1985

The Relative Effects of Acute and Chronic Beta-Blockage on Infarct Size and Hemodynamics After Coronary Occlusion in Canine Hearts

Patrick Hughes
Loyola University Chicago

THE RELATIVE EFFECTS OF ACUTE AND CHRONIC BETA-BLOCKADE ON INFARCT SIZE AND HEMODYNAMICS AFTER CORONARY OCCLUSION IN CANINE HEARTS

by

Patrick J. Hughes

A Thesis Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of

Master of Science

October

1985

ACKNOWLEDGEMENTS

My sincere gratitude is expressed to Dr. David Euler for his enthusiastic support, guidance and most of all for his influence on my training.

I wish to extend my thanks to the other members of my thesis committee, Dr. Walter C. Randall, Dr. Patrick J. Scanlon and Dr. John X. Thomas, Jr. for their advice, consultation and support.

May I also express my appreciation to Dr. John F. Moran for his infectious enthusiasm and to Miss Stephanie Moran for her assistance in the preparation of this manuscript.

Finally, I wish to acknowledge my wife for her patient understanding and many sacrifices which have made this work possible.

VITA

The author, Patrick Joseph Hughes, is the son of Joseph Francis Hughes and Winifred Margaret (Kilduff) Hughes. He was born on February 25, 1955 in New York city.

His elementary education was obtained at Holy Family Grade School and his secondary education at Holy Trinity Diocesan High School, both in Hicksville, New York.

In the fall of 1973 he entered Loyola University of Chicago and in June, 1977 he received the degree of Bachelor of Science with a major in biology.

In July 1977 he entered the Loyola University Stritch School of Medicine and received the Doctor of Medicine degree on June 7, 1980. While attending medical school he was elected a member of Alpha Omega Alpha. On June 8, 1980, he married Jana Hoendervoogt and is now the father of three children, Joseph, Julie and Sarah.

In the summer of 1980 he began his residency in Internal Medicine at the Medical College of Wisconsin in Milwaukee. He completed his residency in June, 1983 and became certified by the American Board of Internal Medicine in September, 1984.

In July, 1983 he began graduate training in the Department of Physiology of Loyola University, under the supervision of Dr. David Euler and Dr. Walter Randall.

In the summer of 1984 he entered the Cardiology Fellowship Program at Loyola University Medical Center.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

With the advent of widespread use of coronary care units in the late 1960's, deaths due to arhythmias in patients with evolving myocardial infarctions were substantially reduced. Left ventricular dysfunction, as manifest by pulmonary edema and cardiogenic shock, became recognized as the chief cause of in-hospital mortality. Autopsy studies revealed that cardiogenic shock occurred when the cumulative amount of myocardial necrosis from old and new infarctions exceeded 40% of the left ventricle (1,2). More recently, residual ventricular function has been shown to be a major predictor of long term survival after myocardial infarction (3). Recognition of these facts has led to a large number of experimental and clinical studies in which pharmacologic, mechanical or surgical interventions have been employed to reduce the amount of myocardial necrosis resulting from coronary occlusion.

Stimulation of myocardial beta-1 receptors by catecholamines increases heart rate and contractile function and thereby oxygen consumption. Some studies have proposed that catecholamines augment oxygen consumption more than is necessary for the increased external work performed, and thus have an additional direct oxygen wasting effect (4). Enhanced cardiac sympathetic nerve activity (5) and marked elevation of circulating catecholamine levels have been demonstrated within minutes of coronary occlusion (6). Beta-blockers were thus an obvious first choice in early experimental studies (7) and more recent clinical trials

(8) designed to limit infarct size after coronary occlusion.

In current clinical practice, patients with coronary artery disease or hypertension, who are at higher than average risk for myocardial infarction, receive beta-blockers for periods of years. There is a wealth of experimental information about the effects of acute intravenously administered beta-blockade on infarct size, hemodynamics and arhythmias resulting from coronary occlusion. In contrast, very little is known about the effects of chronic beta-blockade prior to coronary occlusion on these parameters. Investigations performed in animals which have received chronic beta-blocker therapy (9) or chronic cardiac denervation (10) suggest that long term interruption of beta-adrenergic influences may confer more protection to ischemic hearts than may be achieved with short term adrenergic interruption. This study was designed to compare the effects of acute vs. chronic beta-blockade on myocardial infarct size and hemodynamics in response to coronary occlusion.

LITERATURE REVIEW

Modification of Infarct Size - A Historical Perspective

Experimental studies attempting to measure and modify infarct size have substantially improved our understanding of the basic pathophysiology of myocardial infarction. Many different models of myocardial infarction and methods of measuring infarct size have been employed and later abandoned as knowledge has grown. A few studies are particularly noteworthy because they have demonstrated important new concepts which serve as the foundation for present experimental and clinical investigations.

In 1971 Maroko et al. (7) ligated a branch of the left anterior descending coronary artery and measured the magnitude of ST-segment elevation and CPK depletion at sites within and remote from the area of ischemic injury in anesthetized dogs. They demonstrated; (1) that the magnitude of epicardial ST-elevation 15 minutes after coronary occlusion predicted the magnitude of cell death (as measured by CPK depletion) 24 hours later, (2) that interventions which enhanced myocardial contractility or heart rate (isoproterenol, oubain, glucagon, bretylium, pacing) or depressed coronary perfusion pressure (hemmorhage) substantially increased EKG and enzymatic indices of myocardial injury, (3) that propranolol, which decreases myocardial oxygen consumption by depressing heart rate and contractility, significantly decreased myocardial injury resulting from coronary occlusion, and lastly, (4) that

the ability of isoproterenol and propranolol to alter the extent of injury persisted, but was blunted, when they were applied 3 hours after coronary occlusion. These were the first studies to demonstrate that the extent of injury resulting from coronary occlusion could be altered and suggested a time-dependent evolution of myocardial infarction.

The idea that all cells within a region of evolving myocardial infarction did not die simultaneously was demonstrated by Riemer et al. (11). The extent of transmural myocardial necrosis at the level of the posterior papillary muscle was examined histologically in anesthetized dogs subjected to circumflex coronary occlusions of varying duration. They found that myocyte necrosis progressed in a wavefront from endocardium to epicardium. Irreversible cell death occurred rapidly in the subendocardial region but was seen progressively later in the mid-wall and subepicardial regions. The relative proportion of transmural myocardium which was ischemic but salvageable at 15 minutes, 40 minutes, 3 hours and 6 hours after coronary occlusion were 100%, 55%, 33%, and 16% respectively. When completed, the process of infarction spared a small region of subepicardium. Microvascular injury similarly progressed from endocardium to epicardium but over a longer period of time. The most severe injury occurred in endocardial regions where endothelial swelling and degeneration led to vascular occlusion which prevented reperfusion. The major contribution of these studies was their definition of a very narrow temporal window for therapeutic interventions during evolving myocardial infarctions.

A major problem which hindered early studies was the fact that unequivocal gross identification of myocardial infarcts becomes possible

only after 24 hours of coronary occlusion (12). Thus investigators found it necessary to prolong the duration of their studies until the processes of cell death and inflammation were sufficiently developed to permit visual identification of regions of infarction. Myocardial infarctions can be recognized earlier by light and electron microscopy, but for practical reasons, these methods limit quantitation of necrotic myocardium to progressively more circumscribed regions (i.e. the posterior papillary muscle (11)). Epicardial mapping of ST changes allows early identification of ischemic injury at a particular site but does not discriminate precisely the borders between normal and ischemic tissue and is only an indirect predictor of subsequent infarction. A significant technical advance occurred when macroscopic histochemical stains, as originaly described by Nachlas and Shnitka in 1963 (12), were rediscovered and popularized by Lie et al. (13) in 1975. They recognized that when myocardial muscle was immersed in colorless solutions of certain tetrazolium salts (nitroblue tetrazolium and triphenyltetrazolium chloride), a deeply colored precipitate was deposited on the surface of viable tissue (NBT-blue, TTC-red), while infarcted tissue remained unstained. This technique precisely discriminates between infarcted and non-infarcted tissue as early as 3 hours after coronary occlusion (14). Several studies have shown that both NBT and TTC are as accurate as the "gold standards" of histology and electronmicroscopy in the identification of infarcted tissue (14-16). Klein et al. (17) have shown that failure of myocardial cells to stain with these tetrazolium salts occurs because of the loss of dehydrogenase enzymes or their necessary co-enzymes (NAD/NADH). Loss of co-enzymes is an earlier bio-

chemical event than loss of dehydrogenases and accounts for the ability to identify infarcted muscle within the first hours after coronary occlusion. In comparison with earlier methods of quantitating the size of experimental myocardial infarcts, tetrazollium salts allow experiments of shorter duration and permit the visual identification and measurement of myocardial infarcts with the accuracy of histology and electron-microscopy but with much greater ease.

It had been apparent to early investigators (18) and was later rediscovered (19) that despite ligations of the same coronary artery at a fixed anatomic site in the canine model, the proportion of the left ventricle that became infarcted could vary widely among animals. Lowe et al. (19) were the first to propose that anatomic variation in the size and distribution of a given coronary artery was the major reason for this phenomenon. They defined the myocardium perfused by a coronary artery distal to its site of occlusion as the area at risk for infarction and suggested that infarct size should be expressed in relation to the size of the risk region.

A wide variety of methods to measure the size of the "area at risk" were developed. Ante-mortem techniques included the injection into the left atrium of colored dyes, fluorescent vital stains, or technetium-labelled albumin microspheres which would identify the risk region by its failure to take up the dye, fluoresce, or emit radiation respectively (20-22). Intracoronary injection of microfil, latex, water soluble dyes, or barium sulfate gelatin allowed either direct or "defect" definition of the risk region post-mortem (23, 19, 24, 25).

Regardless of the method employed, a few important new concepts

were consistently demonstrated. Widely recognized was the fact that the amount of the left ventricle infarcted increased linearly as a function of the size of the risk region (19,22,24,25). When the area at risk was less than 10-20% of the canine left ventricle, no infarction occurred after coronary occlusion (22,25-27). Pharmacologic interventions could shift this relation so that for any size risk region the mass infarcted was significantly less (19,28). It was also recognized that as the perfusion bed of the occluded coronary became larger, the proportion infarcted increased (25). However, even with very large risk regions there was always a small region of subepicardium which remained viable. The ability of a pharmacologic intervention to salvage ischemic tissue depends primarily on the size of the hypoperfused zone (22). Experimental animals with the smallest areas at risk had the greatest reduction in damage while those with the largest risk regions had the least reduction in the proportion of the risk region infarcted. Additionally, spatial differences exist such that the most severe injury occurs in basal and subendocardial portions of the risk region (25, 27).

An explanation for these regional differences in infarct size was first proposed by Lowe et al. (19) and actually demonstrated by Jugdutt et al. (25). Using radioactive microspheres it was shown that collateral blood flow within the area at risk was highest when the risk region was small, in samples taken from the subepicardium, and in that part closest to the apex (25,27). Conceptually, the relationship of the size of the area at risk to its collateral blood flow was likened to that of the volume of a sphere to its surface area (19). The relative proportion of the area at risk receiving collateral flow sufficient to

prevent cell death is greatest when the risk region is small (25). In apical and subepicardial regions, which are the surfaces of the sphere interfacing with collateral vessels, the proportion of the risk region which goes on to infarction is least.

An additional interesting finding which arose from these studies was described by Becker et al. (26). They found that in normal dog hearts subjected to coronary occlusion, collateral blood flow to the circumflex bed was consistently greater than that into the left anterior descending bed. Consequently when risk regions of equal size were generated by ligation of a coronary artery the amount infarcted was greater after a left anterior descending occlusion than after circumflex occlusion. By inference one would expect that interventions designed to limit infarct size would be less effective when the left anterior descending is occluded as opposed to the circumflex.

Studies of the type cited thus far have evaluated the effects of ischemia and interventions which modify it on quantitative measures of cell necrosis. However, no real benefit is achieved if histologic salvage of myocardium is unaccompanied by preservation or restoration of contractile function. Tennant and Wiggers (29) first recognized that coronary occlusion caused the immediate loss of contraction within the ischemic region. Even though myocardial function is lost rapidly, cell death does not occur unless coronary occlusion is maintained for more than 20 minutes (29). Several investigators have recognized that recovery of function after brief periods of ischemia (< 20 minutes) requires 2-3 days and is associated with gradual improvement of ultrastructural and biochemical indices of reversible injury (30,31). Reperfusion after

2 hours of coronary occlusion is accompanied by even longer periods of functional recovery (14 days) which, on a regional basis, is never complete (32). Braunwald has described reversibly injured myocardial muscle as having been "stunned" by an ischemic insult (30). Thus, the improvement in regional and global left ventricular function achieved by interventions (i.e. reperfusion) which limit infarct size may not be manifest for weeks.

