Effect of Inhibition of Lipolysis on Infarct Size After Experimental Coronary Artery Occlusion

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ABSTRACT Recent studies have demonstrated a depressant effect of increased delivery of FFA to the hypoxic heart. Because catecholamines are released in acute myocardial infarction, it is likely that lipolytic activity is increased. The purpose of this study was to determine whether inhibition of hormone-sensitive lipases influence the extent and severity of myocardial ischemic injury produced by coronary occlusion. Myocardial infarction was produced by occlusion of the left anterior descending coronary artery in open-chest dogs. 15 min later a surface map of S-T segments was obtained with the use of 10–14 epicardial leads in the distribution area of the occluded artery. Average S-T segment elevation of all sites was used as an index of myocardial ischemic injury. Before coronary occlusion, the average S-T segment elevation was 0.3±0.2, which increased to 4.1±0.7 mV (SEM, 12 dogs) after occlusion. Inhibition of lipolytic activity by β-pyridyl-carbinol before repeated coronary occlusion reduced the occlusion-induced S-T segment elevation to 2.1±0.6 mV ($P < 0.001$). When arterial concentrations of FFA were raised by i.v. infusion of a triglyceride emulsion and heparin, average S-T segment elevation after coronary occlusion increased from 1.2±0.7 to 2.2±0.8 mV ($P < 0.05$) in animals treated with β-pyridyl-carbinol, which suggests an unfavorable effect of circulating FFA in this setting. Isoproterenol given before a repeated occlusion increased the severity and extent of the ischemic injury. The effect of isoproterenol on the occlusion-induced S-T segment elevation was reduced, however, when the lipolytic effect of the drug was inhibited by β-pyridyl-carbinol.

Our study suggests that β-pyridyl-carbinol during acute coronary artery occlusion may be of importance in reducing the extent and severity of myocardial ischemic injury.

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INTRODUCTION

Increasing attention has been given to measures which might protect the heart during acute coronary occlusion and limit the size of the subsequent infarction. There is evidence to suggest that the ultimate extent of myocardial necrosis after acute coronary occlusion is determined by the balance between oxygen supply and demand in the areas of myocardium supplied by the vessel (1). Because myocardial ischemic injury is irreversible (2) long before the extent of collateral flow has increased significantly (3, 4), a reduction in the oxygen requirement of the infarcted area until establishment of collateral flow would be an attempt to limit the extent of myocardial injury. Ideally, the agent of choice should depress oxygen requirement exclusively in the ischemic area. Agents reducing myocardial oxygen consumption ($M\dot{V}O_2$),[1] e.g. β-adrenergic receptor blockade, reduce symptoms of myocardial ischemia and have been shown to reduce the size of an infarction in dogs (5); however, the effect of β-adrenergic receptor blockade is potentially disadvantageous in that it depresses myocardial contractility in general (6).

After acute coronary occlusion, myocardial concentrations of FFA are likely to be high during the infarction, because catecholamines are released in the acutely ischemic myocardium (7), thereby stimulating lipolytic activity (8). It has recently been demonstrated that increased delivery of FFA to the heart augments myocardial oxygen requirements, unrelated to changes in myocardial performance (9). Furthermore, as much as 30% of the rise in $M\dot{V}O_2$ induced by catecholamines was found to be attributable to metabolic stimulation by FFA released through the lipolytic effect of catecholamines, and was independent of the increase in mechani-

[1] *Abbreviations used in this paper:* $\overline{AP}$, mean arterial pressure; CPK, creatine phosphokinase; LAD, left anterior descending (artery); $M\dot{V}O_2$ myocardial oxygen consumption.

cal activity of the heart (10). Marked depression of myocardial contractility has been observed in oxygen-limited hearts receiving high concentrations of FFA (11, 12). This has been attributed to increased oxygen demand (12), suggesting that FFA in high concentrations contribute to the myocardial cell injury.

The purpose of the present investigation was to examine the possibility that inhibition of the catecholamine-induced lipolysis by β-pyridyl-carbinol (Ronicol, F. Hoffmann-La Roche & Co. A. G., Basel, Switzerland) would reduce the oxygen demand of the infarcted area and consequently diminish the magnitude and extent of the myocardial injury after experimental coronary occlusion. The infarct size was assessed quantitatively by examining the extent and magnitude of epicardial S-T segment elevation in areas surrounding the occluded artery. Previous studies have shown that this technique provides rapid and reproducible determinations of ischemic injury in the same animal (5).

The ultimate effect of β-pyridyl-carbinol on the infarction was studied by measuring the reduction in myocardial creatine phosphokinase (CPK) activity in myocardial specimens obtained from sites previously determined with epicardial S-T segment elevation. CPK has earlier been shown to be a quantitative index of the ultimate extent of cell death 24 h after a coronary occlusion (13).

