 15 ± 5 µm in diameter as in the group I experiments. Immediately after completion of the measurements made at 2 hr after coronary occlusion, ventricular fibrillation was induced by an injection of 10–15 ml of concentrated potassium chloride, and the hearts were removed. Eight myocardial biopsies were taken as described in group I experiments, but were not further subdivided except into epicardial and endocardial halves for the measurement of MBF. Venous blood for propranolol assay was collected at 10 min after the end of the bolus and again at 15, 30, 60, 90 and 120 min after LAD occlusion.

Statistical analysis was performed using the Student’s t-test (for paired and unpaired data). All results are expressed as means ± SEM.

**Results**

**Group I Experiments**

Of 28 dogs who had LAD ligation after pretreatment with propranolol, seven (25%) succumbed to ventricular fibrillation (VF) within 30 min of coronary occlusion. One dog died of asystole at 15 min after occlusion, and four were found dead within the next 24 hr. Of ten dogs in the control group one died of early VF, but there were no late deaths. Dogs dying before 24 hr were excluded from the study, so that only results from the 25 surviving dogs were considered. Although there was a tendency for mortality rate to be higher in pretreated than in control animals, this was not statistically significant.

Up until closure of the thoracotomy at approximately 20 min after completion of LAD ligation, control dogs (subgroup A) and dogs pretreated with propranolol (subgroups B and C) showed similar changes in arterial pressure, left atrial pressure, heart rate, and ST-segment elevation as were observed in the group II experiments (described later). When these measurements were repeated at 24 hr (table 1), heart rate was significantly different from the control group only in subgroup C (P < 0.001), and mean arterial pressure (MAP) and left atrial pressure (LAP) were not significantly different comparing control with treated animals. The mean number of areas (out of a possible total of ten areas in the center and border zones, fig. 1) which developed pathological Q waves greater than 0.03 sec in duration was 7 ± 1 in control dogs, 7 ± 1 in dogs pretreated with propranolol, and 6 ± 1 in dogs receiving pretreatment and infusion with the drug.

**Table 1. Hemodynamic Measurements at 24 Hours after Coronary Ligation in Control Dogs, Dogs Pretreated with Propranolol, and Dogs Pretreated and Maintained on a Continuous Infusion of Propranolol (group I)**

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>MAP (mm Hg)</th>
<th>LAP (mm Hg)</th>
<th>MBF (ml/min/100G)</th>
<th>Endo/Epi ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Zone</td>
<td>Border Zone</td>
<td>Center Zone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (Control)</td>
<td>162 ± 7</td>
<td>79 ± 8</td>
<td>6 ± 1</td>
<td>92 ± 5</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>B (Pretreatment)</td>
<td>144 ± 8</td>
<td>96 ± 8</td>
<td>10 ± 2</td>
<td>112 ± 8</td>
<td>76 ± 10</td>
</tr>
<tr>
<td>C (Pretreatment + infusion)</td>
<td>128** ± 2</td>
<td>76 ± 9</td>
<td>6 ± 1</td>
<td>88 ± 8</td>
<td>58 ± 11</td>
</tr>
</tbody>
</table>

1P < 0.05, **P < 0.001, as compared with control group.
Abbreviations: HR = heart rate; MAP = mean arterial pressure; LAP = left atrial pressure; Endo/Epi ratio = endocardial/epicardial flow ratio.

Figures for myocardial blood flow as well as the endocardial/epicardial distribution of flow (endo/epi ratio) at 24 hr after coronary ligation are also shown in table 1. The results of flow measurements at 15 min after ligation were similar to those obtained in the group II experiments (see next section), and for the sake of clarity are not reported in detail. At 24 hr, the transmural MBF and endo/epi ratio did not differ significantly in subgroups B and C compared with the controls in the normal and border zones. In the center zone, however, the treated animals behaved differently; subgroup B showed a significant increase in transmural MBF com-

**FIGURE 2. Serum propranolol levels (top) in nonsurvival experiments (group II, N = 9) and (bottom) in survival experiments (group I, N = 16 up until 15 min; N = 8 from 4–24 hours). Time 0 refers to completion of LAD ligation.**
pared to controls \((P < 0.02)\), and this increase was not found in subgroup C (pretreatment and infusion). Endo-epi ratio was higher in both groups of treated animals, but the difference was significant only in subgroup C \((P < 0.05)\).

Plasma propranolol levels are shown in figure 2. Levels fell from a peak of \(1490 \pm 189\) ng/ml at 10 min after the bolus injection to \(475 \pm 52\) ng/ml at 15 min after coronary liglation; in the dogs which were infused with propranolol from 4-24 hr (subgroup C), the levels ranged from \(461 \pm 197\) ng/ml on commencement of the infusion at 4 hr after liglation to \(811 \pm 166\) ng/ml at 22-24 hr after liglation.

