1985

Effects of calcium channel blockade on catecholamine cardiomypopathy

Virginia Shau Huang
Yale University

EFFECTS OF CALCIUM CHANNEL BLOCKADE ON CATECHOLAMINE CARDIOMYOPATHY

Virginia Shau Shen Huang

1985

Permission for photocopying or microfilming of " Effects of Calcium Channel Blockade on Catecholamine Cardiomyopathy "
(title of thesis)

for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work, or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

Virginia A Huang
(Signature of author)

VIRGINIA HUANG
(Printed name)

March 15, 1985
(Date)

EFFECTS OF CALCIUM CHANNEL BLOCKADE ON CATECHOLAMINE CARDIOMYOPATHY

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Virginia Shau Shen Huang

1985

ABSTRACT

EFFECTS OF CALCIUM CHANNEL BLOCKADE

ON

CATECHOLAMINE CARDIOMYOPATHY

Virginia Shau Shen Huang

1985

The catecholamines have long been known to cause cardiac injury when delivered in high concentrations. Pathogenetic mechanisms have been much debated and remain unresolved. Those receiving most attention include ischemic injury resulting from coronary vasoconstriction or excessive metabolic demand, and direct membrane injury causing breakdown of critical ion exchange mechanisms. Excessive calcium accumulation within the myocyte is frequently found and viewed as a lethal consequence of this process. Whether it is causal, or the result of other pathways leading to membrane damage is presently unresolved. To provide further insight into the role of calcium exchange as a significant factor in myocyte injury, experiments were undertaken to determine if a recognized calcium channel blocking agent (verapamil) was capable of altering the pattern of myocardial damage known to result from norepinephrine administration in a rabbit model.

New Zealand white rabbits were anesthetized with pentobarbital (30 mg/kg), and cannulas placed in the femoral artery and vein. They were then subjected to various 90 minute infusion protocols. These included norepinephrine, 2 or 3 ug/kg/min (NE-2, NE-3). Other groups were given a 50 ug loading dose of verapamil (VE), followed by infusions of NE-2 or NE-3 with concomitant infusions of verapamil, 1 or 2 ug/kg/min (VE-1, VE-2). Two to three days later, the rabbits were sacrificed, the hearts examined microscopically and assigned a histologic score. Administration of verapamil simultaneously with norepinephrine significantly reduced the histologic injury score from 1.24 $\pm$ 0.10 (NE-2) to 0.59 $\pm$ 0.13 (NE-2 + VE-1, $p<0.01$) and 0.65 $\pm$ 0.10 (NE-2 + VE-2, $p<0.01$). VE-2 infusion was also protective against a higher dose of norepinephrine at 3 ug/kg/min (NE-3), reducing the histologic score from 1.44 $\pm$ 0.13 (NE-3) to 0.69 $\pm$ 0.21 (NE-3 + VE-2, $p<0.01$). A higher dose of verapamil did not result in a substantial increase in protection as the histologic scores of the VE-1 and VE-2 groups were not significantly different. Administration of verapamil did not substantially lower the pressure-rate product (an index of metabolic demand) during norepinephrine infusion nor did it significantly diminish the norepinphrine-induced rise in systemic blood pressure. Thus, afterload reduction was not a significant mechanism. Arterial pH, arterial blood gases, plasma glucose, and hematocrit were unaltered by verapamil

infusion. It is concluded that verapamil significantly reduces myocardial damage produced by norepinephrine infusion in the rabbit and the mechanism of protection probably does not involve verapamil-induced reductions in afterload or cardiac metabolic demand. Verapamil may act by prevention of norepinephrine-induced coronary vasoconstriction, inhibition of myocardial alpha receptor activation, or prevention of lethal calcium accumulation within the myocyte.

ACKNOWLEDGEMENTS

As is true with any scientific research project, the end result is never solely the product of one person's effort. Therefore, I would like to acknowledge those people who have been instrumental to the completion of this project.

With special thanks to S. Evans Downing, M.D., whose patience and guidance have been a constant source of encouragement throughout the completion of this thesis and without whom the research could not have been carried out, to Victor Chen, Ph.D., for his thoughtful suggestions regarding this thesis and statistical analysis of data, and to Sandra Rancourt for her excellent technical assistance.

TABLE OF CONTENTS

INTRODUCTION

It has been known since the early 1900's that catecholamines can injure the heart. The agents that have been studied most extensively are epinephrine, norepinephrine, and isoproterenol. Epinephrine was the first one to be found to have cardiotoxic effects. In his review of catecholamine-induced cardiomyopathy, Haft (1) describes a classic 1905 study in which Ziegler (2) found intramyocardial hemorrhage, edema, patchy areas of myofibrillar damage, round cell infiltration, and proliferation of fibrous tissue in the hearts of rabbits injected with epinephrine. Fleisher and Loeb (3) in 1909 produced myocarditis in the rabbit with epinephrine alone or in combination with sparteine or caffeine. Similar cardiac lesions were reported by Christian et al in 1911 in studies involving the effects of epinephrine injections on the production of renal and cardiac injury (4).

Following these earlier studies, numerous investigators have been able to confirm the cardiotoxicity of epinephrine (5-10). It is interesting to note that although the pharmacological properties of norepinephrine were described by Barger and Dale in 1910 (11), and its presence in tissues demonstrated in 1946 when Von Euler (12) isolated it from adrenergic nerve fibers, it was not until 1958 that norepinephrine was demonstrated to be cardiotoxic. Szakacs et al (13) infused norepinephrine into dogs and found large subendocardial and myocardial hemorrhages in both ventricles, the right atrium, and the mitral valve. There were also multi-

ple focal lesions without apparent preferential distribution. The myocardial lesions were identical to those caused by epinephrine in rabbits and manifested edema, endocardial fibroblastic proliferation, degenerating myofibrils, and cellular infiltration (3,4). The cardiotoxic effects of norepinephrine infusion were further documented by Maling (7,14) in 1958 and 1960. They found fatty changes in the myocardium of dogs one to three days after infusion with norepinephrine. Focal necrosis, hemorrhages, and round cell infiltration of the myocardium were also present. Nahas (6) found in 1958 that infusions of norepinephrine in heart-lung preparations of dogs which isolated the heart from all secondary or neural influences caused extensive degeneration and hemorrhagic lesions of the myocardium, cardiac valves, and the appearance of coronary lesions. Rosenblum et al in 1965 (9) reported that three consecutive intraperitoneal injections of norepinephrine made at 24 hour intervals produced interstitial edema and mononuclear cell infiltration that was less severe than damage caused by isoproterenol at comparable doses.

Schenk and Moss (15) in 1966 infused norepinephrine into dogs, cats, and rabbits with doses comparable to those used clinically and found cardiac lesions in all the animals. These consisted of focal degeneration and necrosis of myofibers and subendocardial hemorrhages. Rabbits were most susceptible while dogs were the least susceptible to the development of cardiac lesions. The authors were also able to establish dose-response relationships.

Similarly, Hoak (16) in 1969 found that intravenous

infusions of norepinephrine for six hours in dogs produced elevated plasma free fatty acid (FFA) concentrations and hemorrhagic lesions in the subendocardial portions of the left ventricle with areas of myofibrillar necrosis, edema, and polymorphonuclear infiltration. Electron microscopy showed extensive destruction of myofibrils and mitochondria and the presence of osmiophilic inclusions within the mitochondria.

Shortly after norepinephrine was discovered to be cardiotoxic, Chappel and Rona et al (17) demonstrated in 1959 that isoproterenol, a synthetic adrenergic agent, administered subcutaneously to the rat caused infarct-like myocardial necrosis and that the severity of the lesions was directly proportional to the damage. Thus lesions of predictable severity could be produced. They also showed that the lesions seen after isoproterenol treatment were more severe than those produced by epinephrine and norepinephrine (8). The synthetic and exogenous isoproterenol has since then been used extensively in studies of catecholamine-induced cardiomyopathy (18-26).

Excess catecholamines secreted in stressful situations or in pheochromocytomas have been shown to result in myocardial damage. Szakacs and Cannon (13) found in 1958 that patients who received prolonged norepinephrine infusions for shock, and patients with pheochromocytoma had similar cardiac lesions to those produced in the dog by prolonged norepinephrine infusions in amounts comparable to therapeutic dosages. The lesions consisted of focal myocardial ne-

crosis, inflammatory exudate, and epicardial hemorrhage.

Kline (26) in 1961 reported autopsy findings from patients with pheochromocytomas and found that these myocardial lesions resembled those in experimental animals treated with norepinephrine. These consisted primarily of severe degenerative changes in groups of muscle fibers, foci of necrosis, and chronic interstitial inflammatory exudation.

Van Vliet et al (27) in 1966 compared autopsy findings of patients with pheochromocytomas and those produced by a series of subcutaneous injections of norepinephrine in rats. Fifteen of twenty-six patients manifested "active catecholamine myocarditis". The lesions were characterized by focal degeneration and necrosis of myocardial fibers with foci of inflammatory cells that were predominantly histiocytes, but also included plasma cells and occasional polymorphonuclear leukocytes. Diffuse edema of the myocardium was also found. The focal inflammatory lesions were most numerous in the inner two-thirds of the myocardium and most often appeared around small vessels. They were also found in the rest of the heart but in fewer numbers. Of the eleven patients who did not have active myocarditis, eight exhibited patchy myocardial fibrosis. Two showed marked myocardial fibrosis similar in appearance and distribution to the fibrosis of those with active myocarditis. This pattern was remarkably similar to that found in rats. All of the animals that died or were killed within five days of the initial injection of norepinephrine had active myocarditis. Fifteen of twenty-seven rats killed after three, four, and eight weeks showed increased amounts of fibrous tissue which was present focal-

ly around vessels, primarily in the inner two-thirds of the left ventricular myocardium.