METHODS

Animal preparation. Mongrel dogs weighing between 15 and 30 kg were anesthetized with Nembutal Sodium (Abbott Laboratories, North Chicago, Ill.), (25 mg/kg). Ventilation was maintained with a Cyclator Mk 2 respirator (The British Oxygen Company, Ltd., London, England). The heart was exposed through a left thoracotomy and suspended in a pericardial cradle. Care was taken not to obstruct the caval veins. A branch of the left anterior descending (LAD) coronary artery was dissected free and left in situ with a ligature (3-0) placed loosely around the vessel. A femoral artery and vein were cannulated to obtain aortic pressure and as a route for intravenous infusion. Aortic blood pressure was monitored with a Statham pressure transducer (P23 Gb, Statham Instruments, Oxnard, Calif.) and recorded on a Sanborn two-channel oscillographic recorder (Hewlett-Packard Co., Waltham Div., Waltham, Mass.). Rectal temperature was controlled with an electric heating pad placed beneath the animal, and was not allowed to change by more than 0.3°C.

Experimental procedure. An Elema Schønander (Stockholm, Sweden) electrocardiographic recorder was utilized for recording limb leads and epicardial electrocardiograms. Multiple epicardial electrocardiographic measurements were performed with a cotton wick electrode, as described previously (5). Electrocardiograms from 10 to 14 anatomically recognizable sites of the myocardium supplied by the dissected coronary artery, as well as surrounding left ventricular tissue, were monitored sequentially at a paper speed of 25 mm/s. The sensitivity of the epicardial recordings was set at 1 mV/mm deflection.

Since the appropriate control for these studies is the effect of the occlusion in the absence of the drug to be tested, control occlusion of the vessel was performed by a releasable Schwartz intracranial arterial clip. Repeat recordings from the same sites as those used before occlusion were obtained at 5-min intervals. After 20 min the clip was removed. After restoration of myocardial blood flow, recordings from epicardial leads rapidly reverted to a normal pattern.

Irreversible myocardial injury does not occur within the first 20 min of myocardial ischemia (14), and S-T segment elevation has previously been shown to be reproducible following reocclusion of the vessel after a recovery period of 30–45 min, thus permitting comparison between ischemic electrocardiographic changes during different interventions. All drug interventions were given before the experimental occlusions. When drug administration had been initiated, an equilibration period of 5 min was allowed before another epicardial map of S-T segments was obtained. The dissected coronary artery was then reoccluded and epicardial recordings were repeated at 5-min intervals for a total of 20 min, after which the occlusion was released.

In each animal the sum of S-T elevations from all recording sites at 15 min of coronary occlusion was used as an overall index of the severity of the ischemic injury in any given animal. Changes in the mean S-T elevation ($\overline{S\text{-}T}$) were analyzed by the paired t test technique using differences between paired data.

The animals were separated into four groups. In the first group (27 dogs) we studied the effects of acute interventions on infarct size following three procedures (*a*, *b*, and *c*):

(*a*) In 12 dogs three occlusions were carried out in each animal, 30 min being allowed for recovery between occlusions. β-pyridyl-carbinol, 0.1 mg/kg·min, was given i.v. 10 min before the second or third occlusion in alternate dogs. This allowed testing of possible differences in epicardial electrocardiograms because of reocclusion of the coronary artery.

(*b*) After control occlusion, i.v. infusion of isoproterenol, 0.2 μg/kg·min, was begun before reocclusion, and maintained during occlusion. After release and recovery of the occlusion, a similar infusion of isoproterenol was given after starting administration of β-pyridyl-carbinol. This regimen was maintained during the next experimental occlusion (five dogs).

(*c*) After control occlusion, plasma FFA were elevated by i.v. infusion of a triglyceride emulsion (Intralipid Vitrum,[2] Stockholm, Sweden 4 ml/min), starting 5 min before, and maintained during, reocclusion (four dogs). Sodium heparin, 3 mg/kg of body weight, was given i.v. as a bolus in order to secure a maximum lipolytic effect (15).

In six dogs, β-pyridyl-carbinol, 0.1 mg/kg·min, was given i.v. before occlusion and maintained during subsequent occlusions. After release and recovery, plasma FFA were elevated as described above. Intralipid was infused at a rate of 4 ml/min, and maintained during occlusion.