In summary, these investigations have demonstrated that myocardial infarction is a dynamic process which can be favorably modified within a narrow temporal window. Simple methods exist for accurately assessing the amount of necrosis which occurs in laboratory animals. The amount of the left ventricle which becomes infarcted and the severity of ischemia within the perfusion bed of an occluded coronary artery are largely determined by the amount of muscle served by that artery. Myocardium salvaged by reperfusion (and possibly by other interventions) regains contractile function only after a period of days to weeks.

Effects of Acutely Administered Beta-blockade on Experimental and Clinical Myocardial Infarction

Adequate rationale exists for proposing that the presence of beta-blockade may favorably alter the outcome of myocardial infarction. In numerous experimental studies involving several species, beta-blockers have been administered just before or within hours of the onset of myocardial infarction. As might be expected, variations in methods, experimental models, species utilized and the specific beta-blocker given have been accompanied by results that at times are conflicting.

There are several studies which evaluate the ability of beta-blockers to protect myocardium subjected to prolonged periods of coronary occlusion (i.e. 5 or more hours). As mentioned earlier, Maroko et al. (7) demonstrated that administration of propranolol (0.5-2.0 mg/kg i.v.) just prior to ligation of the left anterior descending in dogs resulted in less ST-elevation at 15 minutes and less CPK depletion at 24 hours than seen in control animals. When they performed ligations of the circumflex in dogs, Rasmussen et al. (33) found that the proportion of the posterior papillary muscle infarcted after 24 hours was reduced from 85% to 52% in animals given 5 mg/kg of propranolol intra-arterially 10 minutes before occlusion. When propranolol therapy was delayed to 3 hours after ligation, it was less than half as effective as pre-treatment. Fox et al. (34) investigated the effects of intravenous propranolol (0.5 mg/kg) administered 30 minutes after left anterior descending occlusion in anesthetized dogs. Five hours after the onset of infarction they noted significantly less CPK release, less QRS change, and more rapid and substantial improvement in radionuclide images of the perfusion deficit caused by coronary occlusion. Downey et al. reported that administration of 2 mg/kg propranolol at 6 hour intervals beginning just after embolization of either the circumflex or anterior descending produced substantial salvage of subepicardial ischemic muscle (35). Recently, the relative effects of propranolol, timolol and metoprolol on infarct size determined 6 hours after left anterior descending ligation were compared (36). When administered 15 minutes after coronary occlusion all three agents reduced infarct size as compared with placebo, but the beta-1 selective agents were twice as effective.

In contrast, a few well done experimental studies are notable for their failure to demonstrate protective effects of beta-blockers in myocardium subjected to long periods of ischemia. Peter et al. (37) gave high doses of propranolol (5 mg/kg iv) just prior to left anterior descending ligation and supplemented this with an infusion at 1.25 mg/kg/hr. Identical degrees of CPK depletion were seen in control and propranolol treated animals 24 hours later. More recently, the comparative effects of metoprolol and pindolol administered at 30 to 90 minutes after left anterior descending occlusion on coronary blood flow, left ventricular function, and infarct size after 6 hours of ischemia were measured (38). Although both beta-blockers decreased LV dP/dt and metoprolol decreased heart rate, neither agent produced changes in infarct size or in coronary blood flow as measured by microspheres. Rogers et al. performed the only experimental study of this type in primates. They investigated the effects of sotalol on LV function and CPK depletion in baboons 24 hours after LAD ligation, and found no benefit (39).

Another group of experimental studies have investigated the effects of acute beta-blockade on infarct size resulting from shorter periods of coronary occlusion (2 hours or less) followed by reperfusion. At least 6 different investigations have been performed in anesthetized dogs given various beta-blockers prior to or up to 2 hours after coronary ligation (40-45). In comparison with reperfusion alone, substantial additional reductions in infarct size varying from 40-90% have been seen. The greatest additional benefit from adding beta-blockade to reperfusion occurs when beta-blockade is administered prior to the onset of ischemia, and when the time to reperfusion is brief. By diminishing

the oxygen requirements of ischemic myocytes beta-blockade delays cell death so that more muscle can be salvaged at the time of reperfusion.

While the literature is more consistent in affirming the salutary effects of acute beta-blockade on infarct size when combined with reperfusion, two exceptions bear mention. Genth et al. (46) found that pindolol, a beta-blocker with partial agonist activity, when administered prior to a 90 minute coronary occlusion with subsequent reperfusion, did not alter infarct size in dogs. Because of its intrinsic sympathomimetic property, pindolol produced no changes in heart rate and may have both blocked and stimulated beta receptors in ischemic muscle. More important is a study by Geary et al. done in baboons (47). Although pretreatment with 2 mg/kg of propranolol produced substantial reductions in heart rate and LV dP/dt, no limitation of infarct size was seen when 2 hours of coronary occlusion were followed by reperfusion.

The value of urgent beta blockade in patients experiencing acute myocardial infarction has been studied in a host of clinical investigations (48-57,8). In contrast to the animal studies cited thus far, a number of uncontrolled variables have hindered these investigations. Among them are; (1) inter-patient variability in the pathologic cause of myocardial infarction, (2) difficulty in accurately measuring the duration of ischemia prior to treatment, (3) differences in the coronary artery involved, (4) variation in the severity of associated atherosclerosis in other coronary arteries, (5) diversity in the presence and adequacy of collateral flow, (6) the variable presence and extent of prior myocardial infarction, (7) pre-existing left ventricular dysfunction, and (8) difference in medications received chronically prior to

the infarct. Additionally, the ante-mortem methods of measuring infarct size in patients that have been employed (serum CPK modelling, surface EKG mapping, and planar radionucleide images) are at best indirect and semi-quantitative measures of infarct size.

Despite these limitations, six clinical trials have reported reductions of enzymatic or EKG indices of infarct size (48-54). In 3 large studies the incidence of primary ventricular fibrillation within the first 48 hours of myocardial infarction was significantly reduced by treatment with a beta-blocker (55-57). These results are particularly remarkable when it is realized that the dose of beta-blockers used have generally been one-tenth of that found to limit infarct size in experimental animals.

Only one major clinical study failed to find a beneficial effect of acute beta-blockade on infarct size (8). In that study, propranolol was given intravenously and later orally to patients presenting up to 18 hours after the onset of chest pain. No improvement in enzymatic, EKG or radionucleide estimates of infarct size was found. The results of that study are most likely explained by the fact that fewer than 2 per cent of the patients studied received propranolol within 4 hours after the onset of symptoms.

Myocardial Ischemia and Infarction After Chronic Cardiac Denervation or Long-term Beta-blockade

Two separate lines of investigation suggest that chronic interruption of adrenergic stimulation may induce secondary adaptive changes in cardiac muscle that would be beneficial during periods of ischemia.

Resting myocardial oxygen consumption 2 weeks after cardiac denervation (58) or ventricular sympathectomy (59) was approximately 50% less than that in sham-operated dogs and was accompanied by significant reductions in myocardial blood flow (59-61). Despite increasing amounts of external work, both myocardial oxygen consumption and coronary blood flow continued to be one-half that of normal animals (59). As a consequence of this enhancement in the efficiency of energy utilization, Barber et al. (61) found that the minimal blood flow needed to prevent the death of ischemic cardiac muscle was substantially lower after chronic cardiac denervation. A major reduction in coronary collateral resistance (62) and a marked increase in collateral blood flow after coronary occlusion (63) were reported after chronic ventricular sympathectomy. Not suprisingly, a number of different studies have described dramatic reductions in infarct size, myocardial dysfunction and arhythmias resulting from coronary occlusion in chronically denervated animals (10,60,61,64-66).

Jones et al. (10) and Barber et al. (61) found that infarct size following mid-LAD ligation was reduced in animals which underwent cardiac denervation immediately before coronary occlusion. However, significant further reductions in infarct size were seen in animals denervated 2-4 weeks prior to coronary occlusion. Thomas et al. (64) compared the effects of coronary occlusion on contractile function in dogs with normally innervated, acutely denervated and chronically denervated hearts. Epicardial contractile force in the ischemic region was minimally depressed in chronically denervated hearts but no similar protection was provided by acute denervation.

With section of cardiac sympathetic nerves, Wallerian degeneration of postganglionic fibers occurs. As a result, myocardial catecholamine levels drop to less than 2% of normal in the first week after cardiac denervation (67). No similar depletion of catecholamines would be anticipated in acutely denervated hearts subjected to coronary occlusion. Local release of norepinephrine in response to ischemia might be responsible for the intermediate preservation of ischemic myocardium and regional function in acutely denervated animals. However, Jones et al. (10) administered propranolol and phentolamine prior to coronary occlusion in their acutely denervated dogs to prevent stimulation of adrenergic receptors by locally released catecholamines. They still found that chronic denervation was significantly more effective in limiting infarct size. This finding implies that metabolic alterations or enhanced collateral flow after prolonged withdrawal of adrenergic neural stimulation are more important mechanisms for protection of ischemic myocardium.

In distinct contrast to previous studies is a recent investigation by Lavallee et al. (68). They reported that ligation of the circumflex produced higher left ventricular end-diastolic pressures, diminished collateral blood flow and larger myocardial infarctions in chronically denervated dogs than was seen in normally innervated animals. They attributed the difference between their findings and those of earlier investigations to the fact that most previous studies were done in anesthetized animals while they produced coronary occlusions in concious animals. However, not all prior studies demonstrating beneficial effects of cardiac denervation on the course of subsequent myocardial in-

farction have been done in anesthetized animals. Schaal et al. (65) found that chronic cardiac denervation abolished the ventricular tachycardia seen in 9 of 10 control animals, after ligation of the anterior descending, in conscious dogs.

Most experimental studies have shown that cardiac denervation favorably affects infarct size, myocardial contractile function and arhythmias after coronary occlusion. In addition, prolonged denervation seems to produce adaptive changes which afford the ischemic heart added protection. The salutary effects of acute denervation during ischemia are similar to that achieved by acute beta-blockade (10). A series of experiments suggest that chronic beta-blockade may induce many of the added benefits seen with chronic denervation.

In rabbits treated with propranolol for 4 or more weeks, electronmicroscopy revealed substantially fewer mitochondria per cell than in untreated animals (69). In addition, the relative volume occupied by capillaries was increased while the volume of the interstitial space was diminished. Besides demonstrating that the distance for diffusion of oxygen from capillary to cell membrane is shortened, this study implies a lower basal oxygen consumption after prolonged beta-blockade. The same treatment regimen in another study (70) was found to markedly depress the activities of dopamine beta-hydroxylase and tyrosine hydroxylase (the rate limiting enzyme in norepinephrine synthesis) in sympathetic ganglia. Depression of norepinephrine synthesis by prolonged beta-blockade might result in lower myocardial norepinephrine content and thus decrease catecholamine mediated myocardial injury during ischemia. Becker et al. (71) have shown that chronic beta-blocker therapy

produces a functional alteration in the relation between beta receptors and adenylate cyclase. They found that prolonged beta-blockade reduced adenylate cyclase activity such that the amount of cyclic AMP produced for any given extracellular concentration of catecholamine was significantly diminished. Wexler (72) found that 7 days after withdrawal of long-term propranolol therapy, massive doses of isoproterenol failed to produce the extensive myocardial necrosis and mortality seen in rats not previously treated with beta-blockers. This protection was present at a time when tissue and serum propranolol levels would be unmeasurable.

A few studies have evaluated the effects of long-term beta-blockade in models of myocardial ischemia. Manning et al. found that both propranolol and oxprenolol, when administered orally for 3 weeks to rats, resulted in an increased incidence of functional recovery after periods of global ischemia in isolated perfused heart preparations (73). Post-ischemic cellular levels of creatine phosphate and glycogen recovered rapidly and more completely in animals previously beta-blocked than in controls. This beneficial action was seen as long as 48 hours after beta-blocker withdrawal when serum drug levels were undetectable. Nayler et al. (9) treated rabbits with propranolol for five days and then subjected their hearts to 60 minutes of hypoxic perfusion in a Langendorf preparation. They found much lower levels of CPK in the coronary effluent and pronounced preservation of mitochondrial function in the hearts pretreated with propranolol as compared with controls. No functional inhibition of the chronotropic response to isoproterenol could be demonstrated more than 18 hours after the last dose of propranolol. However, dramatic protection resulting from beta-blocker

therapy was seen as long as 72 hours after cessation of beta-blocker administration.