Stress is another example in which there is excess endogenous catecholamines. It is well established that all types of stress, e.g., physical, emotional, or sensory, result in increased plasma catecholamines concentration secondary to sympathetic activation (28). The major early experimental studies of Selye showed that various types of stress such as prolonged restraint, caloric, surgical, and bacteriotoxic stress, vagotomy, and injection of norepinephrine or epinephrine elicited the same type of cardiac necrosis in rats that had been preconditioned with corticosteroids (29).

Raab was one of the major investigators in stress-induced cardiomyopathy. In 1961, he confirmed Selye's observations that cardiac necroses occurred in rats pretreated with fluorocortisol and exposed to prolonged restraint, cold stress, or nicotine (known to release catecholamines), with the latter two being less effective in producing lesions (28). He also found that various antiadrenergic agents such as reserpine (catecholamine depleting), dibenamine (adrenergic blocking), mecamylamine (ganglionic blocking), and chlorpromazine (centrally inhibiting), protected the heart muscle of rats preconditioned with corticosteroids from stress-induced cardiac necroses. Since all stressful situations are accompanied by a reflex discharge of adrenosympathogenic catecholamines, Raab postulated that the excess amount of endogenous catecholamines was a common media-

tor in the pathogenesis of stress-induced myocardial lesions in animals preconditioned with corticosteroids.

In a 1963 review of the literature concerning catecholamines and myocardial necroses, Raab (30) cited studies which supported his theory. He noted that accumulation of catecholamines in heart muscle itself has been observed under direct stimulation of cardiac sympathetic nerves, stimulation of the midbrain, and under various catecholamine liberating stresses such as enforced exercise, cold exposure, insulin overdosage, or restraint. Also, direct electrical stimulation of the sympathetic nerves supplying the heart for several hours results in subendocardial hemorrhages and necroses similar to those elicited by prolonged infusion of norepinephrine.

Later animal studies showed that stress can induce myocardial lesions even in the absence of hormonal pretreatment. In 1964, Raab reported finding myocardial necrosis in 69% of wild rats exposed to frightening noises following periods of isolation (31). In contrast, myocardial lesions were elicited by emotional stress in only one-third to one-half of nonisolated, domesticated white rats even after pretreatment with corticosteroids.

The cardiovascular system of the pig is considered to be more comparable to that of man than of other animals mainly used for cardiovascular research such as rats or dogs (32,33). Johansson et al (34) found that "restraint stress," i.e., prevention of escape behavior by injection of a myorelaxant while administering painful electric shocks, produced a severe cardiopathy which resulted in sudden death

in 13% of the animals. Subendocardial hemorrhages with fragmentation, granulation, and necrosis of the heart muscle cells, proliferation of histiocytes and fibroblasts, and slight polymorphonuclear infiltration developed within 24 to 48 hours. The authors noted the similarity of the lesions to those induced by catecholamines.

Cardiomyopathy could also be produced in pigs by the physical stress of high sustained positive G force as described by Mackenzie et al (35) in 1976. Examination of the stressed pig hearts showed myofibrillar degeneration, pooling of mitochondria, and cell death. Lesions occurred in random cells of the subendocardium and papillary muscles.

Meerson (36) describes another experimental model in which emotional and painful stresses are induced as a rat waits intensely for painful electric shocks which it receives at occasional intervals. Morphologically, he found complete contracture of the myocardial cells within 39 to 45 hours. This was followed by muscle cell death with degeneration and formation of fibroblastic "granulomas" in some cases, and the appearance of a cellular infiltrate around necrotic muscle fibers.

Thus, stress cardiomyopathy has been described in the literature characterized by focal myocardial necrosis or myofibrillar degeneration. Specifically, the cardiac lesion in stressed animal experiments consists of scattered individual or small clusters of myocytes showing homogeneous eosinophilic transverse bands alternating with areas of fine granulation. This pattern has been termed myofibrillar

degeneration (37). While the studies cited above support the theory that stressful sensory and emotional stimuli might produce cardiomyopathy in laboratory animals, direct evidence in man is harder to obtain. Cebelin and Hirsch (38) in 1980 did a retrospective review of autopsy findings of homicide victims over 30 years in Cuyahoga County, Ohio and identified fifteen victims who died as a result of direct physical assault without sustaining internal injuries. Eleven showed cardiac changes (myofibrillar degeneration) consistent with the "stress cardiomyopathy" that had been described in animal experiments. The authors interpreted the data as being strongly supportive of the theory of catecholamine mediation of these myocardial changes in man and of the lethal potential of stress through its effects on the heart.

While the damaging properties of catecholamines have been extensively studied, the mechanism by which they result in myocardial injury is not well understood. One of the prominent theories postulates that high concentrations of catecholamines result in myocardial calcium overload with subsequent hypercontraction and myofibrillar necrosis (39). If this is correct, it seems reasonable to assume that administration of a drug which prevents entry of calcium into myocytes, such as one of the calcium channel blockers, should result in reduction of the injury. Calcium channel blockers are known to act as specific inhibitors of the slow transsarcolemmal calcium influx but do not (or only slightly) affect the fast sodium current that initiates normal myocardial excitation. In fact, Fleckenstein (40) has done

experiments in which he found that the calcium channel blocker, verapamil, reduces isoproterenol-induced myocardial necrosis. However, there have been few studies investigating the effects of calcium channel blockers on the naturally-occurring catecholamines, epinephrine and norepinephrine. In this thesis, the possible protective effects of verapamil on norepinephrine-induced cardiomyopathy are examined.

PATHOLOGY

The pathology of catecholamine-induced cardiomyopathy has been well characterized. Before the lesion is described, the normal myocardial ultrastructure will be reviewed. Components of normal myocardium include: 1) cardiac muscle cells, 2) vascular elements - arterial, venous, and lymphatic vessels, and capillary network, 3) connective tissue cells - pericytes, fibroblasts, mast cells, primitive mesenchymal cells, and histiocytes, 4)neural elements, and 5) extracelluar elements of connective tissue such as collagen fibers, elastic fibers, and connective tissue microfibrils and proteoglycans. Within the cardiac muscle cells, sarcomeres form the functional units of contraction. Sarcomeres are attached end to end and packaged in myofibrils which are arranged parallel to the longitudinal axis of the cell. Alignment of the sarcomeres is responsible for the striated appearance of the myofibrils (Z-lines). Sarcomeres consist of A bands which contain myosin in the form of "thick" filaments, I bands containing actin in the form of "thin" filaments, and Z bands which contain alpha-actinin and other incompletely characterized proteins. The thick and thin filaments interdigitate so that each thick filament is surrounded by six thin filaments. The interaction of the thick and thin filaments constitutes the basis for the sliding filament mechanism of muscle contraction (41).

Catecholamine-induced lesions are characterized by a particular form of cardiac cell necrosis termed myofibrillar degeneration by Reichenbach and Benditt (37). This consists

of clumping and disorganization of the cardiac myofibrils followed by degenerative changes in cell cytoplasm and mineralization of mitochondria. The lesions are similar in all species (including man) and show little variation with the catecholamines used or route of administration (1). The nature of the lesion is determined by the amount of time that has elapsed between the administration of the drug and sacrifice of the animal. Severity and extent of the lesion varies directly with dose and rate of administration.

On gross examination, focal and diffuse subendocardial hemorrhages are seen shortly after exposure to catecholamines which are especially prominent in the left ventricle and papillary muscles (7,6,13,15,16). Myocardial hemorrhages are also seen (13). The subendocardial and myocardial hemorrhages occur in both ventricles, right atrium, right atrial wall, and in the mitral valve (13). Hemorrhage has also been observed in the pericardium and subepicardium (13). Within 48 hours, the lesions appear as well demarcated pale greyish-yellow areas involving the apical area of the heart (17,25). The tricuspid, mitral, and aortic valves become edematous and distorted with occasional hemorrhages noted on the valves (6,7). Within 4 to 5 days fibrosis begins and progresses (15,27), resulting in indistinct linear scars or chronic aneurysm formation (20).

By light microscopy, the immediate findings after exposure to catecholamines are interstitial edema, subendocardial congestion and hemorrhages, especially adjacent to Thesbian vessels (15). Myofibrillar degeneration with necrosis of the fibers can be demonstrated as early as 2 to 3

minutes after a single intravenous injection of isoproterenol (37). By 6 to 10 hours an inflammatory response becomes evident consisting of an intense interstitial leukocytic reaction most pronounced in subendocardial and midzonal portions of the left ventricle but occasionally extending to the subepicardial region (9,10,15,16,19), and uniformly involving the papillary muscles (42). The cellular infiltrate is predominantly mononuclear cells in which large histiocytic cells are most numerous (15,42,43,27). The peak of myocardial necrosis is reached by 48 hours (19,20) with complete destruction of muscle fibers evident and a full blown interstitial mononuclear cell reaction. Though cardiac histiocytes are most numerous and tended to concentrate in association with foci of myofiber necrosis (15,42,43), they were occasionally accompanied by lymphocytes, granulocytes (10,15,42,43), and perivascular Anitschkoff myocytes (26,44).

In addition to fragmentation and local myofiber destruction, numerous contraction bands are also seen in the myocardium (22,43). These represent irregular, acidophilic coagulation or condensations of contractile proteins forming transverse bands, and numerous hypercontracted sarcomeres. Also present are zones of granularity consistent with swollen mitochndria (24,43).

By 72 hours thin bands of connective tissue which stained as collagen become evident (15,26) and the necrotic muscle fibers are partly replaced by fibroblasts (19). Early manifestations of healing with proliferation of fibro-

blasts at the margins of necrotic areas are present by 4 to 5 days (9,24,25). In 5 to 6 days, the necrotic muscle fibers have been resorbed and the interstitial reaction becomes prominent with numerous Anitschkow cells seen among the proliferating fibroblasts (17). The involved areas consist of sheets of histiocytes and fibroblasts suggesting a granulomatous pattern which peaks in 5 to 7 days (19,20). By 1 week, there is evidence of continuing phagocytosis of necrotic fibers and healing by fibrosis (15,37). At 2 weeks, only fibrous areas are present (15,27). The "granulomatous" areas are eventually replaced by fibrous tissue (44), ultimately leading to indistinct linear scars or chronic aneurysm formation (20).