In the second group (seven dogs), a study was made of the effect of β-pyridyl-carbinol on the correlation between S-T segment elevation 15 min after occlusion and CPK depletion 24 h later. In each animal a control occlusion was carried out for 15 min, and a mapping of the epicardial electrocardiogram was recorded, as previously described. After release, and return of the electrocardiogram to normal values, drug administration was initiated, and another epicardial map

[2] 100 ml contains: fractionated soya bean oil, 10 g; fractionated egg lecithin, 1.2 g; glycerol, 2.5 g; water added up to 100 ml. Main fatty acid components are linoleate (40%), oleate (24%), palmitate (10%), and linolenate (7%) (12).

of S-T segments was obtained. The dissected coronary artery was then ligated, and epicardial recordings were repeated as before. The thorax was closed, and drug administration was maintained for 24 h by intramuscular injections (1 mg/kg) every 4 h. Three of the animals served as controls and received no drug. The animal was then killed, and the heart quickly removed. Full-wall thickness biopsies were taken for CPK determinations, as previously described (5), from the same sites as those from which epicardial recordings had been taken earlier.

In the third group (six dogs), the effect of β-pyridyl-carbinol on coronary resistance was studied. The experiments were performed in thoracotomized dogs with the left circumflex and the LAD artery perfused separately through a shunt from the left carotid artery, as previously described (12). Coronary flow rates were determined with an electromagnetic square-wave flowmeter on the shunt (Nycotron, Oslo, Norway). The effect of acute occlusion of the LAD artery on coronary flow was studied before and during i.v. administration of β-pyridyl-carbinol (0.1 mg/kg·min). In two of the dogs, an alternative protocol was used: a branch of the LAD coronary artery was ligated and sectioned distal to the ligature. Timed collection of retrograde flow from the distal end of the severed artery was obtained every 30 s, starting 5 min before and continuing for 15 min during the infusion of β-pyridyl-carbinol (0.1 mg/kg·min, i.v.).

Chemical procedures. All chemical were of the highest grade commercially available, and were formulated in glass-distilled water. FFA were determined in arterial plasma, according to the method of Dole (16), as modified by Trout, Estes, and Friedberg (17).

Determinations of myocardial CPK activity were performed as previously described (13). The ventricular myocardium was homogenized, and CPK activity assayed in the 16,000 *g* supernatant fraction spectrophotometrically, according to the method described by Rosalki (18). CPK activity was expressed in IU (μmoles of substrate converted per minute) per milligram of supernatant fraction protein. Reaction rates were linear for at least 15 min after an equilibration period of 5 min, and results of duplicate determinations of enzyme activity agreed to within 5%.

RESULTS

Heart rate did not change significantly after ligation of a branch of the LAD coronary artery. Mean arterial pressure ($\overline{AP}$) remained constant or decreased by less than 5 mm Hg (Table I). Occlusion of the coronary artery was followed by striking changes in epicardial S-T segments. In the center of the infarction, S-T segments were elevated from 2 to 17 mV 15 min after coronary artery occlusion. Near the border of the infarcted area, determined grossly by cyanosis and bulging during systole, S-T segment elevation was less than in the center of the infarcted area, where S-T elevation was maximal.

In 14 dogs, the average $\overline{S\text{-}T}$ segment elevation increased from 0.3±0.2 mV (SEM) before occlusion to 4.1±0.7 mV 15 min after coronary occlusion. In myocardium clearly not supplied by the ligated coronary artery, S-T segments remained unchanged during occlusion.

Effects of β-pyridyl-carbinol on S-T elevation after coronary occlusion. Administration of β-pyridyl-carbinol in doses sufficient to inhibit catecholamine-induced lipolysis was entirely without effect on blood pressure and heart rate. However, occlusion of the coronary artery re-

TABLE I

Effect of Coronary Occlusion on Average S-T Segment Elevation ($\overline{S\text{-}T}$), Mean Aortic Pressure ($\overline{AP}$) and Heart Rate (HR) before and during β-Pyridyl-Carbinol Infusion (12 Dogs)

	$\overline{S\text{-}T}$			$\overline{AP}$			HR		
Dog no.	C	I	R	C	I	R	C	I	R
		mV			*mm Hg*			*min⁻¹*	
1	0	2.2	0.6	110	105	110	140	140	140
2	1.6	9.0	4.8	125	125	125	155	160	160
3	1.0	4.0	1.0	110	110	110	160	165	160
4	0	1.5	0	110	100	100	180	180	180
5	0	2.2	0.6	110	110	90	120	120	120
6	0	1.8	0	130	125	115	125	125	140
7	0	6.6	2.2	150	155	155	180	180	190
8	0	7.6	6.6	85	85	85	120	120	120
9	0	7.9	6.0	85	85	78	130	130	130
10	0	5.4	1.3	135	135	135	210	210	205
11	1.0	4.2	3.6	120	120	120	175	175	170
12	0	2.9	0	105	105	130	152	156	158
Mean	0.3	4.6	2.4	115	113	113	151	155	156
±SEM	0.16	0.8	0.8	5.5	5.8	6.4	9.0	9.0	8.0
P		<0.001	<0.001		NS	NS		NS	NS

C, before occlusion; I, control occlusion; R, occlusion during β-pyridyl-carbinol infusion. Probability values for differences between paired data were obtained with Student's *t* test.