Three investigations have evaluated the effects of chronic beta-blockade on the course of subsequent coronary occlusion. Of these, the only study in large animals was that of Menken et al. (74). They reported that five days of atenolol therapy increased the ventricular fibrillation threshold and decreased the incidence of ventricular fibrillation after proximal circumflex occlusion in dogs. Hearse et al. (75) performed LAD ligations in isolated perfused working rat hearts previously treated with propranolol or acebutolol for 3 weeks. One hour after coronary occlusion, cardiogreen dye was infused into the patent coronaries and the hearts were immediately frozen. In this study, the area at risk was identified by its failure to take up the green dye due to inadequate antegrade or collateral coronary perfusion. They found that chronic beta-blockade resulted in substantially smaller risk regions than seen in controls, presumably on the basis of enhanced collateral flow. However, the severity of ischemia within the ischemic region, as measured by high energy phosphate levels, was not different between groups. Campbell et al. (76) ligated the left main coronary for one hour in rats pretreated with oxprenolol. When coronary occlusion occurred 1 hour after the last dose of oxprenolol, the incidence of ventricular fibrillation and mortality were less than in control animals, but no difference in infarct size was present. When the same experiments were performed 16 hours after the last dose of oxprenolol, no benefit was evident. The failure of chronic beta-blocker therapy to limit infarct size in this model can probably be attributed to the

severity of the ischemic insult. On the average, 48% of the ventricle was infarcted one hour after left main occlusion. In addition to the fact that a very large proportion of the ventricle was ischemic, the severity of cellular injury within this region was undoubtedly augmented by the rapid heart rates in these animals, which often exceeded 400 beats per minute.

Only one clinical study has attempted to evaluate the effects of prior beta-blocker therapy on infarct size in man (76). In an investigation involving 45 patients with acute myocardial infarction, 10 of whom had chronically received any of a variety of beta-blockers, enzymatic measures of infarct size were made. The time to the onset of enzyme release and the time to peak enzyme release were both significantly delayed by prior beta-blockade, although eventual infarct size was not changed. This data was interpreted as suggesting that beta-blockade had delayed the onset of cell death in those patients. In contrast, 3 large multicenter trials involving timolol, propranolol and metoprolol have all documented enhanced survival and a reduced incidence of sudden cardiac death and reinfarction among patients receiving these beta-blockers on a chronic basis after myocardial infarction (78-80). The mechanism for this protection is unknown.

METHODS

Drug Treatment and Confirmation of Beta-blockade

Mongrel dogs employed in the study were divided into three treatment groups; group 1 (N=10) served as controls, group 2 (N=12) was intravenously beta-blocked just prior to coronary occlusion and group 3 (N=11) was chronically beta-blocked prior to coronary occlusion. Group 3 animals received 80 mg of nadolol twice daily for 16 days. They were restricted to a narrow weight range (19-21.5 kg, mean=20.1 kg) to approximate an 8 mg/kg daily dose of nadolol. Nadolol is a non-selective beta-blocker which is 2 to 4 times more potent than propranolol, but lacks any direct myocardial depressant effects (81). It is free of intrinsic sympathomimetic activity and was chosen because its half life of elimination in dogs (4-5 hrs, 82) is longer than that of any other beta-blocker. Sixteen days of oral therapy were given because a similar period produced marked protection after cardiac denervation (10,61,64). In one animal, 24 hours after a single 80 mg oral dose of nadolol, the chronotropic response to a 2 ug/kg bolus of isoproterenol was inhibited by 51%. In 5 additional experiments performed in our lab, this treatment regimen inhibited the chronotropic response to the same bolus of isoproterenol by 93% (71) and 87% when measured 12 hours after the last oral dose of nadolol on days 7 and 14 of therapy, respectively. This test dose of isoproterenol exceeds the chronotropic stimulus produced by

maximal neural stimulation as demonstrated by experiments in our lab and by Ledsome et al. (84).

It was our intention that the degree of beta-blockade in the two drug-treated groups be equivalent at the time of coronary occlusion. In this manner, differences in the degree of salvage of ischemic myocardium in the two groups would not reflect dose-dependent differences in the level of beta-blockade, but rather, effects achieved by different durations of therapy. Therefore, acutely beta-blocked animals received successive boluses of nadolol intravenously until they had less than a 10 beat per minute increase in heart rate in response to 2 ug/kg isoproterenol given intravenously. Coronary occlusion in the chronically beta-blocked dogs were performed approximately 12 hours after the last oral dose of nadolol. These animals were also given incremental doses of nadolol intravenously until they had less than a 10 beat per minute increase in heart rate in response to the test dose of isoproterenol. In both drug-treated groups, titration of the degree of beta-blockade with i.v. nadolol was performed approximately 15 minutes prior to coronary occlusion.

Surgical Preparation

Animals in all treatment groups were anesthetized with pentobarbital and a tracheostomy tube was inserted for ventilation. The animals were ventilated with a Harvard respirator and arterial blood gases were measured periodically to maintain pH in the range 7.35-7.45, with pO2 greater than 60. The right femoral vein and artery were isolated for

venous access and measurement of arterial pressure. The left femoral artery was used for insertion of a Millar transducer-tipped catheter which was advanced into the left ventricle for measurement of LVEDP, left ventricular peak systolic pressure, and dP/dt. ECG leads II, III, and AVF were monitored to reflect changes in the posterior wall. A biotachometer triggered by the ECG measured heart rate. Epicardial temperature was measured and maintained in the range 37-39 degrees celsius. All hemodynamic and ECG measurements were recorded on a Gould 2800S recorder. The first 30 minutes of the post-occlusion period were recorded on magnetic tape for later analysis of arhythmias.

The chest was opened through the fourth left intercostal space, and the pericardium opened with a small incision at the base of the heart. A segment of the circumflex coronary artery was isolated within 1-2 cm of its origin, proximal to the first major marginal branch.

Experimental Protocol

In all animals control hemodynamic and ECG measurements were obtained 5 minutes prior to occlusion. In drug-treated animals, these pre-occlusion measurements were made after administration of i.v. nadolol. Coronary occlusion was achieved by tying a silk ligature about the vessel. In addition, an atraumatic occluder was placed on the circumflex distal to the ligature when animals failed to manifest epicardial cyanosis or ST elevation. Hemodynamic and ECG measurements were made 15 minutes, 30 minutes, 1 hour, and hourly thereafter for the duration of the six hour period. Three hours after occlusion, 0.5 mg/kg

nadolol was given intravenously to drug-treated animals.

During the last hour of the experiment all animals received 5000 units of heparin, and a right carotid to circumflex shunt was placed to permit reperfusion of the previously ischemic region (Figure 1). The carotid end of the shunt was created with a 50 cm length of tygon tubing containing a cannulating-type electromagnetic flow probe. The tip of a 5 cm length of 8F infant feeding tube was inserted into the circumflex distal to the occluding ligature but proximal to any major branches. After six hours of coronary occlusion, reperfusion through the shunt was begun and shunt flow measured.

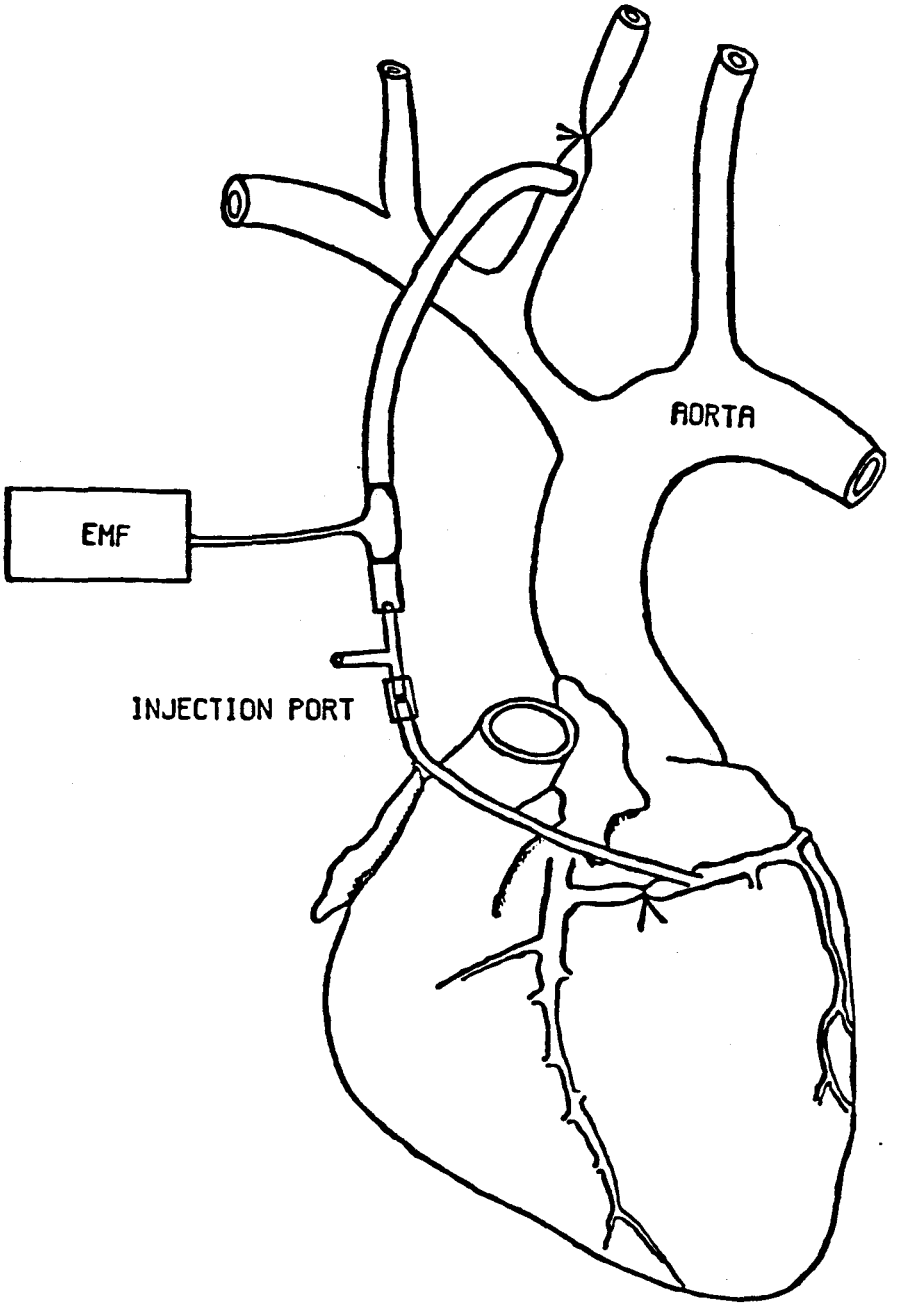
Measurement of Area at Risk and Infarct Size

After 15 minutes of reperfusion 1 cc/kg of a 1.5% solution of Evans blue in Ringer's solution was injected into the left atrium. Because of the longer transit time for blood to reach the circumflex region via the shunt, a brief (2-3 second) interval occurred during which the circumflex bed received dye-free blood while the remainder of the heart was blue stained. At this time the hearts were fibrillated electrically and Ringer's solution was infused into the circumflex cannula (6 cc/min) to keep dye out of the circumflex region while the heart was excised.

Once removed, the heart was sliced in a "bread loaf" manner into 8 to 12 sections, in an apex to base direction. The right ventricle and atria were trimmed away and each slice blotted dry and weighed. The unstained regions within each ring represented the anatomic perfusion

FIGURE 1

THE CAROTID TO CIRCUMFLEX SHUNT



bed of the circumflex artery distal to the site of the occluding ligature, and was designated the area at risk for necrosis. The lateral margins of the area at risk were marked from epicardium to endocardium on both surfaces of each slice with a felt tipped pen (Figure 2).

To measure the size of the infarcted area each slice was soaked for 20 minutes in a 1% solution of triphenyl tetrazolium chloride (TTC) in phosphate buffer (23, 24). Non-infarcted tissue on the surface of each slice was stained a brick red by the tetrazolium dye, while infarcted tissue failed to stain. Although TTC staining masked the Evans blue-stained tissue, the margins of the area at risk, as defined by the felt pen markings, remained visible (Figure 3). Color slides of both surfaces of each slice were made and later projected onto an Apple graphics tablet for planimetry (using an Apple II+ computer and software). From these images the relative sizes of the left ventricular cross sectional area, the area at risk and the area infarcted were identified for both surfaces of each slice. From these areas the absolute weights of each region were calculated and summated for the entire left ventricle.