### Myocardial Tissue CPK at 24 hours

Figure 3 shows the myocardial CPK activity in the normal, border, and center zones of the infarct in the three subgroups. The two normal zone areas (fig. 1) had similar values to the control zone remote from the infarct; therefore these three areas were grouped as the normal zone. In the normal zone and the border zone the three subgroups had similar CPK activity, suggesting similarity in the quantity of viable tissue. In the center zone of the infarct, myocardial CPK was reduced in subgroup C \((P < 0.005)\): A = 13.4 \pm 1 I.U./mg protein; B = 13.8 \pm 1 I.U./mg protein; and C = 9.2 \pm 1 I.U./mg protein).

### Group II Experiments

#### Hemodynamic Changes

Hemodynamic changes observed in the control and pretreated groups of dogs are shown in figures 4, 5, and 6. Before LAD liglation and propranolol the observed measurements as well as the derived indices were similar in both groups. Following propranolol, significant reductions occurred in heart rate \((P < 0.02)\), mean arterial pressure \((P < 0.005)\), cardiac index \((P < 0.001)\), and left ventricular stroke work index \((P < 0.005)\), while left atrial pressure \((P < 0.005)\) and systemic resistance index rose \((P < 0.02)\). These levels of significance refer to measurements made in the same dogs before and after propranolol and before coronary liglation, but the levels were also significantly different comparing control with treated animals after liglation (shown in figures 4, 5, and 6).

Following liglation, the differences between the treated and control groups tended to become less with the passage of time; of the indices measured, only heart rate was significantly different in the treated animals for the full duration of observation (120 min). Mean arterial pressure and left atrial pressure did not differ between the groups at any time from 15 to 120 min; cardiac index was not significantly lower in the treated animals from 60 min onward.

#### Myocardial Blood Flow

Animals pretreated with propranolol had a significantly lower MBF than that of control animals in the normal zone of myocardium both at 15 min and 60 min after coronary liglation (fig. 7). This reduction occurred mainly in the epicardium, so that the endo-epi ratio was higher in the treated dogs. A trend toward lower transmural flow was also seen in the border zone of the treated animals, but this was not statistically significant. Propranolol did not appear to affect total MBF in the center zone, but in contrast to the
normal myocardium, the endo-epi ratio was lower (P < 0.01) in the center of the infarct of the treated dogs at 15 min after ligation.

**Epicardial ST-Segment Elevation**

Pretreated animals showed a marked reduction in ST-segment elevation compared with control animals in the center and border zones of the infarct (table 2). In the control animals, mean ST elevation at the center was in the range 5.8 to 6.7 mV, while in the treated animals it was 1.5 to 1.7 mV (P < 0.01).

**Propranolol Blood Levels**

Levels were similar to those found in the group I experiments, falling from a peak of 1475 ± 263 ng/ml at 10 min after the bolus injection to 350 ± 77 ng/ml at 120 min after LAD ligation (fig. 2A).

**Discussion**

Approaches to the measurement and control of myocardial infarct size in experimental animals have been given major priority in recent years. Of various agents which have been proposed for the limitation of infarct size, beta-adrenergic blockade with propranolol is perhaps the most promising. There is a well-described positive correlation between the level of circulating catecholamines and clinical severity of infarction, and these hormones have an adverse effect on infarcting myocardium. Reduction in oxygen demand and slowing of the heart rate should retard the progress and limit the extent of myocardial necrosis. These predictions have been substantiated by reports that propranolol reduces ST-segment elevation due to acute ischemia, both in the experimental animal and in man. Jennings and his colleagues have shown that propranolol reduces histologic necrosis in the posterior papillary muscle of dogs when given before either temporary or permanent occlusion of the circumflex coronary artery. The object of the present experiments was to reassess the effect of propranolol in our own experimental model in which we have previously demonstrated a close positive correlation between regional collateral MBF and regional myocardial CPK depletion in untreated dogs.

In the group I experiments in the present series, in which propranolol was given before coronary ligation but not infused afterward, no effect on tissue CPK activity at 24 hr after coronary ligation was seen. This result does not appear to be due to failure to maintain adequate beta-adrenergic blockade for the duration of coronary artery occlusion since no improvement was noted in the group I dogs in which the drug was infused for 24 hours. In fact, the center zone of these dogs showed significantly greater loss of CPK compared to the control animals, suggesting that prolonged infusion of high dose propranolol may have a deleterious effect. This is unlikely to be due to marked depression of cardiac...
function since the acute studies demonstrated that apart from a slower heart rate in the treated dogs, the hemodynamic measurements were essentially similar in both groups 2 hr after coronary artery occlusion. This observation that high blood levels of propranolol were well tolerated agrees with that of Reimer and colleagues.

Propranolol is known to reduce blood flow to healthy myocardium, but evidence on its effect on blood flow to ischemic myocardium has been conflicting. Becker and his colleagues using 15 μ radioactive microspheres and 10 g samples of heart muscle showed that propranolol increased the endocardial-epicardial (endo-epi) ratio of flow to ischemic myocardium although it did not change the ratio of flow of ischemic to normal myocardium. Kloner and colleagues using microspheres 8 μ in diameter, found that the endo-epi ratio of flow to the infarct was not altered by propranolol, and that the total collateral flow was diminished. Barcia and colleagues found instead an increased flow to the infarct in conscious dogs in which the blood pressure did not fall after propranolol.