No lesions have been observed in the larger coronary arteries or myocardial arterioles (9,16,43). Occasionally, thrombi are seen in intramural coronary arteries (6,16), but in other studies these have been absent (43). Branches of the coronary arteries and arterioles occasionally show fibrinoid degeneration, even in areas where necrosis of the myocardium is not apparent (44).

Accumulation of lipid droplets within isolated or groups of cardiac cells may also be found. This is seen immediately after exposure to catecholamines (7,10,14,15). Fatty degeneration of the myocardium has been reported (7,16).

Ultrastructural changes involve the myofibrils, sarcoplasmic reticulum, mitochondria, and lysosomes. Cells show a loss of glycogen and accumulation of neutral lipid. Changes in the myofibrils evidenced by marked thickening of

the Z lines have been noted as early as 30 minutes following exposure to catecholamines (10), consistent with hypercontraction of sarcomeres. There is loss of the orderly arrangement of myofilaments as myofibrillar degeneration progresses (24,25). By 6 to 12 hours, the Z line becomes less osmiophilic, and less distinct as the inflammatory response appears (10,37,43).

The ordinarily flattened vesicles of the sarcoplasmic reticulum become swollen (10,37), and lipid droplets can be seen in the sarcoplasm. The mitochondria exhibit marked swelling and loss of cristae. Necrotic cells have the most severely damaged mitochondria (10,16,24,25,37). Osmiophilic inclusions within the mitochondria also appear and probably represent calcium accumulation granules (16,24,37,45).

An increased number of lysosomes in cardiac muscle cells can be seen throughout the myocardium, but these are especially prominent in cells adjoining the necrotic areas (10).

In summary, the main characteristics of the catecholamine-induced mycardial lesion are myofibrillar degeneration, contraction bands, and interstitial mononuclear infiltrate composed primarily of histiocytes, with most of the damage occurring in the subendocardial regions. The acute injury is followed by fibroblastic proliferation and scarring.

PATHOGENESIS

Despite the well characterized pathology of catecholamine-induced cardiomyopathy, the exact mechanism by which catecholamines produce myocardial lesions is uncertain. Numerous theories have been advanced and these may be grouped into two broad categories. The first invokes ischemia or relative hypoxia as the cause of the lesions, and the second presumes that catecholamines have a direct toxic effect on the myocardium leading to membrane permeability alterations.

Many of the early investigators favored ischemia as a major factor in catecholamine-induced cardiomyopathy (21). Raab (30) theorized that adrenergic amines in excessive amounts, whether produced endogenously (as in severe stress or pheochromocytoma) or administered exogenously can elicit disproportions between supply and demand for oxygen in the myocardium causing myocardial necrosis. He concluded that the subendocardial location of catecholamine-induced necroses could be explained by the increased susceptibility of the ventricular inner layers to anoxia secondary to vascular compression when the intraventricular pressure rises. The cardiotoxicity of catecholamines is abetted by a reduction or absence of compensatory coronary dilatation. Based on experimental findings which showed that: 1) increased endogenous or exogenous catecholamines levels in the heart are associated with potassium depletion, 2) loss of myocardial potassium is secondary to hypoxia, and 3) dietary potassium deficiency results in focal necrotic lesions in the myocar-

dium, Raab proposed the following hypothesis:

> "Exaggerated catecholamine action (either due to exogenous administration or to intrinsic liberation) is capable of causing anoxia in vascularly handicapped, and therefore, particularly vulnerable myocardial cell groups. A resulting alteration of cell membrane permeability permits the escape of potassium from the anoxic cell groups and this disturbance in local electrolyte distribution initiates the process of multifocal cell destruction (30)."

In view of findings which showed that adrenal corticoid pretreatment markedly aggravated catecholamine-induced cardiomyopathy, Raab further postulated that exaggerated stimulation of the catecholamine-liberating adrenosympathetic system accompanied by adrenocorticoid overactivation is the common, potentially cardiotoxic denominator of all forms of acute stress, and primarily responsible for all stress-induced cardiac lesions (30).

Rona (8), in his early studies of isoproterenol necrosis, was a proponent of the ischemic theory of catecholamine-induced cardiomyopathy. However, he now feels that a direct toxic effect is the major factor but that alterations in the coronary microcirculation contribute to the necrosis as well. In an early paper comparing the cardiotoxic actions of isoproterenol, epinephrine, and norepinephrine, Rona (8) stated that epinephrine was believed to cause myocardial lesions due to its cardiac stimulating properties, thus increasing the oxygen requirements of the myocardium so that despite coronary vasodilation, a relative insufficiency of oxygen was produced. Therefore, since the study showed that isoproterenol was a more potent cardiac stimulator than the other two catecholamines, it would also

be expected to cause myocardial necrosis by increasing myocardial oxygen requirements and decreasing systemic blood pressure. Handforth (18) also considered isoproterenol necrosis to be ischemic in nature. He injected India ink into the coronary arteries of hamsters sacrificed shortly after injections of isoproterenol and demonstrated that blood flow to the subendocardium of the left ventricular wall was markedly reduced, even prior to the development of myocardial edema or necrosis. The areas affected by ischemia proved to be the sites at which necrosis subsequently developed. Thus, he felt that isoproterenol-induced lesions were infarcts secondary to local myocardial ischemia and suggested that the ischemia may be due to either vasoconstriction or vascular shunts allowing blood to bypass capillary vessels.

Ferrans et al (46) studied isoproterenol-induced myocardial necrosis and found thickened and increased density of Z bands, swelling of mitochondria, and enlargement of the vesicles of the endoplasmic reticulum within the first couple hours of isoproterenol administration. This was followed by myofibrillar degeneration and lipid droplet accumulation in the spaces between myofibrils as well as in the endoplasmic reticulum. These findings as well as the subendocardial distribution of the lesions were attributed to the exaggerated demand for oxygen imposed on the coronary circulation by catecholamine-induced stimulation of myocardial metabolism. These findings support the concept that hypoxia plays a large role in the pathogenesis of catecholamine-induced cardiomyopathy.

Lehr et al (47,48) have recently presented experimental findings supporting their theory of catecholamine-induced cardiomyopathy. They concluded that the electrolyte deranging and necrosis-inducing properties of catecholamines are primarily due to a discrepancy between coronary vascular adaptability of flow and the greatly augmented oxygen demand. This results from either the positive inotropic and pronounced hypotensive effect of the beta adrenergic agonists, or the pronounced increase in peripheral resistance and constriction of the coronary vasculature caused by alpha adrenergic agents. Ischemia may be the ultimate common pathway in the mechanism of myocardial injury elicited by all adrenergic amines, since an identical pattern of ischemic cationic abnormalities was found to occur in the myocardium of both alpha and beta adrenergic amine-induced injury. This pattern starts with a fall in myocardial magnesium concentration, followed by a rise of calcium and sodium, and in severe injury, a reduction in potassium (48).

Lehr has concluded that it is ischemia rather than calcium overloading, as proposed by many other investigators that represents the primary mechanism of myocardial injury. Thus cardiac hypoxia is the main cause of the myocardial electrolyte disturbances which, in turn, may contribute to the initiation of a state of irreversible failure of cellular function. In particular, Lehr emphasized the role of magnesium loss. Myocardial necrosis involves an early obligatory loss of intracellular magnesium, which precedes the loss of potassium and the massive accumulation of calcium

and sodium.

Lehr's (48) studies of coronary artery ligation in the rat demonstrated the occurrence of early magnesium loss. The first significant changes in the myocardial bulk electrolyte content were apparent thirty minutes after ligation and consisted of depletion of magnesium, potassium, and phosphate. Significantly, calcium content was normal at that time. One hour after coronary artery occlusion, the first moderate rise in calcium content coincided with highly significant alterations of the other five electrolytes. Similar observations were made with myocardial injury produced by either alpha or beta adrenergic amines. One hour after injection of a necrotizing dose of either isoproterenol (beta agonist), phenylephrine (alpha agonist), or epinephrine (alpha and beta agonist), i.e., at a point in time when structural injury is as yet not readily demonstrable, significant loss of magnesium is uniformly present as the sole electrolyte disturbance. The calcium concentration begins to rise only subsequently, and is shown to have reached significantly increased levels at the three hour interval when necrosis is clearly apparent.

Further support for a contributory role of electrolyte shifts in the mechanism of cellular injury can be derived from studies indicating that administration of electrolyte solutions containing either magnesium or potassium salts results in protection against catecholamine-induced necrosis.

Lehr's view of the secondary nature of calcium overloading is supported by his studies with parathyroidecto-

mized rats (48). Such animals failed to show any noticeable degree of protection against catecholamine-induced myocardial necrosis despite complete prevention or highly significant inhibition of myocardial calcium accumulation. In addition, Lehr's group was able to confirm Fleckenstein's (40) observation of the propranolol-like protective effect of verapamil in isproterenol-induced myocardial necrosis. However, cardiac injury elicited by phenylephrine was likewise inhibited by verapamil but not by propranolol. Also, verapamil was equally protective in parathyroidectomized rats against isoproterenol-induced necrosis (that is, in the absence of myocardial calcium accumulation). Therefore, Lehr concluded that the beneficial effect of verapamil must be based on a property other than its calcium channel blocking capabilities and might be due instead to its negative inotropic, and thus oxygen sparing effects.

In line with the ischemic theory, Haft et al (49-51) has proposed that intracoronary platelet aggregation may contribute to the mechanism that leads to myocardial ischemia in catecholamine-induced cardiac necrosis. In view of the well known antiaggregating effects of aspirin and dipyridamole on platelets, Haft (50) pretreated ten dogs with aspirin and ten dogs with dipyridamole followed by infusion with epinephrine at 4 ug/kg/min for four hours. He found that only three animals in each group exhibited any myocardial necrosis whereas all animals in the nonpretreated control group exhibited significant myocardial necrosis. Furthermore, they were able to demonstrate platelet aggregates

within the small vessels of the hearts of dogs infused with norepinephrine (50) and in rats given isoproterenol (51). Other investigators, however, have been unable to confirm the finding of platelet aggregates in coronary vessels (27,42,43).