Statistics

Differences between groups in mortality and the incidence of ventricular fibrillation, ventricular tachycardia, or high degree AV block were compared using Fisher's Exact Test. Comparisons between groups in the number of PVCs during the first 30 minutes of coronary occlusion were evaluated using a one-way analysis of variance. This same statis-

FIGURE 2

THE METHOD FOR IDENTIFYING THE

AREA AT RISK

FIGURE 2. This is a photograph of the basal surface of a ring of ventricular muscle at the level of the papillary muscles after infusion of Evan's blue dye. The unstained tissue in the left half of this figure is the perfusion bed of the circumflex coronary artery (i.e. the area at risk). Black lines marking the lateral margins of the area at risk were placed with a felt-tipped pen. Note an extensive region of subendocardial hemorrhage within the area at risk.

FIGURE 3

THE METHOD OF IDENTIFYING THE

AREA INFARCTED

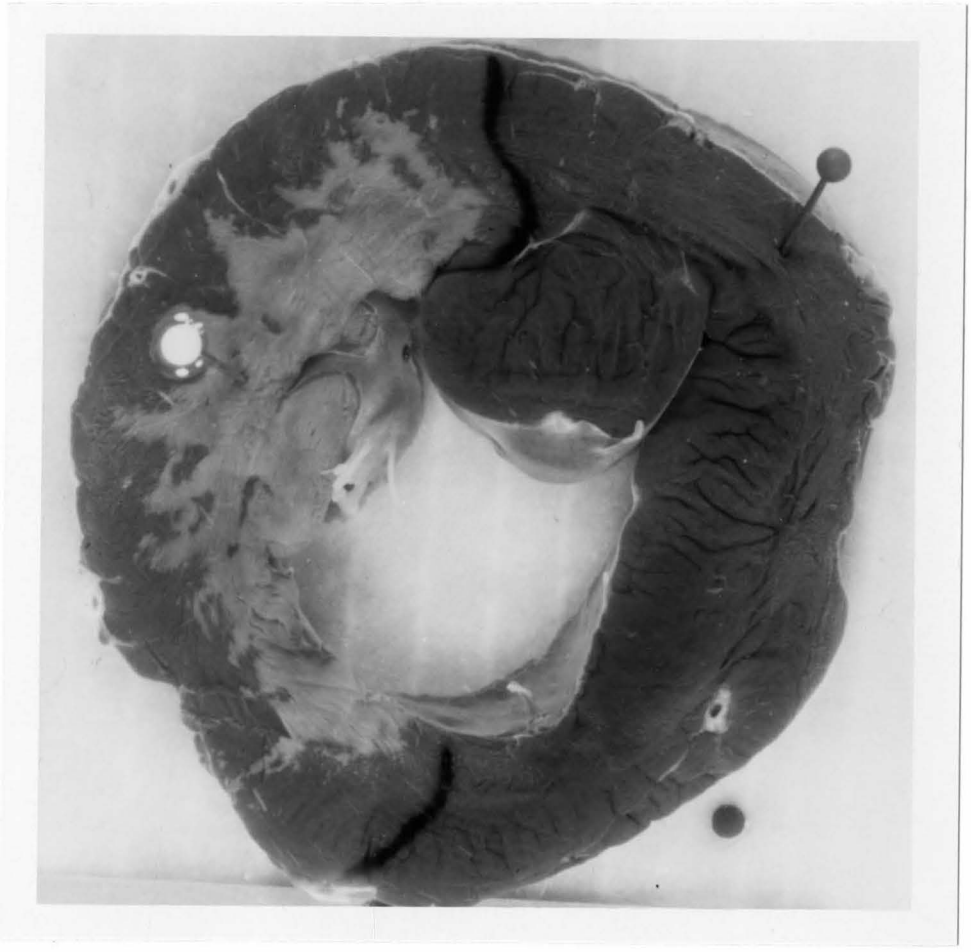


FIGURE 3. This photograph was taken after the same slice of ventricular muscle had been incubated in a solution of TTC. Note that a brick red precipitate was deposited on the cut surface of muscle outside the area at risk. In addition, the precipitate was also deposited on myocardium in the epicardial and lateral portions of the area at risk, indicating that it was not infarcted. A large region of infarcted muscle involving the endocardial half of the area at risk is identified by its failure to deposit the precipitate.

tical analysis was also employed to evaluate differences between treatment groups in body weight, left ventricular mass, and the mass and proportion of the left ventricle at risk. T-tests with a Bonferroni correction were used to determine the significance of differences in infarct size between groups (85, 86). Linear regressions were calculated using the method of least squares. Hemodynamic data were evaluated using a two-way analysis of variance with replications followed by Duncan's Multiple Range Test. This permitted comparisons between groups at any individual time and comparisons within any group overtime. All values are expressed as the mean ± the standard error of the mean.

RESULTS

Mortality and Arhythmias

A total of 33 animals were studied. One control animal and 2 acutely beta-blocked animals developed high degree AV block associated with profound hypotension and died within 2 hours of coronary occlusion. Measurement of the size of the risk region and infarct size were not possible in these animals. Hemodynamic data obtained from these experiments were excluded. One control animal developed ventricular fibrillation 18 minutes after circumflex ligation and was defibrillated within 25 seconds, with prompt restoration of hemodymanics to the levels seen prior to fibrillation. The incidence of ventricular tachycardia (defined as 3 or more consecutive ventricular extrasystoles at a rate greater than 100 beats per minute) was 70%, 36% and 60% in the three groups, respectively. These differences were not statistically significant. Similarly, no difference was seen between groups in the number of ventricular extrasystoles recorded during the first half-hour following circumflex occlusion.

Effects of Acute and Chronic Beta-blockade on Infarct Size

Measurement of the size of the risk region and of the myocardium infarcted was possible in 30 of the dogs studied. The data obtained is presented in Table 1. No difference was present between groups in body

Table 1

RISK REGION AND INFARCT SIZES

	Controls (n=9)	Acutely β-blocked (n=10)	Chronically β-blocked (n=11)
Infarct mass (g)	33.1±3.0 (18.9-45.1)	24.7±3.7 (2.4-39.8)	21.4±4.3 (0.4-38.0)
Risk region mass (g)	48.2±2.9 (38.0-59.1)	46.1±2.9 (28.6-59.7)	47.1±2.0 (37.9-56.9)
LV mass (g)	103.0±4.5 (88.6-117.7)	105.7±4.6 (82.6-133.6)	103.6±2.6 (91.9-114.4)
Infarct/LV (%)	32.0±2.6 (21-45)	22.7±3.2 (3-38)	20.6±4.2 (0-41)
Risk region/LV (%)	46.9±2.4 (35-59)	43.4±1.4 (35-51)	45.5±1.8 (34-54)
Infarct/risk region (%)	67.7±2.9 (50-77)	51.5±6.6 (8-79)	43.5±8.3* (1-80)

Values are expressed as the mean ± s.e.m. (range in parentheses)

*$p < .05$ as compared with the control group.

weight (group I = 19.2 ± 0.2 kg, group II = 20.4 ± 1.0 kg, group III = 20.1 ± 0.3 kg), left ventricular mass, or in the mass of the risk region. While the proportion of the left ventricle at risk in each group was similar, a stepwise decrease in the mass infarcted and in the percent of the left ventricle infarcted was seen in groups II and III respectively. When expressed as a proportion of the risk region, infarct size was reduced 24% by acute beta-blockade and 36% by chronic beta-blockade. The latter result was statistically significant as compared with the control group. However, differences in infarct size between the acutely beta-blocked animals and either of the other two groups did not reach statistical significance.

In Figure 4 the percent of the left ventricle infarcted is plotted as a function of the percent of the ventricle at risk, for the control group. A linear correlation with an R value of 0.90 was found ($Y=.96x-12.98$, $P < .01$). By extrapolation, no infarction would be predicted with risk regions smaller than 14% of the left ventricle in this group. Similar linear correlations were performed for acutely ($Y=1.58x-45.71$, $R=0.70$, $P < .05$) and chronically ($Y=1.72x-57.38$, $R=0.69$, $P < .05$) beta-blocked animals. In general, the lines for these two groups were shifted rightward, such that the mass of risk region necessary to produce an infarct of a given size was successively greater after acute and chronic beta-blockade. However, the much lower correlation coefficients highlight the wide variability of infarct size in the drug treated groups.

Degree of Beta-blockade after Prolonged Oral Therapy and Infarct Size in Chronically Beta-blocked Animals

All animals in group 3 received the same daily dose of nadolol for the same duration of time, and were of similar weights. However, variability was present in the chronotropic response to the test dose of 2 ug/kg of isoproterenol (0-30 bpm, mean=22 bpm) 12 hours after the last oral dose of nadolol. Similar variation was present in the supplemental dose of i.v. nadolol (0-1.3 mg/kg, mean=0.5 mg/kg) given just prior to circumflex ligation to inhibit the increase in heart rate after isoproterenol to less than 10 bpm. On the average, this supplemental dose of i.v. nadolol was less than half that given to acutely beta-blocked animals (1-2.5 mg/kg, mean=1.3 mg/kg). When the percent of the left ventricle infarcted was plotted as a function of the chronotropic response to isoproterenol, a statistically significant linear correlation was found ($Y=0.76x + 4.27$, $R=0.60$, $P < .05$). Thus, the ability of nadolol to limit infarct size after chronic oral therapy was in part a function of the degree of beta-blockade at the end of the 16 day treatment period. This relationship was seen despite the fact that supplemental doses of i.v. nadolol were given just prior to coronary occlusion to ensure equivalent degrees of beta-blockade in all drug treated animals.

Hemodynamics Before and After Coronary Occlusion

Several measured and derived hemodynamic parameters obtained before and at three different times after circumflex ligation are presented in Table 2 and Figures 5-7. At all times heart rate in either of

the drug treated groups was significantly less than in control animals. Over the duration of the experiment heart rate averaged 25 bpm higher in control animals. Six hours after coronary ligation heart rate was 21 bpm greater than prior to coronary occlusion ($p < .05$) in this group. In contrast, heart rate was unchanged or minimally increased during the experiment in groups II and III, respectively.

Pre-occlusion left ventricular end diastolic pressures were similar in all groups. However, by 6 hours after circumflex occlusion it was significantly elevated in control and acutely beta-blocked animals, as compared with pre-occlusion values. LVEDP rose minimally in chronically beta-blocked animals and was significantly less than in either of the other two groups at 6 hours ($p < .05$).

There was no difference in dP/dt among groups prior to coronary occlusion. Over the duration of the experiment, it rose in control animals and declined in drug treated animals. Six hours after circumflex ligation it was 36% and 26% lower, in groups II and III respectively, than in group I ($p < .05$).

Mean arterial pressure and left ventricular peak systolic pressure were similar in all groups and essentially unchanged during the experiment as compared with pre-occlusion values. Changes in rate pressure product paralelled those in heart rate. At 6 hours it was 49% and 35% greater in control animals than in acutely or chronically beta-blocked animals, respectively ($p < .05$).