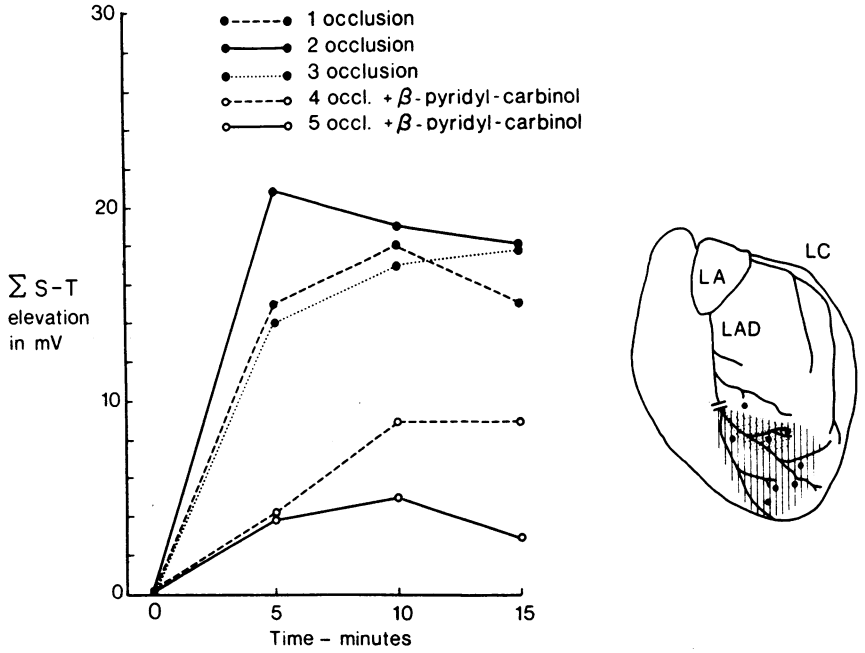

FIGURE 1 Effects of coronary occlusion alone and occlusion during infusion of β-pyridyl-carbinol (0.1 mg/kg·min). Right panel: lined area, area of injury after 15-min occlusion; stippled area, area showing S-T elevation under the influence of β-pyridyl-carbinol. LAD, left anterior descending coronary artery; LA, left atrial appendage; LC, left circumflex artery. Left panel: S-T in the same experiment after three simple occlusions and after two occlusions under the influence of β-pyridyl-carbinol. Time is in minutes after occlusion.

sulted in a much smaller S-T segment elevation than in preceding control occlusions. A representative experiment is presented in Fig. 1. In several sites with reproducible S-T segment elevation after three control occlusions at 30-min intervals, a marked reduction occurred in S-T segment elevation during drug infusion. At some sites S-T segment changes disappeared completely. In all 14 dogs the average S-T segment elevation 15 min after occlusion was 2.1±0.6 mV, and the number of sites of ischemic injury was 3.5±1.0. Both values were significantly smaller than those observed in control experiments before β-pyridyl-carbinol administration: 4.1±0.7 mV ($P < 0.001$), and 7.3±0.8 sites ($P < 0.02$), respectively.

The difference in S-T segment elevation could not be ascribed to temporal changes induced by simple reocclusion, since the differences between S-T segment elevations caused by sequential LAD artery occlusions before (six dogs) and during (eight dogs) continuous infusion of β-pyridyl-carbinol were not statistically significant ($P > 0.05$) (Fig. 2).

In two dogs, nonischemic changes in epicardial S-T segments produced by injections of KCl (6 meq/liter saline) directly into a coronary artery were not influenced by the doses of β-pyridyl-carbinol in the present experiments.

Influence of isoproterenol and β-pyridyl-carbinol on S-T segment elevation. Infusion of isoproterenol, 0.10–0.25 μg/kg·min, increased heart rate from 166±5 to 209±10, and reduced $\overline{AP}$ from 104±5 to 91±5. Coronary occlusion or infusion of β-pyridyl-carbinol did not cause further changes in either blood pressure or heart rate (Table II).

Fig. 3 illustrates results of occlusions of a branch of the LAD coronary artery. Isoproterenol increased the number of sites of myocardial ischemia and increased S-T segment elevation at any particular affected site. β-pyridyl-carbinol reduced the extent and magnitude of S-T segment elevation, when compared with control occlusion. When isoproterenol infusion was repeated during β-pyridyl-carbinol administration, the subsequent increase in S-T segment elevation was clearly smaller than that obtained with isoproterenol alone in all sites examined.