Hoak (16) found that following norepinephrine infusions in dogs only a few of the small myocardial vessels were noted to be occluded by platelet aggregates. However, these were not seen often enough to represent the sole cause of the myocardial necrosis and in no instance were coronary arteries found to be occluded. Therefore, it does not appear that platelet aggregation is a major factor in catecholmine-induced myocardial necrosis.

Several studies are inconsistent with the ischemia hypothesis. Ostadel et al (52) found that isoproterenol induces myocardial necrosis in the turtle. This is significant because the turtle does not have coronary arteries, but relies on the blood in the ventricular chambers to sustain the myocardium. These findings do not support Lehr's hypothesis that myocardial necrosis is precipitated by isoproterenol-induced decreased peripheral vascular resistance, decreased systemic blood pressure, and hence lower coronary blood flow resulting in ischemic cardiac injury. Ostadel's study does not rule out the possibility, however, that catecholamines increase the demand for oxygen so much that demand outstrips supply and causes relative anoxia.

Downing's (42,43) studies of norepinephrine-induced cardiomyopathy in rabbits indicate that a supply-demand mismatch causing ischemic injury to the myocardium is un-

likely. He found that although mean arterial blood pressure rose, the heart rate fell, and calculations of the pressure-rate product suggested no increase in myocardial oxygen consumption throughout the course of norepinephrine infusion. Also, the histological pattern and leukocytic response differed from that expected with myofiber necrosis following an ischemic insult. An ischemic insult results in a predominantly polymorphonuclear infiltrate. However, in catecholamine-induced cardiomyopathy, the infiltrate consists primarily of histiocytic mononuclear cells. Morever, ischemia in which coronary flow does not remain interrupted frequently is accompanied by capillary damage and interstitial hemorrhage. These were never observed in Downing's studies nor was coronary vascular injury or thrombus formation identified by light microscopy.

The second category of theories concerning pathogenesis of catecholamine-induced cardiomyopathy postulate that catecholamines have a direct cardiotoxic effect. The calcium overload concept of Fleckenstein is a major theory in this group. Isoproterenol-induced excessive calcium influx into the myocardial cell is thought to cause injury and necrosis by exaggerated activation of calcium-dependent ATPases and the consequent exhaustion of ATP and creatine phosphate reserves (39). For a better understanding of this theory, the normal mechanism of catecholamine action and salient features of myocardial energy metabolism will be presented.

Durret and Adams (53) recently reviewed the mechanisms by which catecholamines exert their effects. These agents

interact with beta-1 adrenergic receptors on the extracellular surface of the sarcolemma to elicit their positive inotropic and chronotropic effects on the heart. The mechanism responsible for the positive inotropic effect appears to be enhancement of the slow inward calcium current and increased intracellular calcium concentration. Activation of the beta receptor stimulates the enzyme adenylate cyclase, resulting in an increase in the intracellular concentration of cAMP. The exact mechanisms by which cAMP leads to increased contractility are not known. It is established that cAMP-dependent protein kinases phosphorylate proteins associated with myofibrils, the sarcoplasmic reticulum, and the sarcolemma. It may therefore affect contractility via several cellular mechanisms: 1)cAMP may act directly on the myofibrils by phosphorylation of myofibril components, F actin and troponin; 2)cAMP may increase calcium availability by enhancing calcium uptake by the sarcoplasmic reticulum; or 3)cAMP may also modulate calcium entry into the myocardial cell by inducing the phosphorylation of a specific protein in the sarcolemma by protein kinase, thereby, opening up membrane "channels" to allow the influx of calcium. The result is that the increase in calcium concentration inactivates the troponin-tropomyosin system and permits enhanced interaction of the actin and myosin filaments.

Bloom (54) noted that a special feature of myocardial energy metabolism is the existence of a large amount of creatine phosphate (CP), the alternative storage form to ATP for high energy phosphate. CP is also important to the myocardium as it serves to buffer the ATP concentrations,

i.e., as ATP is converted to ADP through normal metabolic processes such as contraction, the ATP can be resynthesized either by electron-transport related processes, or at the expense of creatine phosphate through the action of creatine phosphokinase. Thus, a fundamental function of oxidation and glycolysis consists of maintaining a sufficiently high level of ATP and CP in the myocardial fiber for two important functions. The first is to supply energy for contraction and to drive the "ion pumps" (active transport of sodium, potassium, and calcium ions connected with bioelectric and mechanical performance). And the second is to meet the energy expenditure for various synthetic reactions that are necessary for continuous cellular repair.

According to Fleckenstein (39,40,55,56), severe myocardial cell damage, as well as contractile failure, will occur when the high-energy phosphate stores are exhausted, as in excessive ATP consumption by the heart due to beta adrenergic stimulated mechanical hyperactivity. Physiologically, catecholamines increase myocardial tension by enhancing the utilization of high energy phosphates for contraction. Overdoses of catecholamines, however, stimulate the splitting of ATP in the contractile machinery so excessively that not enough ATP is left for the various synthetic processes which are involved in regeneration of the living structures. Fleckenstein was able to demonstrate that high energy phosphate becomes critical when the ATP concentration in myocardium is lowered by more than 50% and the corresponding CP concentration falls by more than 80% (40). He showed that

injections of isoproterenol into rats caused reductions in ATP and CP below the critical levels and this was associated with myocardial fiber necrosis (40).

In a series of studies, Fleckenstein et al (39,40,55,56) showed that the cardiotoxic effects of beta adrenergic catecholamines, including isoproterenol, are mediated by calcium ions, and that these ions play a key role in the production of cardiac necroses. After isoproterenol administration to rats, there is an increased movement of $^{45}Ca^{2+}$ from plasma into heart muscle, and the myocardial content of calcium can be observed to increase. In addition, the ATP content was found to fall. These workers also demonstrated that calcium ions are highly cardiotoxic if they are taken up excessively into the myocardial fibers; and that intracellular calcium overload initiates a breakdown of ATP and CP. Morever, calcium-induced high energy phosphate exhaustion is crucial in the etiology of myocardial fiber necroses produced by a number of cardiotoxic agents, including large doses of beta adrenergic catecholamines, vitamin D, dihydrotachysterol, cardiac glycosides, etc., or conditions of extreme physical or emotional stress.

The mechanism by which calcium overload leads to myocardial necrosis is somewhat complex. Catecholamines increase transmembrane calcium influx into the excited myocardial fibers. This leads to activation of the calcium dependent myofibrillar ATPase that transforms phosphate-bond energy into mechanical work as well as the calcium transport ATPases of the sarcoplasmic reticulum and mitochondria. With increasing doses of these drugs, continued calcium

uptake and excessive splitting of ATP and CP occurs. In addition, the increase in intracellular calcium leads to structural damage and calcification of the mitochondria, impairing their phosphorylating capacity. Active extrusion of calcium from the calcium-overloaded myocytes occurs as long as ATP is availabe for this purpose. But in the advanced stages of ATP deficiency, the calcium extrusion seems to decline rather rapidly, so that a steep rise of the intracellular calcium concentration occurs. The calcium overload initiates a breakdown of ATP and CP, with the result that cardiac function and structural integrity cannot be maintained.

The intracellular sites where myocardial calcium overload induces exhaustive ATP consumption include the myofibrils, sarcoplasmic reticulum, and mitochondria. The first reaction of the contractile system to excessive splitting of ATP by the calcium activated myofibrillar ATPase consists of a supercontraction. Abundant transsarcolemmal calcium influx produces a state of excitation-contraction "overcoupling" by which the myofilaments are eventually destroyed if the myocardial fiber cannot reduce the calcium overload in time. Apart from myofibrillar destruction, intracellular calcium overload is most injurious to the mitochondria. This is signaled by the appearance of intramitochondrial calcium phosphate precipitates. Fleckenstein (56) found that cardiac mitochondria in situ incorporate large amounts of calcium when overdoses of isoproterenol are administrated. Mitochondrial swelling, vacuolization, and cristolysis occur

probably as a self-defense mechanism (calcium-buffer) against a disproportionate rise in the cytoplasmic free calcium concentration that exaggerates ATP consumption. When, however, necrotizing doses of isoproterenol are administered, transsarcolemmal calcium inflow is so dramatically stimulated that there is a toxic accumulation of calcium.

Rona et al (57-60) have incorporated Fleckenstein's calcium overload concept into their own theory. While retaining the importance of coronary microcirculatory factors in the evolution of catecholamine-induced cardiomyopathy, the early sarcolemmal membrane permeability alterations demonstrated by their studies with horse radish peroxidase (HRP), suggest a direct toxic effect of catecholamines. In their studies, isoproterenol, norepinephrine, and epinephrine were administered to rats followed by injection of HRP. In control rats given HRP, the tracer became uniformly distributed in the myocardial interstitium and transverse tubular system with pinocytotic vesicles noted at the border of the sarcolemma of myocytes. However, the sarcolemma was never penetrated by the tracer. With norepinephrine and epinephrine infusion, HRP reached the interstitium more rapidly than in the controls and also appeared within some myocytes as early as ten minutes after infusion in the case of norepinephrine. Significantly, many of the cells containing HRP were ultrastructurally normal. Infusion of isoproterenol produced similar findings except they occurred after the isoproterenol-induced drop in blood pressure had returned to normal. The subsequent appearance of the tracer in the myocardial interstitium was followed by its presence

in some of the myocytes.

Rona attributed these differences in the temporal course of the appearance of HRP to the varying pressor and depressor properties of the catecholamines and their subsequent effect on the coronary microcirculation. Although norepinephrine and epinephrine constrict large coronary vessels, they dilate small coronary branches. When coupled with their pressor effect on systemic blood pressure, coronary blood flow is improved, thus accounting for the early interstitial appearance of HRP. In contrast, isoproterenol, through its vasodilating effects on the peripheral vasculature, causes an initial, transient hypotension which offsets the coronary vasodilation produced both directly and indirectly by increased metabolic demand and thus results in the delay in appearance of HRP.