Table 2

HEMODYNAMIC DATA

TIME	GROUPS	HR (BPM)	MAP (mmHg)	LVEDP (mmHg)	LVPSP (mmHg)	dP/dt (mmHg/sec)	RPP/1000
Prior to	I	146.4±2.9	112±8.1	4.4±1.2	117.7±6.5	1803±105	17.3±1.1
CFX	II	129.7±4.2*	109.8±4.1	6.1±1.7	119.6±5.0	1764±142	15.4±0.4
Occlusion	III	128.1±3.8*	110.6±3.9	4.1±0.8	121.7±4.1	1758±115	15.6±0.8
15 min	I	148.7±2.5	96.7±7.7	8.3±2.0	108±8.9	1778±189	16.5±2.2
after	II	124.6±4.0*	92.7±5.3	10.0±2.2+	101.8±4.8+	1549±108	12.6±0.7*
CFX Occl	III	130.5±4.1*	99.3±3.6	8.2±1.1	105.6±3.8	1598±123	14.1±0.7
2 hrs	I	158.9±6.2	112.8±5.4	8.7±1.5	121.8±6.1	1976±125	19.5±1.6
after	II	120±5.5*	113.6±6.3	12.4±2.3	120.7±5.3	1517±89*	14.6±1.1*
CXF Occl	III	138.1±3.5#*	110.4±3.7	9.0±1.3	119.9±3.2	1649±81	16.5±0.6
6 hrs	I	167.4±6.8+	107.4±6.1	15.9±1.9+	124.7±5.5	2020±110*	21.1±1.5+
after	II	129.6±6.5*	97.9±4.6	15.8±2.6+	108.9±3.7	1297±95+	14.2±1.0*
CXF Occl	III	142.1±5.3*	99.5±6.4	9.5±1.2#*	111.4±5.3	1491±142*	15.8±0.8*

* $p < .05$ as compared with the control group
+ $p < .05$ as compared with the preocclusion value
# $p < .05$ as compared with the acutely β-blocked group

HR = Heart Rate, MAP = Mean Arterial Pressure, LVEDP = Left Ventricular End Diastolic Pressure, LVPSP = Left Ventricular Peak Systolic Pressure, dD/dT = The Rate of Rise of Left Ventricular Pressure, RPP/1000 = Rate Pressure Product ÷ 1000

FIGURE 4

INFARCT SIZE AS A FUNCTION
OF THE AREA AT RISK

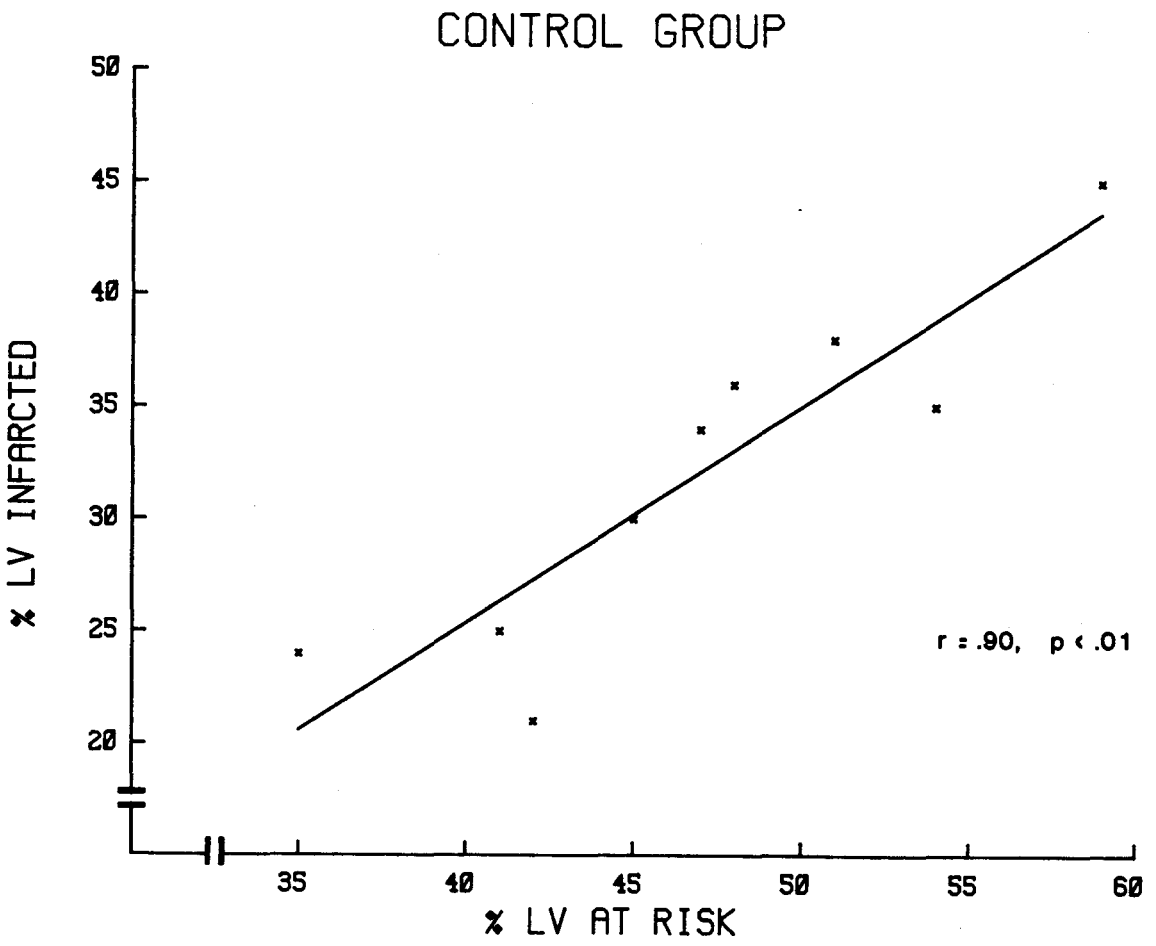
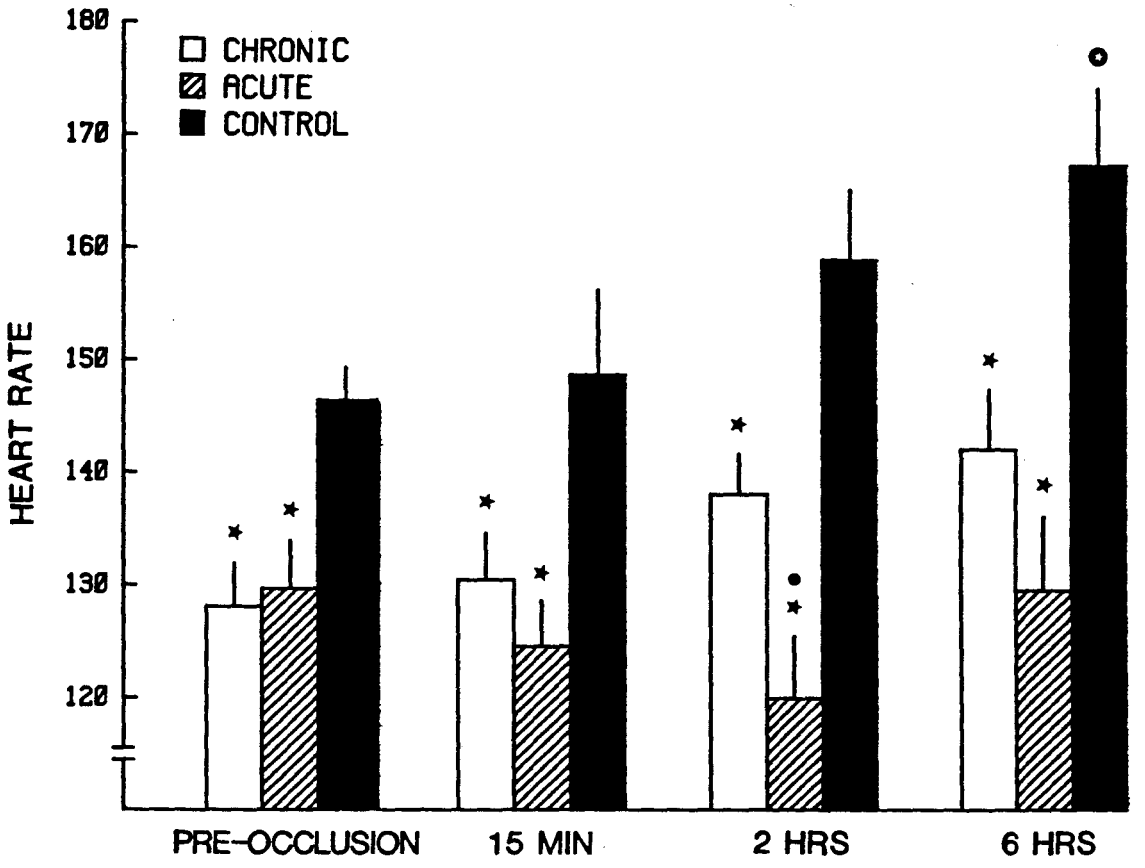


FIGURE 4. The percent of the left ventricle infarcted increased as a linear function of the size of the area at risk. Extrapolation of this line to the X intercept indicates that no infarction would be expected with risk regions smaller than 14% of the ventricle.

FIGURE 5

HEART RATE PRIOR TO AND AFTER

CORONARY OCCLUSION

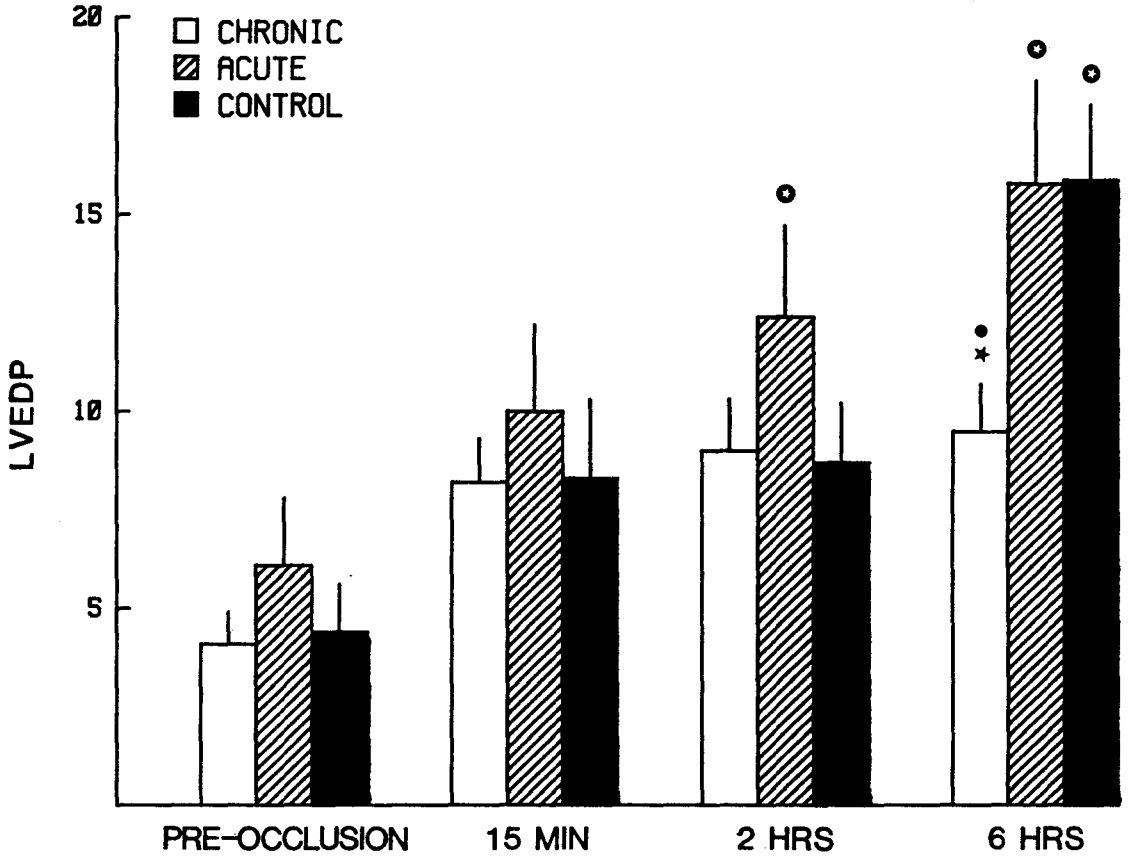


(⊙P ‹.05 as compared with the pre-occlusion value, *p‹ .05 as compared with the control group, •p ‹.05 as compared with the chronically beta-blocked group.)

FIGURE 6

LVEDP PRIOR TO AND AFTER

CORONARY OCCLUSION

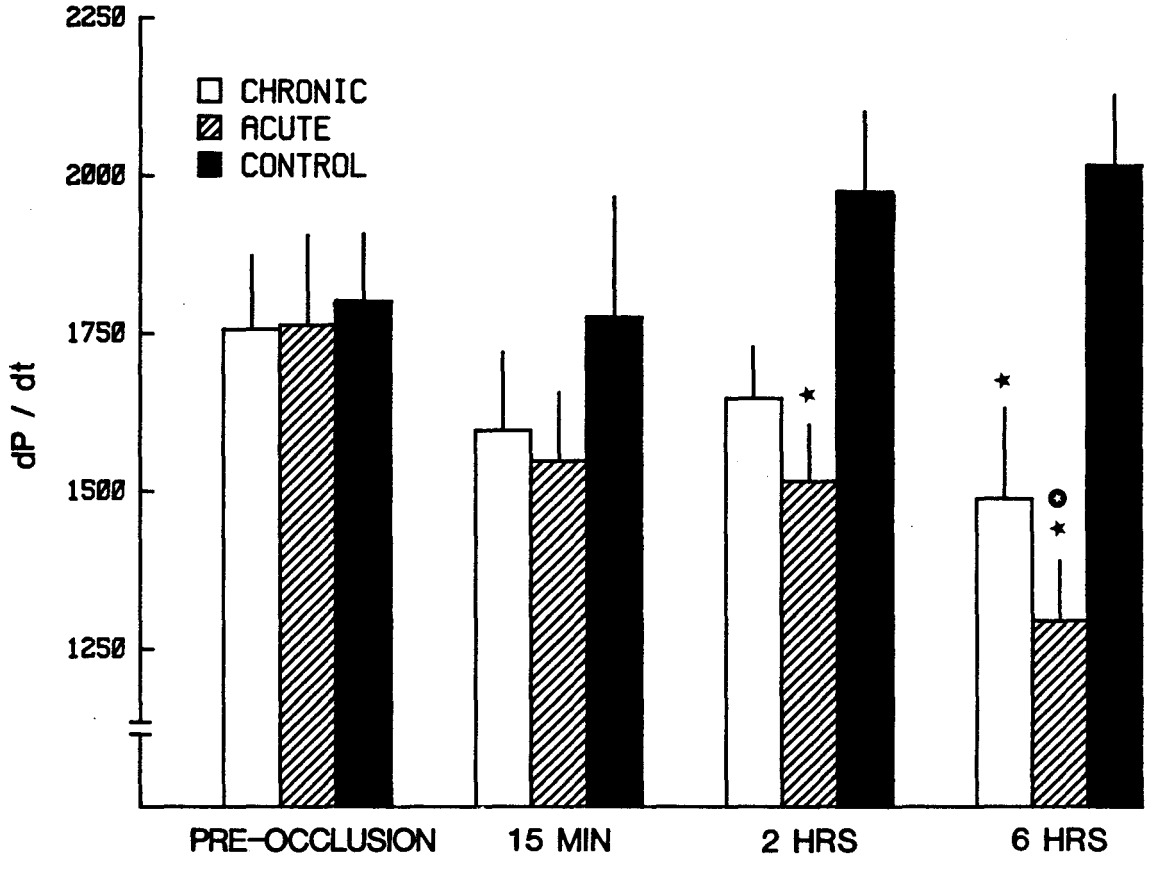


(⊙P< .05 as compared with the pre-occlusion value, *p < .05 as compared with the control group, •p< .05 as compared with the acutely beta-blocked group.)