Infusion of isoproterenol increased occlusion-induced S-T segment elevation from an average of 1.7±0.6 mV to 6.3±0.6 mV ($P < 0.005$), and increased the number of sites with ischemic injury from 4.2±1.3 to 7.8±0.6 ($P < 0.05$). When β-pyridyl-carbinol infusion was started before isoproterenol infusion and a third coronary occlusion, S-T segment elevation and number of sites with ischemic injury associated with administration of

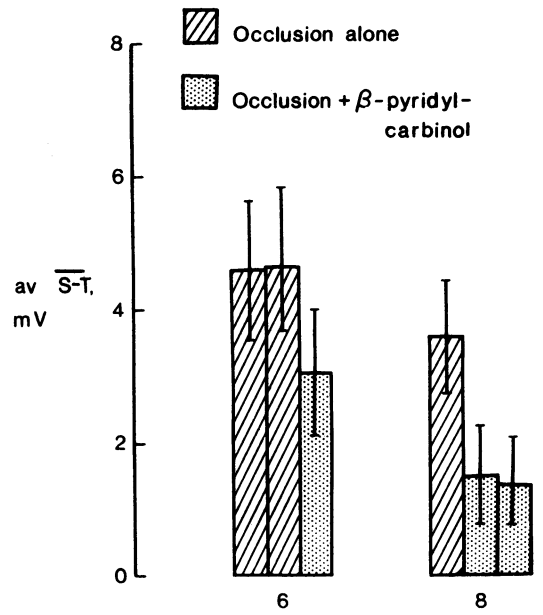

FIGURE 2 Effects of repeated occlusions on average S-T elevation ($\overline{S\text{-}T}$) during occlusion alone and under the influence of β-pyridyl-carbinol. Figures below columns indicate number of animals.

isoproterenol were markedly reduced, averaging 2.8±0.6 mV ($P < 0.02$) and 4.7±0.7 sites ($P < 0.05$), respectively. As shown in Table II, isoproterenol increased FFA concentration in plasma from 450±95 μeq/liter to 1400±160 μeq/liter, but when administered after β-pyridyl-carbinol effected no change in plasma concentration of FFA (340±49 μeq/liter), indicating abolished effect of catecholamine-induced lipolysis.

Effects of Intralipid-heparin on S-T segment elevation. Coronary occlusions were followed by essentially similar ischemic changes in the epicardial electrocardiograms before and during increased arterial concentrations of FFA induced by infusion of Intralipid-heparin (i.v.), S-T segment elevation averaging 4.5±0.7 and 4.3±0.6 mV, respectively ($n = 4$) ($P > 0.05$). It should be noted, however, that the number of sites with S-T segment elevation of 2 mV or more increased from 5.8±1.2 to 7.8±0.8 ($P < 0.05$), which suggests that areas in the periphery of the infarction were adversely affected by high levels of FFA, while more central areas were not. When arterial concentrations of FFA were similarly increased (from 118±11 to 3126±723 μeq/liter) in animals treated with β-pyridyl-carbinol, S-T segment elevation increased from 1.2±0.7 to 2.2±0.8 mV ($P < 0.02$), and the number of sites with ischemic S-T segment elevation increased from 2.0±1.1 to 4.8±1.3 ($P < 0.05$).

Influence of β-pyridyl-carbinol on epicardial S-T segment elevation and myocardial CPK depletion. 24 h after coronary occlusion, in nonischemic tissue from three animals treated with β-pyridyl-carbinol, CPK activity averaged 22.8±0.39 ($n = 15$) IU/mg protein, compared to 21.5±0.62 IU/mg ($n = 15$) in nonischemic tissue from five dogs not receiving the drug ($P > 0.1$). Thus, administration of β-pyridyl-carbinol alone did not lead to depression of myocardial CPK activity.

Administration of β-pyridyl-carbinol reduced the extent and magnitude of S-T segment elevation after coronary artery occlusion, compared with that resulting from coronary artery occlusion alone, and led to a proportionately smaller depression of myocardial CPK activity. With β-pyridyl-carbinol, the extent of depression of myocardial CPK activity was significantly less than anticipated from the magnitude of S-T segment elevation produced by coronary artery occlusion alone (Fig. 4). The regression line relating CPK depression to S-T segment elevation in control animals differed significantly ($P < 0.001$) from that relating CPK depression after β-pyridyl-carbinol to S-T segment elevation before administration of the drug. Thus, after administration of β-pyridyl-carbinol, tissue damage was less compared to what would have been expected from the S-T segment elevation after the control occlusion.