Following infusion of all three catecholamines, one of the earliest changes noted was the passage of HRP across the sarcolemma of normal appearing cells to be deposited on myofibrils. This suggested that some form of injury resulting in early permeability had occurred. In addition, HRP showed a marked affinity for hypercontracted or necrotic myofilaments in structurally damaged cells. A later finding is HRP deposition along mitochondrial cristae indicating that the mitochondrial membrane had become permeable to the macromolecular tracer.

In several recent papers, Rona et al (59,60) have noted the similarity between reperfusion injury and catecholamine-induced injury, particularly that which follows isoprotere-

nol administration. They both exhibit contraction band formation and mitochondrial calcium phosphate deposits. Furthermore, permeability alterations of myocardial cells in reperfusion injury parallel those seen in catecholamine-induced injury. Cardiac cells made ischemic by coronary artery ligation for ten minutes followed by reperfusion for sixty minutes were structurally normal except for abundant lipid droplets. Sixty minutes of ischemia followed by reperfusion induced HRP deposition resembling that found with isoproterenol infusion in which there was contraction band necrosis with heavy deposition of HRP on hypercontracted myofilaments. These findings suggest a common causal pathway consisting of microcirculatory derangement plus direct cardiac muscle cell stimulation, possibly mediated by the release of catecholamines. Thus, Rona concluded that the pathogenesis of catecholamine-induced lesions involves relative hypoxia, altered membrane permeability, and primary myofilament stimulation. These act together to produce calcium overload which activates calcium dependent myofibrillar ATPase. Depletion of ATP and CP levels results in myofilament hypercontraction and loss of structural integrity as described by Fleckenstein.

Other mechanisms which have been suggested include increased serum free fatty acid (FFA) levels in view of the lipid deposits observed in hearts following administration of catecholamines (7,14). Hoak (16) found elevated serum FFA in dogs following infusions of norepinephrine. He theorized that the stimulatory effect of catecholamines on lipid metabolism plays an important part in the production of

cardiac lesions. With the mobilization of lipid from adipose tissue induced by catecholamines, the plasma level of FFA increases and may lead to their accumulation within the myocardial cell. Accumulation of long chain fatty acids can cause uncoupling of oxidative phosphorylation and direct tissue damage. However, catecholamine infusions in rabbits do not cause a concomitant increase in FFA unlike the dog or rat (15). In this species a mechanism of injury that does not involve an increase in plasma FFA must be present. Oxidation of FFA by the myocardial cell may certainly be inhibited during catecholamine injury resulting in cellular overload with fatty acids.

Mallov (61) also concluded that FFA are involved in the production of myocardial necrosis by catecholamines. Catecholamines increase FFA levels by stimulating lipolysis in depot fat. He suggested that high concentrations of catecholamines cause increased influx of calcium and FFA into myocardial cells, and that these are deposited as soaps. Soaps may produce alterations in plasma membrane permeability, permitting increased rates of influx and deposition to continue, ultimately causing cell damage and death.

Other investigators have concentrated on the direct cardiotoxic effects of catecholamine metabolites. Yates et al (62) proposed that oxidation products of catecholamines such as adrenochrome, rather than catecholamines per se, may play a role in the pathogenesis of catecholamine injury by causing cell necrosis and contractile failure. This was based on studies (62-64) in which perfusion of isolated rat

hearts with fresh isoproterenol or epinephrine failed to induce necrosis in the myocardium. But lesions were found in hearts that had been perfused with isoproterenol that had undergone spontaneous oxidation in solution. Furthermore, perfusion of rat hearts with adrenochrome produced similar lesions along with of contractile failure. The hemodynamic effects of catecholamines, including reduced endocardial perfusion resulting from lower diastolic blood pressure, shortened diastole, and/or coronary vascular changes, were thought to cause stagnation of blood flow and allow accumulation of catecholamine oxidation products. These factors, by potentiating the deleterious effects of catecholamine oxidation products on oxidative phosphorylation and glucose metabolism, could also produce relative hypoxia. Thus, they concluded that catecholamine-induced lesions resulted from the combined effects of catecholamines and their oxidation products, including the adrenochromes.

Free-radicals are produced during autoxidation of catecholamines, and Singal et al (25) suggested that catecholamine-induced changes in the heart may also involve an increase in free-radical activity. They found that pretreatment of rats with either vitamin E or zinc prevented isoproterenol injury. Since vitamin E is an antioxidant, it could act as a free-radical scavenger to prevent the cell membrane damaging effects of free radicals. Cell membranes contain high concentrations of highly unsaturated fatty acids and thus are susceptible to free-radical induced lipid peroxidation. This would result in membrane permeability alterations and allows the occurrence of intracellular cal-

cium accumulation and its deleterious consequences. Consistent with this is the fact that zinc has a membrane stabilizing effect probably by reducing free-radical induced membrane changes (24).

Peroxidation of membrane lipids is a concept proposed by Meerson (36) to explain the pathogenesis of cardiac lesions caused by excess release of catecholamines in severe emotional and painful stress. This theory is based on a model of emotional and painful stress in which rats wait intensely for painful electric shocks for six hours and actually receive them at occasional intervals. Hearts were studied at various periods following termination of stress induction ranging from 2 hours to 4 days. Accumulation of hydroperoxides of lipids (products of lipid peroxidation) and labalization of lysomal enzymes occurred in those animals subject to emotional/painful stress. Morphologically, there was hypercontraction and necrosis of myocardial fibers. Meerson suggested that the hydroperoxides of lipids and proteolytic lysosomal enzymes interfere with the membrane calcium transport apparatus and result in hypercontraction. In further experiments, he demonstrated that stress resulted in a significant decrease in capacity of sarcoplasmic reticulum membranes to accumulate calcium. This was thought to contribute to increased concentrations of calcium in the cytosol. Simultaneously, disturbances of oxidation and phosphorylation developed in mitochondria.

Based on the above findings, Meerson proposed a theory of stress-induced cardiomyopathy. Emotional/painful stress

causes excitation of the higher vegetative centers which leads to an increased concentration of catecholamines in the blood. The catecholamines, in addition to acting on adrenoceptors in the sarcolemma to activate adenylate cyclase, induce lipid peroxidation. These products (hydroperoxides) cause the release of proteolytic enzymes by lysis of lysosomes. The action of both the lysosomal enzymes and hydroperoxides of lipids results in damage to the sarcoplasmic reticulum membranes and sarcolemma. The mechanism for calcium transport is affected and excessive amounts of calcium accumulate in the cardiac cells causing what Meerson refers to as the "calcium triad." This includes contracture of myofibrils, damage to mitochondria with uncoupling of oxidation-phosphorylation, and activation of phospholipases and proteases. The result is hypercontraction and necrosis of myocardial fibers.

With respect to norepinephrine-induced cardiomyopathy, Downing et al have demonstrated that short term (ninety minute) infusion of norepinephrine given in relatively modest doses (2 to 3 ug/kg/min) elicits a consistent pattern of myocardial injury in the rabbit (42). Measurements of cardiac function have revealed significant impairment of left ventricular performance when studied with afterload curves (65) or standard ventricular function curves (66). In studying the possible mechanisms of norepinephrine-induced injury, this group showed that insulin significantly reduced the extent of myofiber injury when rabbits were infused with norepinephrine (42). Previous studies in the isolated muscle preparation as well as the intact swine heart showed

that insulin substantially reduces contractility responses to norepinephrine (67,68). This indicates that there may exist a relationship between inotropic stimulation and catecholamine cardiomyopathy. Downing suggested that insulin may in larger doses exert other effects perhaps similar to the membrane stabalizing action of steroids. In a recent study (43) designed to assess the receptor system predominantly involved in the pathogenesis of norepinephrine-induced lesions, Downing found that beta adrenergic blockade with practolol or propranolol failed to significantly reduce cardiac injury with norepinephrine. However, alpha adrenoceptor blockade with phentolamine alone or in combination with either of the beta antagonists, markedly reduced lesion formation. Administration of the alpha agonist methoxamine produced dose-related increases in the intensity of myocardial injury morphologically identical with those resulting from norepinephrine. The alpha blocking agent, phentolamine, markedly reduced methoxamine injury. Downing concluded that the norepinephrine cardiomyopathy results in large part from activation of the alpha adrenergic system in the rabbit model. He also speculated that alterations in myofiber calcium translocation, uptake, and binding induced by alpha-1 receptor activation may contribute to membrane damage. This is based on evidence for the existence of alpha receptors in cardiac muscle of several species, which, when activated, elicit inotropic changes. In contrast to the beta adrenergic system, activation of alpha receptors induces little reduction of time to peak tension, and relaxa-

tion time is lengthened, thus suggesting that altered myocardial calcium translocation is a primary event.

While the exact mechanism of catecholamine-induced cardiomyopathy remains to be established, experimental evidence indicates a major role for membrane permeability alterations leading to myocardial calcium overload. Ischemia due to coronary vasoconstriction appears unlikely to be an important pathogenetic mechanism. This is suggested by the fact that whereas catecholamines cause myocardial necrosis in the turtle, this species has no coronary arteries (52). Morever, the catecholamines probably do not evoke a net increase in myocardial oxygen demand (42,43) that might contribute to ischemia. The importance of calcium overload was suggested by investigations showing that verapamil protects against isoproterenol and epinephrine-induced myocardial necrosis (40,48). The mechanism of verapamil protection may be more complex than simple blockade of calcium influx, however. This is indicated by studies in which this agent also protected against isoproterenol-induced damage in parathyroidectomized rats. In this circumstance, myocardial calcium accumulation does not occur but myocyte injury does appear (48). It was therefore suggested that verapamil was acting through its negative inotropic and oxygen-sparing effects. However, this seems unlikely in view of the experimental evidence against the importance of ischemia in this process. The present study explores the question as to whether verapamil protects against norepinephrine-induced cardiomyopathy in the rabbit model, and examines the contri-

butions of afterload and cardiac metabolic demand as potential contributory mechanisms.