FIGURE 7

dP/dt PRIOR TO AND AFTER

CORONARY OCCLUSION



(⊙P< .05 as compared with the pre-occlusion value, *p < .05 as compared with the control group.)

DISCUSSION

Differences in Infarct Size and Potential Sources of Variability within Groups

In this study acute and chronic beta-blockade resulted in incremental decreases in the proportion of the area at risk which infarcted after circumflex ligation. While a modest degree of variation in infarct size was seen in control animals, drug treated animals exhibited much less uniformity. Some dogs in both drug-treated groups evolved infarcts as large as any in control animals while others derived marked protection from beta-blockade. As a result, only the reduction in infarct size achieved by prolonged beta-blockade was statistically significant as compared with the control group. In addition, infarct sizes in acutely and chronically beta-blocked animals did not significantly differ from each other.

The reason for the wide variability in the extent of myocardial injury among animals in groups II and III is not clear. In a recent co-operative study, a group of investigatiors found that much of the variation in infarct size in canine models of myocardial infarction could be attributed to three independent variables (90). The size of the area at risk, collateral blood flow, and myocardial oxygen consumption (estimated by the rate-pressure product) accounted for roughly 70%, 15%, 5% of this variation, respectively. To control for the most important of these three variables, infarct size is commonly normalized

for the size of the risk region, as was done in the present study. Inconsistencies in accurate measurement of the risk region could produce sufficient experimental error to make comparisons of normalized infarct size between groups difficult. Two pieces of data suggest that this was not the case. Figure 3 demonstrates that the technique employed in this study accurately reproduces the finding of other investigators; (19,22,24,25) the amount of the left ventricle infarcted increases linearly as a function of the size of the perfusion bed of the occluded coronary artery. In addition, the proportion of the left ventricle perfused by the circumflex, as defined by this technique, is very similar to previously reported values (24, 87-90). Therefore, variation in infarct size within or between groups cannot be ascribed to errors in measuring the risk region. Moreover, the average size of the area at risk and variability about these means were similar in all three groups.

The fact that collateral blood flow into an ischemic region can differ considerably between experimental animals has been observed repeatedly (25,26,90,91). Its relative importance in determining the size of experimental myocardial infarctions has only recently been determined. Once the area at risk has been controlled for, 50% of the remaining variability in infarct size in canine hearts is due to differences in collateral blood flow (90). In the present study collateral blood flow was not measured. It would be anticipated that biologic variation in collateral flow would affect all three groups equally if the number of animals studied was sufficiently large. The much wider range in infarct size in drug-treated animals suggests that substantial reductions in infarct size may only have occurred when collateral blood

flow exceeded a minimal critical value. In the absence of significant collateral flow, neither acute nor chronic beta-blockade would be sufficient to maintain cellular viability for 6 hours after coronary occlusion. This hypothesis is supported by the finding that beta-blockers have been unsuccessful in reducing infarct size in species which lack a significant coronary collateral circulation (39,47).

The severity of myocardial injury resulting from coronary occlusion depends upon the mismatch between oxygen supply and demand. In combination, the area at risk, collateral blood flow and the rate-pressure product have been found to account for 90% of the variability in infarct size seen in animals (90). However, of these three, variability in myocardial oxygen consumption was the least important. In this experiment variation within groups in rate-pressure product was not different and fails to account for the wider range of infarct size in groups II and III.

By design, differences between animals in the degree of beta-blockade immediately prior to and during the 6 hour period of circumflex occlusion were minimized. A large dose of a very potent beta-blocker was chosen and a supplemental dose was given before one half-life of elimination. Additionally, circumflex ligation in groups II and III were performed only when sufficient beta-blockade was present to inhibit the chronotropic responses to a large test dose of isoproterenol. It therefore seems unlikely that variation in the degree of beta-blockade during the ischemic period contributed significantly to differences in infarct sizes among drug-treated animals.

However, despite equivalent beta-blockade during coronary oc-

clusion, infarct size in chronically beta-blocked animals was significantly correlated with the degree of beta-blockade (i.e. the inhibition of the chronotropic response to isoproterenol) at the end of the 16 day treatment period. Differences between animals in the absorption, metabolism and elimination of nadolol are likely to have resulted in disparity in the magnitude of beta-blockade during this 16 day period. This was illustrated by the range of responses to the test dose of isoproterenol. This finding suggests that "dose dependent" adaptive changes may have occurred in the hearts of chronically beta-blocked animals which conferred additional protection from ischemia induced injury. Whether these adaptive changes were favorable alterations in metabolism or in collateral blood flow is unknown.

In summary, although a number of potential sources for the wide variation in infarct size among acutely and chronically beta-blocked animals exist, two appear to have been particularly important. In both groups variability in collateral blood flow may have served a permissive function such that significant reduction in infarct size may only have occurred when collateral flow exceeded a critical minimal value. In the chronically beta-blocked animals the degree of beta-blockade during the 16 day treatment period was found to be an independent predictor of infarct size.

Mechanisms for the Protective Effect of Acute and Chronic Beta-Blockade

In animal models of myocardial infarction, dramatic increases in cardiac sympathetic nerve activity (5) and in circulating levels of

plasma catecholamines (6) have been demonstrated shortly after coronary occlusion. Striking elevations of plasma norepinephrine and epinephrine concentrations have similarly been reported in patients with evolving infarcts (92). By enhancing the disparity between myocardial oxygen supply and consumption, catecholamines have been shown to augment the severity of cellular injury in ischemic tissues (7,93). Beta-blockers have been shown in experimental and clinical myocardial infarction to decrease myocardial oxygen consumption and to favorably shift myocardial metabolism from lactate production to lactate extraction (94-96).

Catecholamine mediated stimulation of beta-1 receptors leads to increases in both heart rate and contractility. If decreasing heart rate alone was the only important action of beta-blockers, they would still achieve the dual benefits of decreasing myocardial oxygen consumption and increasing the time available for diastolic perfusion of the coronary circulation. However, beta-blockers have been shown to protect ischemic tissue independent of their negative chronotropic effects. Hilis et al. (97) observed that when propranolol was administered to dogs subjected to LAD occlusion, the resulting rise of intramyocardial pCO2 was 43% less than in control experiments. Virtuallly the same protection was seen in animals paced to prevent beta-blocker mediated decreases in heart rate. Similarly, Armstrong et al. (98) found that when atrial pacing was performed in a group of patients with coronary artery disease the threshold to angina and the electrocardiographic and metabolic indices of ischemia were all attenuated.

The enhanced sympathetic activity that accompanies acute myocardial infarction may also effect the severity of myocardial ischemia

through indirect, extracardiac actions. Catecholamines are lipolytic and elevated levels of circulating free fatty acids have been demonstrated during myocardial infarction (92,99). With elevation of free fatty acids, substrate utilization within ischemic tissue shifts unfavorably from the anaerobic metabolism of glucose towards the oxidation of free fatty acids (100). Pharmacologic inhibition of catecholamine-sensitive lipases has been shown to reduce infarct size in a canine model (101). By inhibiting the action of epinephrine on adipocytes, beta-blockers have been shown to reduce circulating free fatty acids during myocardial infarction in man (92). The ability of beta-blockers to reduce oxygen consumption in ischemic tissues may partially be due to this action.

Another proposed effect of beta-blockers is that they improve oxygen supply to ischemic myocardium by increasing collateral blood flow (102). While some studies have reported enhanced collateral flow (102) and an increase in the ratio of endocardial to epicardial flow (103), others have found no beneficial changes in regional myocardial blood flow when beta-blockers were administered during experimental myocardial infarction (37, 38).

In this experiment, measurement of the major determinants of myocardial oxygen consumption were made before and after coronary occlusion. Pre-occlusion values of left ventricular end diastolic pressure, mean arterial pressure, and dP/dt were not different between groups. Heart rate, however, was lower in beta-blocked animals. Over the course of the 6 hour experiment heart rate rose progressively in control animals while changing little in the other groups. This sug-

gests that the control group was exposed to progressively increasing adrenergic stimulation in response to circumflex ligation.

Similarly, coronary occlusion produced directionally opposite changes in dP/dt in control and beta-blocked animals. Despite the fact that 32 ± 2.6% of the left ventricle was infarcted in control animals, the expected decline in dP/dt was not seen. Instead dP/dt rose and was significantly greater than in drug treated animals at 6 hours. This suggests that a marked augmentation of contractility occurred in the non-ischemic myocardium of control animals. It was of sufficient magnitude that dP/dt, an index of overall left ventricular contractile function, failed to decline as anticipated. Higher heart rates, elevated left ventricular end diastolic pressures, and greater levels of sympathetic tone in the control group, together may account for this phenomenon. In combination, they also resulted in significantly larger myocardial infarcts as compared with chronically beta-blocked animals.

All the potential mechanisms cited above for the salutary effects of beta-blockade in acute myocardial infarction are pertinent to both groups of drug-treated animals evaluated in this study. As discussed earlier, a series of studies have demonstrated alterations of myocardial ultrastructure, metabolism efficiency of energy utilization and collateral blood flow afer prolonged interruption of cardiac adrenergic stimulation. These effects could potentially confer added protection from ischemia induced injury to chronically beta-blocked hearts. Although infarct size was not statistically different between drug treated animals in this study, left ventricular end diastolic pressure was significantly less in chronically beta-blocked animals 6 hours after cir-

cumflex ligation.

In the setting of acute myocardial infarction, loss of contractile function by ischemic cardiac muscle leads to diminished ventricular emptying during systole and elevated end diastolic volumes. In addition, impaired relaxation of ischemic myocardium during diastole results in diminished left ventricular compliance. Together systolic and diastolic dysfunction result in elevation of left ventricular end diastolic pressure. In the present study, systolic ventricular function in chronically beta-blocked animals, as measured by dP/dt, was no different than that in acutely beta-blocked animals and was less than that in the control group 6 hours after coronary occlusion. The finding that left ventricualar end diastolic pressure was significantly lower in chronically beta-blocked animals than in either of the other two groups suggests that left ventricular compliance may have been substantially better in this group. However, since left ventricular compliance was not directly measured in this study, differences in compliance between groups can only be inferred.

Comparisons with Earlier Studies

No previously published study has compared the effects of immediate and long-term beta-blockade on infarct size and hemodynamics resulting from experimental coronary artery occlusion. In two studies, isolated, working hearts of small animals have been subjected to periods of global ischemia or hypoxia at various times after cessation of prolonged beta-blocker therapy. Manning et al. (73) found that the number

of hearts that recovered mechanical function and the post-ischemic cellular levels of creatine phosphate and glycogen were significantly enhanced in rats previously treated with either propranolol or oxprenolol. Prolonged pre-treatment with propranolol resulted in less CPK release, less mitochondrial calcium accumulation and enhanced mitochondrial function in rabbit hearts subjected to 60 minutes of hypoxic perfusion by Nayler et al. (9). In both of these studies, the protective effects of prior beta-blockade were seen at times when residual drug levels or functional beta-blockade could not be demonstrated. They suggest that there are secondary consequences of long-term beta-blockade which are beneficial during myocardial ischemia.