Addition of β-pyridyl-carbinol (0.5 mg/ml homoge-

TABLE II

Effect of Repeated Occlusions under the Influence of Isoproterenol (ISO) on the Average S-T Segment Elevation ($\overline{S\text{-}T}$), Mean Aortic Pressure ($\overline{AP}$), Heart Rate (HR), and Arterial FFA Concentrations (FFA_a) before and during β-Pyridyl-Carbinol Infusions (six Dogs)

	$\overline{S\text{-}T}$			$\overline{AP}$			HR			FFA_a		
	I	ISO	ISO + R	I	ISO	ISO + R	I	ISO	ISO + R	I	ISO	ISO + R
	mV			*mm Hg*			*min*$^{-1}$			*μeq/liter*		
Mean	1.7	6.3	2.8	104	91	88	166	209	216	450	1400	340
±SEM	0.6	0.6	0.6	4.6	5.4	5.3	5.1	10.4	8.8	95	160	49
P	<0.005		<0.02	<0.01		NS	<0.005		NS	<0.001		<0.001

I, control occlusion; ISO, occlusion during isoproterenol infusion; ISO + R, occlusion during infusion of isoproterenol and β-pyridyl-carbinol. Probability values for differences between paired data were obtained with Student's *t* test.

TABLE III

Effect of β-Pyridyl-Carbinol I.V. (0.1 mg/kg·min) (R) on Cardiac Hemodynamics before (C) and during (I) Occlusion of LAD Artery (four Dogs)

	AP				HR				CF			
	C	C + R	I	I + R	C	C + R	I	I + R	C	C + R	I	I + R
	mm Hg				*min⁻¹*				*ml/min*			
Mean	98	96	95	92	148	148	149	149	60	60	43	40
±SEM	9.7	10.7	10.6	13.2	8	8	8	8	6.9	7.7	2.8	4.8
P	NS		NS		NS		NS		NS		NS	

$\overline{AP}$, mean aortic pressure; HR, heart rate; CF, coronary flow. Probability values for differences between paired data were obtained with Student's *t* test.

nate) or nicotinic acid (0.5 mg/ml homogenate) to homogenates of normal and ischemic hearts did not influence CPK activity.

Effects of β-pyridyl-carbinol on coronary flow. Coronary flow was unchanged in four dogs receiving β-pyridyl-carbinol at infusion rates of 0.1 mg/kg·min (Table III). When coronary flow was acutely reduced by occlusion of the LAD artery, it remained constant for more than 20 min. Furthermore, subsequent occlusion after return of coronary flow to control level and after start of infusion of β-pyridyl-carbinol demonstrated identical reduction of coronary flow to the left ventricle. Aortic pressure and heart rate were similar at both occlusions.

Retrograde flow from the severed end of the artery serving the infarcted area was not influenced by β-pyridyl-carbinol. In two dogs, retrograde flow averaged 7.8 and 16.5 ml/min before and 7.2 and 16.4 ml/min during drug administration.

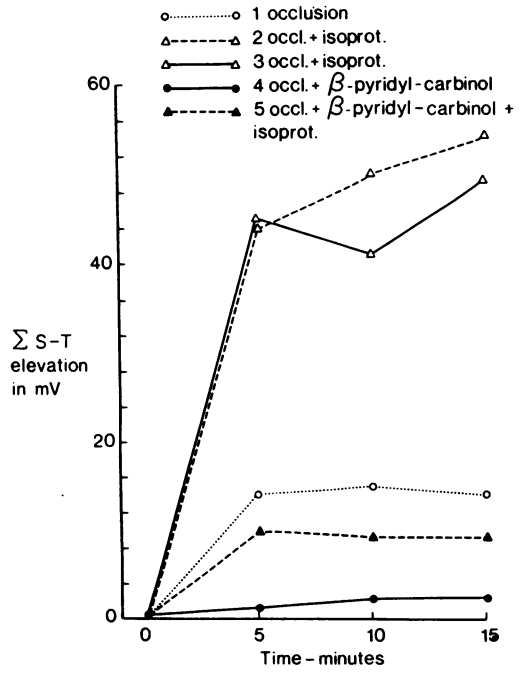

FIGURE 3 Effects of isoproterenol (0.2 μg/kg·min) before and during infusion of β-pyridyl-carbinol. ΣST = sum of S-T elevations.