MATERIALS AND METHODS

A total of 50 New Zealand white rabbits were used in this study. All animals were anesthetized with pentobarbital, 30 mg/kg, and polyethylene catheters were placed in a femoral artery and vein. Arterial pressure was measured continuously with a Sanborn transducer, and heart rate was determined with a Sanborn cardiotachometer. The latter was verified by manual assessment of pulse frquency from pressure traces inscribed by a Sanborn recorder. Arterial blood samples were obtained at 15 minute intervals and analyzed for PO_2, PCO_2, and pH, with an Instrumentation Laboratories analyzer. Hematocrit and glucose concentrations (Glucostat, Worthington Biochemical) were also determined.

The animals were divided into four groups. One group of thirteen rabbits was infused with norepinephrine (Levophed, Winthrop) at 2 ug/kg/min (NE-2) for ninety minutes. A second group of ten rabbits received a 50 ug loading dose of verapamil about five minutes before beginning infusion with NE-2 and verapamil at 1 ug/kg/min (VE-1) for 90 minutes. A third group of eight rabbits was also pretreated with 50 ug of verapamil five minutes prior to infusion with NE-2 and verapamil at 2 ug/kg/min (VE-2). Ten rabbits were treated with norepinephrine at 3 ug/kg/min (NE-3), and four were given NE-3 in combination with VE-2. A group of five control animals was infused with saline for ninety minutes.

After infusion, the catheters were removed, the femoral wound surgically closed, and the animals returned to their cages after recovery from anesthesia. They were fed a

standard rabbit chow diet and given water ad libitum. All animals were sacrificed 2 to 3 days later by overdose of pentobarbital via ear vein. The hearts were immediately removed, emptied, and weighed. The atria and right ventricular free wall were dissected and weighed, and the left ventricle and septum separately weighed. Transverse "ring" sections of left ventricles were obtained from the basal and mid-portions and fixed in 10% buffered formalin. They were prepared by standard histological methods and stained with hematoxylin and eosin for the subsequent analysis.

Morphological evaluation employed a semiquantitative histological scoring system first described by Downing and Lee in 1978 (42). Each section was graded by 2 observers according to the extent and intensity of leukocytic response without prior knowledge of the procedures used in a given animal. A maximum score of 2.0 was given when the lesions were florid, extensive, and transmural. Those with definite but sparse lesions were scored 1.0. Equivocal focal lesions were scored 0.5. Those judged to manifest injury more extensive than 1.0, but less than 2.0 (e.g., nontransmural) were assigned a score of 1.5. A score of 0 was given when no histological abnormality was present. Values for each of the 2 sections were taken.

RESULTS

HISTOLOGICAL DATA

DESCRIPTIVE RESULTS: The characteristic histologic findings in rabbits sacrificed 48 hours after a 90 minute infusion of norepinephrine at 3 ug/kg/min (NE-3) are shown in figure 1. This received a histological score of 2.0. There is an intense cellular infiltrate confined mostly to the interstitium especially in association with foci of myofiber necrosis. The predominant cell population is mononuclear with large histiocytic cells being the most numerous accompanied by few lymphocytes. Polymorphonuclear cells including some eosinophils are occasionally present but in much smaller numbers. Extensive myofiber damage is present focally and numerous contraction bands and zones of granularity consistent with swollen mitochondria can also be seen. The Z lines are generally indistinct, and myofiber nuclei often are lost in the more active inflammatory foci.

These changes characteristic of norepinephrine-induced damage were most pronounced in the inner half of the ventricular wall (subendocardial and mid-zonal portions), however, transmural involvement was occasionally seen, especially in those hearts exposed to higher doses of norepinephrine. The papillary muscles were uniformly involved but there did not appear to be a difference in intensity of free wall or septal involvement. With regard to the coronary vasculature, no discernible histopathological changes were evident in either the larger coronary arteries or myocardial arterioles.

Figure 2 shows a section typical of the verapamil treated animals. There are definite but sparse lesions with damage limited to the subendocardium. Scattered foci of myofiber necrosis, cellular infiltration, and vacuolization are seen. It was given a score of 1.0. Figure 3 was taken from a saline control and illustrates the normal histologic appearance of myocardium.

QUANTITATIVE RESULTS: As described in the materials and methods section, basal and mid-level transverse slices from each heart were assigned an individual histologic score. All of the scores from a particular treatment group were averaged together into the overall score for that group.

All groups treated with norepinephrine only exhibited significantly higher ($p<0.05$) histological scores than the saline control group. Average histologic scores of the norepinephrine and verapamil treatment groups are shown in figure 3. The value of the group treated with norepinephrine at 2 ug/kg/min (NE-2) was 1.24 ± 0.10 (S.E.). Treatment of the rabbits with a 50 ug loading dose of verapamil followed by infusion of verapamil at either 1 ug/kg/min or 2 ug/kg/min (VE-1; VE-2) simultaneously with NE-2 infusion resulted in a significant reduction in myocardial injury as shown by a score of 0.59 ± 0.13 ($p<0.01$) in the VE-1 group, and 0.65 ± 0.10 ($p<0.01$) in the VE-2 group.

The 50 ug loading dose of verapamil followed by infusion of VE-2 also was protective against a higher dose of norepinephrine, i.e., 3 ug/kg/min infusion of norepinephrine (NE-3). As shown in figure 3, lower panal, the mean histo-

logic score of the NE-3 group was 1.44 $\pm$ 0.13. Simultaneous infusion of NE-3 and VE-2 resulted in a significantly lower score of 0.69 $\pm$ 0.21 ($p<0.01$).

Figure 5 shows the relationship of norepinephrine and verapamil dosages to histologic scores. There does not appear to be a substantial increase in protection with a higher dose of verapamil as there was no significant difference between the VE-1 and VE-2 groups (both of which were infused with NE-2). Also, the histologic score of the group given NE-3 along with VE-2 was not significantly different from the scores of the other two verapamil treated groups. The histologic scores of the NE-2 and NE-3 treatment groups were not significantly different from one another.

HEMODYNAMIC AND METABOLIC RESPONSES TO NOREPINEPHRINE AND THE EFFECT OF VERAPAMIL INFUSION

HEMODYNAMIC RESPONSES: Despite its protective effects against norepinephrine-induced histological damage, verapamil did not have a significant effect on the hemodynamic responses of the rabbit to norepinephrine. Figures 6 and 7 show the mean arterial pressure (MAP), heart rate (HR), and MAP x HR (P x R) product responses of the various treatment groups. In the control animals, saline infusion did not result in any significant changes in MAP, HR, or P x R product.

Figure 6 compares the hemodynamic responses of the NE-2 group to the NE-2 + VE-2 group (as well as the saline controls). The NE-2 + VE-1 group showed basically similar trends in each parameter and was omitted from the figure for

the sake of clarity. Initial MAP just prior to the beginning the drug infusions was 94 ± 5.0 (NE-2), 80 ± 3.2 (NE-2 + VE-1), and 95 ± 4.3 (NE-2 + VE-2). Initial HR was 249 ± 9.4 (NE-2), 243 ± 4.5 (NE-2 + VE-1), and 254 ± 9.1 (NE-2 + VE-2). Ten minutes after initiation of either norepinephrine infusion or norepinephrine + verapamil infusion, MAP rose in all three groups to 128 ± 5.6 (NE-2), 101 ± 5.6 (NE-2 + VE-1), and 118 ± 7.4 (NE-2 + VE-2). This was accompanied by a reflex bradycardia as the HR dropped to 145 ± 12.3 (NE-2), 186 ± 5.8 (NE-2 + VE-1), and 195 ± 9.4 (NE-2 + VE-2). During the 90 minute infusion period, MAP gradually declined and HR increased toward baseline values. Ten minutes after cessation of infusion, MAP had dropped below baseline values in all three groups to 62 ± 3.2 (NE-2), 65 ± 4.6 (NE-2 + VE-1), and 72 ± 3.4 (NE-2 + VE-2), while the mean HR had nearly reached baseline levels at values of 238 ± 6.5 (NE-2), 231 ± 9.4 (NE-2 + VE-1), and 253 ± 12.1 (NE-2 + VE-2).

Figure 7 illustrates the hemodynamic responses of NE-3 and NE-3 + VE-2 treatment groups and shows basically the same pattern as described above with the MAP rising to a peak value at ten minutes after initiation of the infusion accompanied by reflex bradycardia. There followed a gradual decline in MAP towards baseline values and accompanying rise in HR, followed by a drop below baseline values by ten minutes after cessation of the infusion.

It should be noted that all of the verapamil treated groups received a 50 ug loading dose of verapamil five minutes prior to beginning the infusion of norepinephrine

and verapamil. This resulted in a slight fall in MAP presumably due to the vasodilating effects of verapamil on the peripheral vasculature with a reflex tachycardia (see figures 6 and 7).

In view of its vasodilating effects on the peripheral vasculature, it is conceivable that verapamil may protect the myocardium from norepinephrine-induced damage via reduction of afterload. In order to assess this possibility, the effects of verapamil on MAP responses to norepinephrine were examined. Table 1 summarizes the blood pressure (BP) changes in the various groups at zero and ten minutes. It also shows the values for the difference between BP at ten minutes (the time at which maximal BP rise had occurred) and control BP and the difference between the integrated BP over the ninety minute infusion period and control BP. Initial BP of the NE-2 and NE-2 + VE-2 groups were essentially the same at values of 94 $\pm$ 5.0, and 95 $\pm$ 4.3 mmHg, respectively. The NE-2 + VE-1 group had a slightly lower initial BP of 80 $\pm$ 3.2 ($p<0.05$). BP rose in all three groups ten minutes after initiation of infusion. While the two groups treated with verapamil attained lower absolute BP than the NE-2 group, only the NE-2 + VE-1 was significantly lower at 101 $\pm$ 5.6 ($p<0.01$). This group also started out at a control BP that was about 15 mmHg lower than the other 2 groups. The NE-2 + VE-2 group had a BP at ten minutes of 118 $\pm$ 7.4 which was not significantly different from the NE-2 group's BP of 128 $\pm$ 5.6.