Two studies, both performed in rats, have evaluated the effects of chronic beta-blockade on injury resulting from coronary ligation. Hearse et al. (75) occluded the LAD in isolated, working hearts of rats previously treated with acebutolol or propranolol for 2-3 weeks. After 1 hour of ischemia cardiogreen dye was infused into the aortic root to identify all myocardium perfused by native or collateral vessels. They found that the ischemic region, defined by the absence of green dye, was substantially smaller in the hearts of chronically beta-blocked animals. This implies that collateral blood flow in these hearts was enhanced. However, high energy phosphate content within the ischemic region was not different between groups and infarct size was not measured. Recently Campbell et al. (76) reported that chronic oxprenolol treatment did not reduce the size of myocardial infarcts after ligation of the left main coronary artery in rats. It is doubtful that any intervention would favorably alter infarct size with such a large proportion of the

ventricle ischemic or in the presence of the marked tachycardia they reported (400+ bpm).

Direct comparison between the present study and those cited above are difficult. None of these studies have investigated the effects of acute and chronic beta-blockade on infarct size in the same model. In addition, heart rate, left ventricular internal diameter and wall thickness (all important determinants of myocardial oxygen consumption) are drastically different in rat and rabbit models from that seen in the canine heart. In experimental design, the present study is much closer to those which have evaluated the effects of cardiac denervation on infarct size and myocardial function in anesthetized dogs.

Jones et al. (10) ligated the apical portion of the anterior descending and compared the proportion of the left ventricle infarcted in control animals, those denervated just prior to coronary occlusion, and those denervated 2 weeks previously. They found sequential substantial reductions in infarct size with acute and chronic cardiac denervation. Using a similar model with coronary occlusion at the same site, Barber et al. (61) reproduced these findings. In contrast, the abilities of acute and chronic beta-blockade to limit infarct size in the present study were less dramatic. This difference might be attributed to the substantially larger risk regions in this study. The area at risk was not measured in the former studies but it appears to have been no larger than 20-25% of the left ventricle. In comparison, the area at risk was nearly twice as large in this investigation. It has previously been demonstrated that even in control animals no infarction frequently occurs with risk region less than 15-20% of the ventricle (22,25-27).

Additionally, the ability of an intervention to limit infarct size increases substantially as the area at risk becomes smaller (22).

The relative effects of acute and chronic cardiac denervation on myocardial contractile function after coronary occlusion were described by Thomas et al. (64). They found substantial equivalent reductions in epicardial contractile force after coronary ligation in control and acutely denervated hearts. However, the depression of regional myocardial function seen in chronically denervated hearts was only one third that seen in other groups. Although regional myocardial function was not measured in the present study, left ventricular end-diastolic pressure was significantly lower in chronically beta-blocked animals than in other groups 6 hours after coronary occlusion.

Recently Lavallee et al. (68) reported larger myocardial infarcts and higher left ventricular end diastolic pressures after proximal circumflex ligation in concious, chronically denervatd dogs. The reason for the disparity between these results and earlier investigations of the effects of cardiac devervation on infarct size, myocardial function and arhythmias in anesthetized animals is not clear. However, the work of Lavallee et al. suggests that an avenue for future investigation might include the repitition of the present study in conscious animals.

SUMMARY

In the present study, the relative effects of acute and chronic beta-blockade on infarct size and hemodynamics after circumflex ligation in anesthetized dogs were investigated. In comparison with control animals, mean infarct size was smaller in both groups of drug-treated animals, but only the reduction in infarct size achieved by chronic beta-blockade was statistically significant. While infarct size among the two groups of beta-blocked animals were similar, left ventricular end diastolic pressure was significantly less 6 hours after coronary occlusion in chronically beta-blocked animals. The salutary effects of beta-blockade in this study can partially be attributed to protection from enhanced adrenergic tone after coronary occlusion. Despite the fact that all drug treated animals had similar and substantial degrees of beta-blockade during coronary occlusion, infarct size in chronically beta-blocked animals was independently correlated with the degree of beta-blockade during the previous 16 days.

From these findings we conclude that long term beta-blockade results in at least as great a reduction in infarct size as does acute beta-blockade. However, left ventricular hemodynamics during myocardial infarction, as evidenced by lower end-diastolic pressures, appear to be better preserved after chronic beta-blockade. In addition, the protective effect of prolonged beta-blockade during subsequent periods of myocardial ischemia is related to the degree of beta-blockade during the

previous treatment period.

These findings support the current clinical practice of prolonged beta-blocker therapy in patients at high risk for subsequent ischemic events. In addition, they suggest that patients may benefit from receiving the largest dose of beta-blockers tolerated without side effects.

BIBLIOGRAPHY

1. Page DL, Caulfield JB, Kastor JA, DeSanctis RW, Saunders CA: Myocardial changes associated with cardiogenic shock. N Engl J Med 285:133, 1971

2. Harnarayan C, Bennett MA, Pentecost BL, Brewer DB: Quantitative study of infarcted myocardium in cardiogenic shock. Br Heart J 32:728, 1970

3. Sanz G, Castaner A, Betriu A, Magrina J, Roig E, Coll S, Pare JC, Navarro-Lopez F: Determinants of prognosis in survivors of myocardial infarction. N Engl J Med 306:1065, 1982

4. Raab W: Key position of catecholamines in functional and degenerative cardiovascular pathology. Am J Cardiol 5:571, 1960

5. Gillis RA: Role of the nervous system in the arhythmias produced by coronary occlusion in the cat. Am Heart J 81:667, 1971

6. Karlsberg R, Penkoske PA, Cryer PE, Corr PB, Roberts R: Rapid activation of the sympathetic nervous system following coronary artery occlusion: relationship to infarct size, site, and haemodymamic impact. Cardiovascular Research 13:523, 1979

7. Maroko PR, Kjekshus JK, Sobel BE, Watanabe T, Covell JW, Ross J, Braunwald E: Factors influencing infarct size folllowing experimental coronary artery occlusions. Circulation 43:67, 1971

8. Roberts R, Croft C, Gold HK, Hartwell TD, Jaffe AS, Muller JE, Mullin SM, Parker C, Passamani ER, Poole WK, Raabe DS, Rude RE, Stone PH, Turi ZG, Sobel BE, Willerson JT, Braunwald E, MILIS study group: Effect of propranolol on myocardial-infarct size in a randomized blinded multicenter trial. N Engl J Med 311:218, 1984

9. Nayler WG, Yepez CE, Fassold E, Ferrari R: Prolonged protective effect of propranolol on hypoxic heart muscle. Am J Cardiol 42:217, 1978

10. Jones CE, Devous MD, Thomas JX, DuPont E: The effect of chronic cardiac denervation on infarct size following acute coronary occlusion. Am Heart J 95:738, 1978

11. Reimer KA, Lowe JE, Rasmussen MM, Jennings RB: The wavefront phenomenon of ischemic cell death. Circulation 56:785, 1977

12. Nachlas MM, Shnitka TK: Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. Am J Pathol 42:379, 1963

13. Lie JT, Pairolero PC, Holley KE, Titus JL: Macroscopic enzyme--mapping verification of large, homogeneous, experimental myocardial infarcts of predictable size and location in dogs. J Thorac Cardiovasc Surg 69:599, 1975

14. Fishbein MC, Meerbaum S, Rit J, Lando U, Kanmatsuse K, Mercier JC, Corday E, Ganz W: Early phase acute myocardial infarct size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. Am Heart J 101:593, 1981

15. Kloner RA, Darsee JR, DeBoer LWV, Carlson N: Early pathologic detection of acute myocardial infarction. Arch Pathol Lab Med 105:403, 1981

16. Schaper W, Frenzel, Hort W: Experimental coronary artery occlusion. Basic Res. Cardiol. 74:46, 1979

17. Klein HH, Puschmann S, Schaper J, Schaper W: The mechanism of the tetrazolium reaction in identifying experimental myocardial infarction. Pathol Anat 393:287, 1981

18. Blumgart HL, Gilligan DR, Schlesinger MJ: Experimental studies on the effect of tempory occlusion of coronary arteries. Am Heart J 22:374, 1941

19. Lowe JE, Reimer KA, Jennings RB: Experimental infarct size as a function of the amount of myocardium at risk. Am J Pathol 90:363, 1978

20. Darsee JR, Kloner RA: Dependency of location of salvageable myocardium on type of intervention. Am J Cardiol 48:702, 1981

21. Simson MB, Harden W, Barlow C, Harken AH: Visualization of the distance between perfusion and anoxia along an ischemic border. Circulation 60:1151, 1979

22. Ribeiro LGT, Cheung W, Maroko PR: Influence of the extent of the zone at risk on the effectiveness of drugs in reducing infarct size. Circulation 66:181, 1982

23. Geary GG, Smith GT, McNamara JJ: Defining the anatomic perfusion bed of an occluded coronary artery and the region at risk to infarction. Am J Cardiol 47:1240, 1981

24. Bush LR, Romson JL, Ash JL, Lucchesi BR: Effects of diltiazem on extent of ultimate myocardial injury resulting from temporary coronary artery occlusion in dogs. J Cardiovasc Pharmacol 4:285, 1982

25. Jugdutt BI, Hutchins GM, Bulkley BH, Becker LC: Myocardial infarction in the conscious dog: three-dimensional mapping of infarct, collateral flow and region at risk. Circulation 60:1141, 1979

26. Becker LC, Schuster EH, Jugdutt BI, Hutchins GM, Bulkley BH: Relationship between myocardial infarct size and occluded bed size in the dog: difference between left anterior descending and circumflex coronary artery occlusions. Circulation 67:549, 1983

27. Koyanagi S, Eastham CL, Harrison DG, Marcus ML: Transmural variation in the relationship between myocardial infarct size and risk area. Am J Physiol 242:H867, 1982

28. Melin JA, Becker LC: Salvage of ischemic myocardium by prostacyclin during experimental myocardial infarction. J Am Coll Cardiol 2:279, 1983

29. Tennant R, Wiggers CJ: The effect of coronary occlusion on myocardial contractions. Am J Physiol 112:351, 1935

30. Braunwald E, Kloner RA: The stunned myocardium: prolonged, postischemic ventricular dysfunction. Circulation 66:1146, 1982

31. Henyndrickx GR, Baig H, Nellens P, Leusen I, Fishbein MC, Vatner SF: Depression of regional blood flow and wall thickening after brief coronary occlusions. Am J Physiol 234:H653, 1978

32. Ellis SG, Henschke CI, Sandor T, Wynne J, Braunwald E, Kloner RA: Time course of functional and biochemical recovery of myocardium salvaged by reperfusion. J Am Coll Cardiol I:1047, 1983

33. Rasmussen MM, Reimer KA, Kloner RA, Jennings RB: Infarct size reduction by propranolol before and after coronary ligation in dogs. Circulation 56:794, 1977

34. Fox K, Welman E, Selwyn A: Myocardial infarction in the dog: effects of intravenous propranolol. Am J Cardiol 45:769, 1980

35. Downey JM, Chambers D, Wilkerson RD: The inability of isoproterenol or propranolol to alter the lateral dimensions of expermentally induced myocardial infarcts. Basic Res Cardiol 77:486, 1982

36. Vik-Mo H, Maroko PR, Ribeiro LGT: Comparative effects of propranolol, timolol and metoprolol on myocardial infarct size after experimental coronary artery occlusion. J Am Coll Cardiol 4:735, 1984

37. Peter T, Heng MK, Singh BN, Ambler P, Nisbet H, Elliot R, Norris RM: Failure of high doses of propranolol to reduce experimental myocardial ischemic damage. Circulation 57:534, 1978

38. Lange R, Nieminen MS, Kloner RA: Failure of pindolol and metoprolol to reduce the size of non-reperfused infarcts in dogs using area at risk techniques. Cardiovasc Res 18:37, 1984

39. Rogers GG, Rosendorff C, Coull A, Warner SJC, Jarvis AC: Sotalol and infarct size after coronary ligation in the baboon. J Cardiovasc Pharmacol 5:28, 1982

40. Reimer KA, Rasmussen MM, Jennings RB: Reduction by propranolol of myocardial necrosis followling temporary coronary artery occlusion in dogs. Circ Res 33:353, 1973

41. Kloner RA, Fishbein MC, Cotran RS, Braunwald E, Maroko PR: The Effect of propranolol on microvascular injury in acute myocardial ischemia. Circulation 55:872, 1977

42. Burmeister WE, Reynolds RD, Lee RJ: Limitation of myocardial infarct size by atenolol, nadolol and propranolol in dogs. European J Pharmacol 75:7, 1981