DISCUSSION

Results obtained in this investigation demonstrate that the extent and magnitude of early elevation of S-T seg-

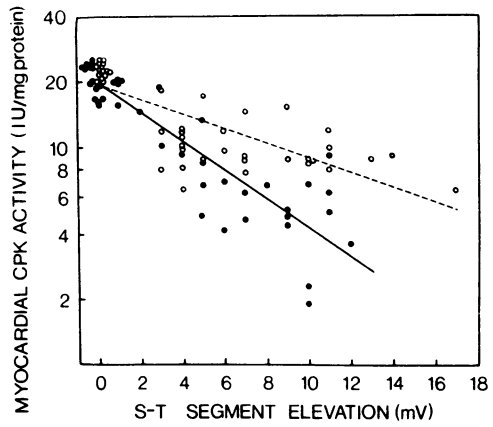

FIGURE 4 Depression of myocardial CPK activity after administration of β-pyridyl-carbinol in animals with coronary artery occlusions. Epicardial recordings were obtained 15 min after coronary artery occlusion from anatomically identifiable sites. The artery was then released, and permanently ligated. CPK activity, expressed on a logarithmic scale, was measured in homogenates from full-wall specimens obtained from the same sites 24 h later. Data from multiple samples from three animals receiving β-pyridyl-carbinol before ligation and maintained for 24 h (open circles) and from five untreated animals (control) (closed circles). Solid line: regression line for control study (log CPK = 1.300 − 0.067 S-T; $r = 0.89$). Dotted line: regression line relating S-T segment elevation after coronary artery occlusion before administration of β-pyridyl-carbinol and log CPK from corresponding sites 24 h later (log CPK = 1.278 − 0.033 S-T; $r = 0.78$). Animals receiving β-pyridyl-carbinol showed less depression of myocardial CPK activity than would have been expected from S-T segment elevation occurring before drug administration ($P < 0.001$).

ments in epicardial recordings after acute coronary artery occlusion are reduced when the experimental animals are pretreated with β-pyridyl-carbinol.

Although S-T segment changes have been widely used in diagnosing myocardial infarction, their use as a quantitative index for assessment of infarct size needs some qualification. As shown in previous studies, epicardial S-T segment elevation reflects ischemia of the myocardial cells (19, 20) and correlates with a diminution of myocardial oxygen tension and an asynergistic myocardial contraction in experimental animals subjected to coronary artery occlusion (21). Prinzmetal and associates (22) found close correspondence during hypoxia between elevation of S-T segments in epicardial electrocardiograms and changes in myocardial membrane potentials which occurred in the underlying myocardium. By using depression of myocardial CPK activity as an index of necrosis after coronary artery ligation, it was recently demonstrated that a good correlation existed between acute epicardial S-T segment elevation and subsequent development of necrosis (5). Accordingly, although the electrical changes are reversible for several hours if coronary flow is restored, they nevertheless effectively presage the extent of tissue damage after a given and sustained occlusion. Furthermore, since the present and previous studies (5) have shown that the extent and magnitude of epicardial S-T segment elevations are reproducible in repeated occlusions, alterations in S-T segment produced by pharmacologic interventions can be used as indices of eventual augmented or diminished cell damage related to the interventions.

The present data showing that β-pyridyl-carbinol reduced the extent and magnitude of epicardial S-T segment elevation therefore strongly suggest that this agent may be useful in reducing the ultimate extent of myocardial necrosis. Although nonischemic alterations of S-T segment elevation are known to occur (23), it is unlikely that S-T segments were spuriously affected, since alterations in S-T segments were always confined to the grossly identifiable infarction and were never observed in obviously nonischemic areas. Furthermore, nonischemic elevation of S-T segments produced by coronary injection of KCl was not inhibited by β-pyridyl-carbinol.

Administration of β-pyridyl-carbinol significantly reduced the extent to which myocardial CPK activity was depressed with respect to S-T segment elevation observed after occlusion of the coronary artery before administration of the drug. Depletion of CPK activity correlates with the amount of necrosis in rabbits subjected to coronary artery occlusion (13), and with local myocardial blood flow 24 h after coronary occlusion and consequently myocardial ischemia (24). Accordingly, infusion of β-pyridyl-carbinol resulted in less cellular necrosis than anticipated from simple occlusion of the coronary artery, thus corroborating findings on alterations in early S-T segments obtained with this drug.

β-pyridyl-carbinol is readily converted to nicotinic acid in the liver (25), and in both drugs the pharmacologic properties in the intact organism are essentially similar (26). β-pyridyl-carbinol therefore acts as a vasodilator on terminal vessels and is an inhibitor catecholamine-induced lipolysis. Although the doses used in the present experiments had no discernible effect on blood pressure, and coronary resistance was unchanged by the drug before and after coronary occlusion, there remains the possibility of intramyocardial redistribution of flow. However, β-pyridyl-carbinol did not improve retrograde flow from the infarcted area. Even though a beneficial effect on infarct size through an improvement in coronary collateral circulation is therefore unlikely, this possibility should not be completely excluded.