The change in BP from the control value to the ten

minute value was compared between the NE-2 group and the two verapamil treatment groups (Table 1). The rise in MAP in the verapamil treatment groups was lower than the NE-2 group. However, there was no statistically significant difference between the NE-2 group and the NE-2 + VE-2 group which had rises in BP of 34 $\pm$ 2.2 and 23 $\pm$ 4.0 mmHg respectively at ten minutes. The NE-2 + VE-1 group had a smaller rise in BP of 21 $\pm$ 5.9 mmHg ($p<0.05$). In rabbits treated with NE-3 or NE-3 + VE-2, the initial blood pressures were nearly identical at 102 $\pm$ 4.8 and 100 $\pm$ 3.3 mmHg, respectively. There was no statistically significant difference between the two groups in terms of the absolute BP achieved at ten minutes or the change in BP over ten minutes.

The difference between initial BP and the average BP integrated over ninety minutes was also compared. There was no significant difference found between the NE-2 group and the NE-2 + VE-1 group or the NE-2 group and the NE-2 + VE-2 groups. There was also no significant difference between the NE-3 and NE-3 + VE-2 groups. Thus, while verapamil treatment did appear to cause some blunting of the rise in MAP in response to norepinephrine infusion, this reduction in afterload was probably not enough to be a major factor in its protective effect on the myocardium. In support of this is the fact that the NE-2 + VE-2 and NE-3 + VE-2 had MAP values and changes in BP that did not differ significantly from their respective norepinephrine infusion groups, but exhibited significantly lower histologic damage scores. Also against the role of a certain absolute BP leading to myocardial damage is the fact that the BP at ten minutes

after initiation of infusion for the NE-3 + VE-2 group was higher than the BP of the NE-2 group (134 ± 5.8 vs. 128 ± 5.6 mm Hg, respectively). Despite the tendency for a higher average BP (though the difference was not statistically significant), the histologic score of the NE-3 + VE-2 group was significantly lower than that of the NE-2 group ($p < 0.01$).

Further evidence against excessive hemodynamic loading as a main factor in norepinephrine-induced cardiac damage is presented in figures 8 and 9. In these figures, the rabbits in the NE-2 group are separated into 2 groups based on their histologic scores: those with scores less than or equal to 1.0, and those with scores greater than or equal to 1.5. In figure 8, the maximum BP attained during the ninety minute infusion period is compared between the groups. Figure 9 is a comparison between the groups of the integrated mean BP over the ninety minute infusion period. There was no significant difference in maximum BP between the high score and low score groups which had maximum BP's of 134 ± 5.9 and 123 ± 9.6 mmHg respectively. There was also no significant difference in the integrated BP over the ninety minute infusion period between the groups. The high score groups had an integrated BP of 116 ± 5.2 mmHg, and the low score had a value of 107 ± 7.7 mmHg.

CARDIAC METABOLIC DEMAND: The MAP X HR (P x R) product for each group is shown in the lowermost panels of figures 6 and 7; and values at 0, 10, 30, 60, and 90 minutes are summarized in table 2. For all the groups, the P x R remained

nearly constant throughout the infusion and in no instance was the initial control value exceeded. The initial values ($x10^3$) were 29.3 $\pm$ 1.8 (saline controls), 23.7 $\pm$ 1.8 (NE-2), and 25.7 $\pm$ 1.8 (NE-3). The P x R values at 0, 10, 30, 60, and 90 minutes for the saline controls were compared to the NE-2 and NE-3 groups. The P x R values for the NE-3 groups did not differ significantly from the saline cntrols, while the P x R values for the NE-2 group did not differ significantly from the saline controls except at the 60 and 90 minute points where the NE-2 P x R product was significantly lower ($p<0.01$). At no point did the P x R product of either norepinephrine group rise or exceed the values of the saline controls. Thus, it appears unlikely that excessive metabolic demand was a significant factor in the pathogenesis of the myocardial lesions caused by norepinephrine. Furthermore, when the P x R product at each interval was compared between the norepinephrine infusion groups and the norepinephrine + verapamil infusion groups, the only statistically significant difference was between the control P x R product of NE-2 + VE-1 which was lower than that of NE-2 ($p<0.05$). However, this value was obtained prior to the infusion of verapamil + norepinephrine. Thus, verapamil treatment did not result in significantly lower P x R products, and therefore it is unlikely that verapamil could have exerted its protective effect by decreasing metabolic demand.

ARTERIAL pH, BLOOD GASES, SERUM GLUCOSE, AND HEMATOCRIT CHANGES

The pH in all treatment groups showed a tendency towards a slight decrease during the infusion followed by return to baseline values ten minutes after the infusion was stopped. This may be a reflection of a slight respiratory acidosis which is indicated by the increase in pCO_2 values that occurred in all five treatment groups during the infusion period. There was a return towards baseline values ten minutes after cessation of the infusion. In contrast, the saline controls exhibited a slight rise in pH which was probably a result of the slight fall in pCO_2 values during the infusion period. Arterial pO_2 values in all five treatment groups showed minimal change during the infusion period with only a slight increase observed ten minutes after cessation of infusion.

Figures 10 and 11 show the plasma glucose values of all the groups. It can be seen that the dramatic rise in plasma glucose caused by norepinephrine infusion was unaltered by simultaneous infusion of verapamil. The curves for all treatment groups show nearly identical rises in plasma glucose in contrast to the flat saline control curves. The plasma glucose in the NE-2, NE-2 + VE-1, and NE-2 + VE-2 groups rose progressively from about 168 mg/dl to a maximum value of about 375 mg/dl ten minutes after cessation of infusion. The NE-3 and NE-3 + VE-2 groups had an initial plasma glucose of about 123 mg/dl, which rose to a maximum of about 371 mg/dl. The saline controls showed minimal changes in serum glucose during infusion and averaged about

155 mg/dl.

The hematocrit values of all groups are also shown in figures 10 and 11. The norepinephrine and verapamil treatment groups showed an average drop in hematocrit of about 4%, presumably due to hemodilution by fluid infusion. This probably was not physiologically significant. Thus, the changes in the measured physiological parameters induced by norepinephrine infusion were essentially unchanged by verapamil.

DISCUSSION

The results of this study indicate that verapamil is capable of reducing the severity of norepinephrine-induced cardiomyopathy. It was found that administration of VE-1 or VE-2 simultaneously with NE-2 significantly reduced the histologic score from 1.24 to 0.59 (VE-1) and 0.65 (VE-2). The VE-2 infusion was also protective against a higher dose of norepinephrine (3 ug/kg/min) reducing the score from 1.44 to 0.69. There did not appear to be an increased amount of protection with a higher dose of verapamil as the scores of the VE-1 and VE-2 groups were not significantly different. All of the verapamil treated animals received a 50 ug bolus of verapamil prior to the infusion to rapidly achieve a therapeutic level which could be maintained by the infusion.

These findings are in agreement with previous studies which showed that verapamil is able to reduce the myocardial damage caused by catecholamines (48,69). Fleckenstein (69), in a review of his studies of catecholamine cardiomyopathy, cited a experiment in which he demonstrated that verapamil was capable of protecting the rat heart against structural damage if given in appropriate dosage (50 mg/kg) simultaneously with isoproterenol (30 mg/kg) subcutaneously. Based on a number of studies involving myocardial uptake of radioactive calcium, Fleckenstein found that subcutaneous administration of 30 mg/kg of isoproterenol resulted in an increase in the uptake of $^{45}Ca^{2+}$ by a factor of six to ten with a maximum level in six hours. Simultaneous administration of verapamil significantly inhibited the excessive

isoproterenol-induced radiocalcium uptake. Fleckenstein also described experiments in which verapamil (50 mg/kg) was effective in inhibiting the isoproterenol-induced breakdown of creatine phosphate fraction in the left ventricular myocardium of rats. Based on these data, he attributed the protective effect of verapamil against isoproterenol to its calcium channel blocking abilities by preventing calcium overload induced by high doses of catecholamines. The latter initiates breakdown of ATP and creatine phosphate leading to high-energy phosphate exhaustion via activation of the calcium dependent myofibrillar ATPase responsible for transforming phosphate bond energy into mechanical work.

The mechanism of verapamil-induced protection is probably not due simply to its calcium channel blocking activities to prevent high-energy phosphate depletion. Lehr (48), in a review of his studies on catecholamine cardiomyopathy found that verapamil was protective against isoproterenol. But he also found that verapamil was equally effective in parathyroidectomized rats in which myocardial calcium accumulation does not occur. Parathyroidectomy alone does not protect against isoproterenol-induced injury. Based on these findings, he concluded that calcium overloading is not a mechanism directly responsible for the cardiac injury produced by catecholamines, and that the beneficial effect of verapamil is based on a property other than the prevention of ATP depletion by calcium dependent ATPases. He suggested that verapamil may be protective via an oxygen sparing effect resulting from its negative inotropic and vasodilator actions.

Lehr also found that identical myocardial injury could be produced by phenylephrine, an alpha adrenergic agonist, and that verapamil protected against myocardial necrosis elicited by this drug. He postulated that at cardiotoxic doses, epinephrine acts primarily as an alpha adrenergic agonist. Thus, rats pretreated with theophylline, which inhibits cAMP breakdown by phosphodiesterase, show aggravated myocardial injury induced by isoproterenol. But myocardial injury induced by both phenylephrine and epinephrine are significantly reduced. This was based on the assumption that the stimulation of adenylate cyclase with subsequent formation of cAMP is a mechanism specific for beta, and not alpha receptors. Beta blockade with propranolol prevented isoproterenol-induced injury but had no effect on damage caused by phenylephrine. It was only marginally effective against tissue damage by epinephrine. Furthermore, alpha blockade significantly inhibited the development of epinephrine-induced necrosis. He suggested that ischemia may be a common denominator in myocardial necrosis elicited by alpha and beta adrenergic amines but that alpha agonists create a myocardial oxygen debt. This likely results from enhancement of the workload resulting from increased peripheral resistance (hypertension), probably in the face of impeded blood supply to the myocardium due to coronary vasoconstriction. In contrast, beta agonists cause ischemia by a combination of reduced coronary blood flow (CBF) from hypotension, and oxygen wastage by the intense positive inotropic and chronotropic effects of excessive stimulation of

myocardial beta receptors.