43. Reynolds RD, Burmeister WE, Gorczynski RJ, Dickerson DD, Mathews MP, Lee RJ: Effects of propranolol on myocardial infarct size with and without coronary artery reperfusion in the dog. Cardiovasc Res 15:411, 1981

44. Lange R, Kloner RA, Braunwald E: First ultra short acting beta blocker: its effects on size and segmental wall dynamics of reperfused myocardial infarcts in dogs. Am J Cardiol 51:1759, 1983

45. Hammerman H, Kloner RA, Briggs LL, Braunwald E: Enhancement of salvage of reperfused myocardium by early beta-adrenergic blockade (timolol). J Am Coll Cardiol 3:1438,1984

46. Genth K, Hofmann M, Hofmann M, Schaper W: The effect of beta-adrenergic blockade on infarct size following experimental coronary occlusion. Basic Res Cardiol 76:144, 1981

47. Geary GG, Fenton L, Cheng G, Smith GT, Siu B, McNamara JJ: Failure of pretreatment with propranolol to reduce the zone of myocardial infarction after 2 hours of coronary occlusion in the primate heart. Am J Cardiol 52:615, 1983

48. Norris RM, Sammel NL, Clarke ED, Brandt PWT: Treatment of acute myocardial infarction with propranolol. Br Heart J 43:617, 1980

49. Gold HK, Leinbach RC, Maroko PR: Propranolol-induced reduction of signs of ischemic injury during acute myocardial infarction. Am J Cardiol 38:689,1976

50. Yusuf S, Rossi P, Ramsdale D, Peto R, Furse L, Motwani R, Parish S, Gray R, Bennett D, Bray C, Sleight P: Reduction in infarct size, arhthymia, chest pain and morbidity by early intravenous beta-blockade in suspected acute myocardial infarction. Drugs 25:303, 1983

51. Herlitz J, Emanuelsson H, Swedberg K, Vedin A, Waldenstrom A, Waldenstrom J, Hjalmarson A: Goteborg metoprolol trial: enzyme-estimated infarct size. Am J Cardiol 53:15D, 1984

52. Herlitz J, Ejdeback J, Swedberg K, Waagstein F, Hjalmarson A: Goteborg metoprolol trial: Electrocardiographically estimated infarct size. Am J Cardiol 53:22D, 1984

53. Gold HK, Leinbach RC, Harper RW: Usefulness of intravenous propranolol in predicting left anterior descending blood flow during anterior myocardial infarction. Am J Cardiol 54:264, 1984

54. Jurgensen HJ, Frederiksen J, Hansen DA, Perdersen-Bjergaard O: Salvage of ischaemic myocardium by alprenolol. Acta Med Scand 680:27, 1984

55. Herlitz J, Edvardsson N, Holmberg S, Ryden L, Waagstein F, Waldenstrom A, Swedberg K, Hjalmarson A: Goteborg metoprolol trial: effects on arhythmias. Am J Cardiol 53:27D, 1984

56. Sharpe, DN: Prevention of ventricular fibrillation during acute myocardial infarction by intravenous propranolol. Lancet Oct 20. 2, 1984

57. Pedersen F, Rasmussen SL: Prophylactic effect of alprenolol on ventricular tachyarhythmias during the in-patient phase of acute myocardial infarction. Acta Med Scand 680:34, 1984

58. Gregg DE, Khouri EM, Donald DE, Lowensohn HS, Pasyk S: Coronary circulation in the conscious dog with cardiac neural ablation. Circ Res 31:129, 1972

59. Jones CE, Hurst TW, Randall JR: Reduced oxygen and blood flow demands in the chronically sympathectomized heart. Circulatory Shock 9:469, 1982

60. Barber MJ, Euler DE, Thomas JX, Randall WC: Changes in blood flow and S-T segment during coronary arterial occlusion in denervated and nondenervated canine hearts. Am J Cardiol 45:973, 1980

61. Barber MJ, Thomas JX, Jones SB, Randall WC: Effects of sympathetic nerve stimulation and cardiac denervation on MBF during LAD occlusion. Am J Physiol 243:H566, 1982

62. Jones KW, Jones CE: Reduced coronary collateral resistances after chronic ventricular sympathectomy. Am J Physiol 238:H196, 1980

63. DuPont E, Jones CE, Luedecke RA, Smith EE: Chronic ventricular sympathectomy: effect on myocardial perfusion after ligation of the circumflex coronary artery in dogs. Circulatory Shock 6:323, 1979

64. Thomas JX, Randall WC, Jones CE: Protective effect of chronic versus acute cardiac denervation on contractile force during coronary occlusion. Am J Heart 102:157, 1981

65. Schaal SF, Wallace AG, Searly WC: Protective influence of cardiac denervation against arhythmias of myocardial infarction. Cardiovasc Res 3:241, 1969

66. Ebert PA, Vanderbeek RB, Allgood RJ, Sabiston DC: Effect of chronic cardiac denervation on arhythmias after coronary artery ligation. Cardiovasc Res 4:141, 1970

67. Potter LT, Cooper T, Wilman VL, Wolfe DE: Synthesis, binding, release, and metabolism of norepinephrine in normal and transplanted dog hearts. Circ Res 16:468, 1965

68. Lavallee M, Amano J, Vatner SF, Manders WT, Randall WC, Thomas JX: Adverse effects of chronic cardiac denervation in conscious dogs with myocardial ischemia. Circ Res 57:000, 1985

69. Vaughan Williams EW, Tasgal J: Morphometric changes in rabbit ventricular myocardium produced by long-term beta-adrenoceptor blockade. Lancet Oct 22. 1, 1977

70. Raine AEG, Chubb IW: Long term beta-adrenergic blockade reduces tyrosine hydroxylase and dopamine beta-hydroxylase activities in sympathetic ganglia. Nature 267:265, 1977

71. Becker D, Euler DE, Scanlon PJ: The effects of chronic beta-blockade on beta receptors and adenylate cyclase activity in the canine heart. In press

72. Wexler BC: Prolonged protective effects following propranolol withdrawal against isoproterenol-induced myocardial infarction in normotensive and hypertensive rats. Br J Exp Path 66:143, 1985

73. Manning AS, Keogh JM, Shattock MJ, Coltart DJ, Hearse DJ: Long-term beta-blockade: prolonged protective action in the ischaemic myocardium. Cardiovasc Res 15:462, 1981

74. Menken U, Wiegand V, Bucher P, Meesmann W: Prophylaxis of ventricular fibrillation after acute experimental coronary occlusion by chronic beta-adrenoceptor blockade with atenolol. Cardiovasc Res 13:588, 1979

75. Hearse DJ, Keogh JM, Manning AS: Long term beta-blockade: effects on size of zone at risk and contractile function during ischaemia in rat isolated hearts. Br J Pharmacol 76:180, 1982

76. Campbell CA, Parratt JR, Kane KA, Bullock G: Effects of prolonged administration of oxprenolol on severity of ischaemic arhythmias, enzyme leakage, infarct size, and intracellular cardiac muscle action potentials. J Cardiovasc Pharmacol 6:369, 1984

77. Welman E, Fox KM, Selwyn AP, Carroll BJ: The effect of established beta-adrenoreceptor-blocking therapy on the release of cytosolic and lysosomal enzymes after acute myocardial infarction in man. Clin Sci Mol Med 55:549, 1978

78. The Norwegion Multicenter Study Group: Timolol-induced reducton in mortality and reinfarction in patients surviving acute myocardial infarction. N Engl J Med 304:804, 1981

79. Goldstein S: Propranolol therapy in patients with acute myocardial infarction: the beta-blocker heart attack trial. Circulation 67:I53, 1983

80. Hjalmarson A, Herlitz J, Holmberg S, Ryden L, Swedberg K, Vedin A, Waagstein F, Waldenstrom A, Waldenstrom J, Wedel H, Wilhelmsen L, Wilhelmsson C: The Goteborg metoprolol trial: effects on mortality and morbidity in acute myocardial infarction. Circulation 67:I26, 1983

81. Lee RJ, Evans DB, Baky SH, Laffan RJ: Pharmacology of nadolol (SQ 11725), a beta-adrenergic antagonist lacking direct myocardial depression. Eur J Pharmacol 33:371, 1975

82. Wong KK, Dreyfuss J, Shaw JM, Ross JJ, Schreiber EC: A beta-blocking agent (SQ 11,725) that is not metabolized extensively by dogs and monkeys. Pharmacologist 15:245, 1973

83. Hayes A, Cooper RG: Studies on the absorption, distribution and excretion of propranolol in rat, dog and monkey. J Pharmacol Exp Ther 176:302, 1971

84. Ledsome JR, Kellett RP, Burkhart SM: The ability of propranolol to antagonize isoproterenol induced changes in heart rate. J Pharmacol Exp Ther 188:198, 1974

85. Denenberg, VH: Some statistical and experimental considerations in the use of the analysis-of-variance procedure. Am J Physiol

246:R403, 1984

86. Browm BW, Hollander M: Statistics: a biomedical introduction. New York, John Wiley & Sons, 1977

87. Reimer KA, Jennings RB: The changing anatomic reference base of evolving myocardial infarction. Circulation 60:866, 1979

88. Jolly SR, Kane WJ, Bailie MB, Abrams GD, Lucchesi BR: Canine myocardial reperfusion injury. Circ Res 54:277, 1984

89. Kirlin PC, Romson JL, Pitt B, Abrams GD, Schork MA, Luccesi BR: Ibuprofen-mediated infarct size reduction: effects on regional myocardial function in canine myocardial infarction. Am J Cardiol 50:849, 1982

90. Reimer KA, Jennings RB, Cobb FR, Murdock RH, Greenfield JC, Becker LC, Bulkley BH, Hutchins GM, Schwartz RP, Bailey KR, Passamani ER: Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Circ Res 56:651, 1985

91. Mizutani T, Katada Y, Maekawa K, Yokoyama M, Fukuzaki H: Coronary collateral circulation as an important factor to modify the ischemic injury of the myocardium in coronary ligated dogs. Jap Heart J 20:485, 1979

92. Mueller HS, Ayres SM: Propranolol decreases sympathetic nervous activity reflected by plasma catecholamines during evolution of myocardial infarction in man. J Clin Invest 65:338, 1980

93. Shell WE, Sobel BE: Deleterious effects of increased heart rate on infarct size in the conscious dog. Am J Cardiol 31:474, 1973

94. Mueller HS, Ayres SM: The role of propranolol in the treatment of acute myocardial infarction. Progr Cardiovasc Dis 19:405, 1977

95. Haneda T, Lee T, Ganz W: Metabolic effects of propranolol in the ischemic myocardium studied by regional sampling. Circulation 48:174, 1973

96. Mueller HS, Ayres SM, Religa A, Evans RG: Propranolol in the treatment of acute myocardial infarction. Circulation 49:1078, 1974

97. Hillis LD, Khuri SF, Braunwald E, Maroko PR: The role of propranolol's negative chronotropic effect on protection of the ischemic myocardium. Pharmacology 19:202, 1979

98. Armstrong PW, Chiong MA, Parker JO: Effects of propranolol on the hemodynamic, coronary sinus blood flow and myocardial metabolic response to atrial pacing. Am J Cardiol 40:83, 1977

99. Kurien VA, Oliver MF: Serum free fatty acids after acute myocardial infarction and cerebral vascular occlusion. Lancet 11:122, 1966

100. Opie LH, Thomas M: Propranolol and experimental myocardial infarction: substrate effects. Postgraduate Medical Journal 52:124, 1976

101. Kjekshus JK, Mjos OD:Effect of inibition of lipolysis on infarct size after experimental coronary artery occlusion. J Clin Invest 52:1770, 1973

102. Vatner SF, Baig H, Manders WT, Ochs H, Pagani M: Effects of propranolol on regional myocardial function, electrograms, and blood flow in conscious dogs with myocardial ischemia. J Clin Invest 60:353, 1977

103. Becker LC, Fortuin NJ, Pitt B: Effect of ischemia and antianginal drugs on the distribution of radioactive microspheres in the canine left ventricle. Circ Res 28:263, 1971

APPROVAL SHEET

The thesis/dissertation submitted by Patrick Hughes has been read and approved by the following committee:

Dr. Walter C. Randall
Professor, Physiology, Loyola

Dr. Patrick J. Scanlon
Professor, Medicine, Loyola

Dr. David E. Euler
Assistant Professor, Medicine, Loyola

Dr. John X. Thomas
Associate Professor, Physiology, Loyola

The final copies have been examined by the director of the thesis/dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis/dissertation is now given final approval by the Committee with reference to content and form.

The thesis/dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

6/30/86
Date

Walter C. Randall
Director's Signature