The present results are also conflicting in showing a higher endo-epi ratio in the center zone of the infarct at 24 hr after ligation in the pretreated animals but a lower ratio in the pretreated animals at 15 min. A further unexpected result was that total flow to the center zone was significantly higher at 24 hr in dogs which were pretreated without infusion, but not in those which were pretreated and had a subsequent infusion of propranolol. The reason for this is not clear but should not be interpreted to mean that a beneficial effect occurred since the improvement in blood flow did not occur at 15 or 60 min after ligation, but at 24 hr in the animals which had both pretreatment and infusion.

The present results are disappointing in showing no salutary effect of propranolol on tissue CPK depletion, and they are apparently at variance with those of Reimer and colleagues who have found a reduction in histologic necrosis using the same pretreatment dose of the beta-blocking drug. Variations in experimental design may account for this difference. In most but not all of Reimer's experiments the circumflex coronary artery was only temporarily occluded and then reperfused, whereas in our experiments the anterior descending artery was permanently ligated. Another possible difference may relate to the fact that the posterior papillary muscle after circumflex coronary ligation is relatively well-preserved histologically despite a very low collateral blood flow. This might be explained by the theory that severe ischemia causes decremental conduction and the papillary muscle fails to contract; thus what little oxygen that is available can be used for the maintenance of cellular integrity. It is possible that beta blockade may have varying effects on the myocardium according to the degree of contraction which is occurring in the ischemic muscle.

Differences between our own results and those of other workers relating to the effect of propranolol on indices of severity of ischemia and necrosis but must also be explained, as must the absence of a beneficial effect of pretreatment on the incidence of ventricular fibrillation following acute coronary occlusion. This latter finding agrees with that of Reimer et al. but is at variance with those of earlier workers. The conflict may be accounted for by differences in experimental design; our method does not measure infarct size in the sense that total CPK depletion from the infarct is not measured, but merely CPK loss from selected parts of the infarct. Our method has the advantage that variations in severity of infarction due to differences in coronary arterial anatomy can be allowed for to some extent, and any inaccuracies in sampling technique should be similar in treated and control dogs. On the other hand the effect of propranolol on total CPK loss should probably also be investigated. It is possible that propranolol caused in-

![Graph](https://example.com/graph.png)

**Figure 7.** Myocardial blood flow and endocardial-epicardial flow ratio in control and pretreated dogs (group II experiments, see text). Total flow was significantly reduced by propranolol in the normal zone (P < 0.02 at 15 min and P < 0.05 at 60 min after coronary ligation.) Endo-epi flow ratio was increased by propranolol in the normal zone (P < 0.01 at 15 and 60 minutes), but was decreased in the center zone at 15 minutes (P < 0.01).

### Table 2. Mean ST-Segment Elevation in Control Dogs and Dogs Pretreated with Propranolol (Group II)

<table>
<thead>
<tr>
<th>Time after coronary ligation (min)</th>
<th>Control dogs</th>
<th>Mean ST-segment elevation (mV)</th>
<th>Treated dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Center Zone</td>
<td>Border Zone</td>
<td>Center Zone</td>
</tr>
<tr>
<td>15</td>
<td>5.8 ± 0.7</td>
<td>1.7 ± 0.5</td>
<td>1.5 ± 0.3*</td>
</tr>
<tr>
<td>60</td>
<td>6.8 ± 0.9</td>
<td>2.2 ± 0.5</td>
<td>1.7 ± 0.9*</td>
</tr>
<tr>
<td>120</td>
<td>6.7 ± 1.0</td>
<td>1.4 ± 0.4</td>
<td>1.6 ± 0.9*</td>
</tr>
</tbody>
</table>

*P < 0.01, compared to control.
increased washout of CPK from the ischemic tissue at least in the dogs which had pretreatment only, since center zone blood flow was increased at 24 hr. The explanation is unlikely for those having both pretreatment and infusion because central zone flow in these animals was unchanged compared with the controls. A further possibility is that the membrane effect of propranolol may have caused increased leakage of CPK independent of any effect on infarct size. Finally the large dose may have influenced our results in an unexpected way. The dose of propranolol which we used was very large by clinical standards, but this dose was used because it was most likely to reduce the severity of ischemic damage, and in our own and others’ experience, it does not appear to cause undue hemodynamic depression in anesthetized dogs.

The present results do emphasize that several indices of myocardial ischemia should be studied in different experimental models before the efficacy of any intervention can be assumed. Thus it can be said that propranolol reduces ST elevation, (also confirmed in the present study) and improves the histological appearance of the infarct, although it does not favorably affect myocardial blood flow (at least in the anesthetized animal) nor the intensity of CPK depletion. Possibly the use of more refined methods including a closer analysis of the border zone of jeopardized myocardium may throw further light on the effect of propranolol. In the meantime, the present conflict of evidence should not discourage the progress of clinical trials, since beta-adrenergic blockade has been shown to be safe and probably effective when used in uncomplicated infarction in man.

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