Various interventions increasing $M\dot{V}O_2$ have previously been shown to be accompanied by corresponding changes in the magnitude and extent of the infarct size after coronary occlusion (5). Conversely, interventions reducing $M\dot{V}O_2$ decrease the ultimate extent of cell injury after a similar occlusion. Doses of isoproterenol of the same order of magnitude as that used therapeutically in patients with myocardial infarction did not influence epicardial electrocardiogram or CPK activity in nonischemic myocardium; doses in this range, however, clearly increased the extent and magnitude of S-T segment elevation and augmented the area of depressed myocardial CPK activity after coronary artery occlusion (5). Other studies have shown that β-receptor blockade effectively reduced the extent and magnitude of experimental infarction (5), probably through a reduction in myocardial oxygen demand. The effect of β-pyridyl-carbinol might be secondary, involving a similar mechanism, since preliminary investigations indicate that the effect of β-pyridyl-carbinol on infarct size is dependent on the presence of intact catecholamine stores. There is no evidence, however, that β-pyridyl-carbinol or nicotinic acid exert β-receptor-blocking effects on the heart or reduce $M\dot{V}O_2$ in the normally perfused heart (10). However, when $M\dot{V}O_2$ is increased by catecholamines, administration of β-pyridyl-carbinol abolishes, on average, 30% of the increase, in spite of unchanged myocardial mechanical activity (10). This effect has been attributed to an inhibition of the catecholamine-sensitive lipolytic activity, thus counteracting release of FFA from triglyceride stores. Recent studies in dogs by Mjøs (9) have shown that elevated concentrations of plasma FFA, probably accompanied by increased tissue concentrations of FFA, effected a marked increase in myocardial oxygen requirement, thus confirming earlier studies in the isolated rat heart (27). The rise in $M\dot{V}O_2$ was not asso-

ciated with any change in the mechanical activity of the heart, as long as the oxygen supply was unrestricted. When coronary flow was fixed at an ischemic level, however, with restricted oxygen supply, a depression of myocardial function was observed when essentially similar high concentrations of FFA were presented to the heart (12). This might suggest that high concentrations of FFA increase the oxygen requirement in the hypoxic heart, in competition with requirements for mechanical activity. Catecholamines markedly enhance myocardial lipolysis (8). Accordingly, it was found that the extent and magnitude of S-T segment elevation obtained with a given infusion rate of isoproterenol during acute coronary occlusion was reduced after inhibition of lipolysis with β-pyridyl-carbinol. Aortic pressure and heart rate were similarly affected by isoproterenol before and after administration of β-pyridyl-carbinol and consequently not responsible for the difference. If we assume that a smaller increase in oxygen requirement is obtained due to inhibition of lipolysis, it is conceivable that the magnitude of the ischemic injury after coronary occlusion becomes smaller than with isoproterenol alone.

Because, in the acutely infarcted area, catecholamines are released from endogenous stores at a rate sufficient to activate myocardial phosphorylase and promote glycogenolysis (7), one might anticipate that lipolytic activity is similarly activated (8). Evans (28) found a marked increase in FFA in the isolated heart subjected to anoxia, and recent reports indicate an increased ratio of FFA to triglycerides in myocardial infarction (29). Direct proof that β-pyridyl-carbinol inhibits lipolytic activity in the myocardium has not yet been obtained, but is the subject of continuing investigation. The data suggest that the mechanism of action of β-pyridyl-carbinol is to inhibit release of FFA in the acutely ischemic area, thus precluding an increase in local oxygen requirement.

The finding in this and in previous experiments (30), that elevation of arterial concentrations of FFA by Intralipid-heparin infusion during acute coronary occlusion does not increase the severity of the infarction, is not contrary to the hypothesis proposed. Assuming increased rate of triglyceride hydrolysis in the acutely ischemic myocardium, it is conceivable that raising the level of arterial FFA will hardly increase myocardial FFA concentrations more than can be produced by endogenous triglyceride stores. This suggestion is supported by the present findings in dogs in which lipolysis has been inhibited by β-pyridyl-carbinol. In these animals the extent and magnitude of epicardial S-T segment elevation distinctly increased when the coronary artery occlusions were repeated during raised arterial concentrations of FFA. The present data suggest that β-pyridyl-carbinol may be useful by reducing the ultimate extent of myocardial necrosis, thereby offering a therapeutic approach in the prevention of "pump failure" after myocardial infarction. From a clinical point of view, it is particularly interesting that the improvement in myocardial survival is obtained without a depression of myocardial performance in general, as is the case with β-receptor blocking agents.

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