Norepinephrine, like epinephrine, also has both alpha and beta adrenergic activities, and Downing et al (43) demonstrated a major role of the alpha adrenergic system in norepinephrine-induced cardiomyopathy. They found that beta adrenergic blockade with practolol or propranolol failed to significantly reduce cardiac injury with norepinephrine. However, alpha receptor blockade with phentolamine markedly reduced lesion formation by norepinephrine. Furthermore, administration of the alpha agonist, methoxamine, produced myocardial injury which was morphologically identical to that of norepinephrine. Phentolamine sharply reduced methoxamine-induced myocardial injury. Arterial pressure and heart rate changes caused by methoxamine were the same as those caused by norepinephrine. In both norepinephrine and methoxamine treated animals, phentolamine prevented the rise in arterial pressure and reduced the extent of reflex cardiac slowing.

According to Lehr's theory, alpha agonists induce myocardial injury by causing ischemia. This is secondary to increased peripheral vascular resistance and probably impaired blood supply to the myocardium due to coronary vasoconstriction. Verapamil acts via its oxygen sparing effect, by reducing metabolic demand and afterload, thereby protecting the myocardium from the damaging effects of norepinephrine. The results of the present study indicate that these two mechanisms are probably unlikely. The P x R product (blood pressure x heart rate) has been shown to be the hemodynamic parameter that correlates best with myocardial

oxygen consumption (r = 0.86) (70). However, verapamil did not significantly lower the P x R product at any time during norepinephrine infusion in this study. This is in agreement with previous observations in patients with ischemic heart disease in which the administration of verapamil had no significant effect on MVO_2 (71,72). It suggests that verapamil does not exert its protective effects by decreasing metabolic demand. In addition, this study confirms previous findings that norepinephrine infusion does not result in an increase in metabolic demand (43,73). The NE-2 and NE-3 groups had P x R products that at no point rose or exceeded the values obtained in the saline controls. Thus, it is unlikely that excessive metabolic demand plays a major role in norepinephrine-induced cardiomyopathy.

Another way in which verapamil might decrease norepinephrine-induced damage is via afterload reduction. It is well established that verapamil has profound vasodilatory effects on vascular smooth muscle, especially the arteriolar beds (74). The present study showed a slight drop in systemic blood pressure five minutes after administration of the loading dose of verapamil. However, if verapamil was to protect against the effects of norepinephrine by reducing peripheral resistance, it should be expected to significantly diminish the rise in systemic blood pressure. This mechanism appears unlikely because in the verapamil treated groups, hemodynamic parameters followed the same patterns as the norepinephrine treated groups. Blood pressure rose to a peak value about ten minutes after initiation of the infu-

sion and this was accompanied by a reflex bradycardia. This was followed by a gradual decline in blood pressure towards baseline values and an accompanying rise in heart rate. While the blood pressure rise and absolute blood pressure at ten minutes was slightly lower in the verapamil treatment groups, the differences were not significant between the NE-2 + VE-2 and NE-2 groups, or between the NE-3 + VE-2 and NE-3 groups. Morever, when the average rise in blood pressure over the ninety minute period was compared between the norepinephrine treatment groups and the verapamil treatment groups, no significant differences were found. Thus, it seems probable that the slight reduction in the norepinephrine-induced rise in blood pressure caused by verapamil was not a major factor in the protective effect of verapamil.

While both the NE-2 + VE-2 and NE-3 + VE-2 groups exhibited blood pressure alterations that did not differ significantly from their respective norepinephrine treatment groups, the verapamil treated rabbits had significantly lower histological scores. Furthermore, the absolute blood pressure attained after ten minutes of infusion for the NE-3 + VE-2 groups tended to be higher than that attained by the NE-2 group (though not statistically significant). But the histological score of the NE-3 + VE-2 group was sharply lower than that of the NE-2 group ($p<0.01$). Further evidence against excessive hemodynamic loading as an important mechanism in norepinephrine-induced cardiomyopathy was obtained by comparing the maximum blood pressure and the integrated blood pressure over ninety minutes in a high histological score group (greater than or equal to 1.5) and a low

score group (less than or equal to 1.0). No difference was demonstrated by either analysis.

Lehr (48) also suggested a possible pathogenetic role of impaired blood supply to the myocardium caused by coronary vasoconstriction. Simons (73) recently found that norepinephrine causes an increase in coronary blood flow (CBF) in the rabbit from 2.66 ml/g/min initially to 3.46 after three minutes of norepinephrine infusion ($p<0.05$). CBF declined to baseline values (2.33) after ten minutes, but showed a sharp decline to 1.51 after forty minutes of infusion. Coronary resistance (CR) rose progressively from baseline values of 40.9 units to 74.8 at forty minutes ($p<0.05$). Animals given phentolamine manifested none of these changes in CBF and CR and also had significantly less norepinephrine-induced damage compared to norepinephrine alone ($p<0.01$). He concluded that norepinephrine induces sustained coronary vasoconstriction in the rabbit and that reduced CBF may contribute to the pathogenesis of norepinephrine cardiomyopathy in the rabbit. Since it is well established that the coronary bed is exquisitely sensitive to the vasodilating effects of calcium channel blockers (75), it is possible that verapamil exerts a protective effect via this mechanism. These agents exert a potent coronary vasodilator effect in the isolated rabbit heart (76). In dogs, CBF increases and CR decreases, though no change occurs in the caliber of the large coronary arteries. The resistance vessels are too small to be seen by coronary arteriography (77).

While stimulation of alpha receptors to induce coronary vasoconstriction may contribute to norepinephrine-induced cardiomyopathy, the possible role of myocardial alpha receptors must also be considered. The calcium channel blockers have recently been found to have alpha receptor blocking properties. Hence, this is another mechanism by which verapamil may exert its protective effects. In a recent review, Benfey (78) summarized studies which show that myocardial alpha receptors alter myocardial contractility. Alpha receptor mediated positive inotropic effects have been observed in isolated heart preparations from animals and humans (79). In the absence of beta receptor blockade, alpha blockade with phentolamine potentiates the inotropic effect of epinephrine and norepinephrine on rat ventricle strips. In the presence of beta blockade, however, it inhibits the effect of the catecholamines (80). Phentolamine, and not propranolol, inhibits the inotropic effect of low concentrations of epinephrine and phenylephrine in rabbit atrium (81). Methoxamine in suitable concentrations elicits substantial increases in force development in rat right ventricular papillary muscle and rat atrium (82,83). Methoxamine elicits dose-related increases of left ventricular contractility in the lamb as well (84). Aass et al (85) demonstrated that the myocardial alpha receptors in rabbit heart ventricle can be activated by norepinephrine to produce an inotropic effect. However, beta blockade with propanolol was required to unmask the alpha response. These effects were eliminated by alpha-1 blockade with prazosin. In view of these several findings, it is likely that norepi-

nephrine-induced cardiomyopathy involves activation of the myocardial alpha receptor system.

Recent studies have indicated that selected calcium channel blockers, notably verapamil, are capable of antagonizing binding to myocardial alpha receptors (86-90). Both Endoh et al (86) and Siegl (91) have shown that D600 (the methoxy derivative of verapamil) inhibits alpha adrenoceptor-mediated positive inotropic effects of phenylephrine. Sensitivity to isoproterenol was only partly inhibited, with no decrease in maximal effect. It is interesting to note that Motulsky et al (89) found that of all the calcium channel blockers, only verapamil and its analogues could block binding to alpha receptors. They attributed this to its greater structural similarity to epinephrine than other calcium channel blockers such as nifedipine or diltiazem. There also exists wide species variability in myocardial adrenergic receptor numbers. Mukherjee et al (90) found markedly decreased numbers of alpha and increased numbers of beta receptors in the canine as compared to rabbit or rat myocardium. They also found differences in the degree to which various calcium channel blockers competed with alpha antagonists for binding in different species. Notably, only in rabbit myocardium does verapamil antagonize alpha receptor binding at moderate concentrations (half maximal binding value of 5×10^{-7} M) whereas verapamil in canine and rat myocardium, and D600 in all three species antagonize alpha receptors only at relatively high concentrations. Thus, it is likely that verapamil exerts a protective effect against

norepinephrine-induced cardiomyopathy in the rabbit by inhibiting activation of the myocardial alpha receptor system.

The calcium channel blocking effects of verapamil may also be an important mechanism. Influx of calcium is likely involved in the inotropic effects of alpha receptors. Alpha receptors, in contrast to beta receptors, induce little reduction of time to peak tension, and relaxation time is lengthened. These mechanical events are consistent with stimulation by calcium ions. Indeed, altered calcium translocation may be a final common pathway of cell death (42). It has been recently suggested that accelerated calcium-induced membrane damage may be an important mechanism in the sequence of cell death (92,93). Regardless of the type of initial injury (ischemic or "toxic" damage), the myocyte undergoes calcium accumulation, either by impaired energy metabolism and/or by plasma membrane alterations. Elevated intracellular calcium concentrations are responsible for cytoskeletal modifications that alter cell shape, activate phospholipases that perpetuate membrane damage, and finally lead to mitochondrial calcification. The relationship of norepinephrine-induced cardiomyopathy to this scheme is of course speculative. But it represents a potential mechanism through which verapamil may act to interrupt calcium-induced injury.

In summary this study has demonstrated that verapamil causes a substantial reduction in the severity of damage to the myocardium caused by norepinephrine. It appears unlikely that verapamil exerts its protective effects via reductions in afterload or metabolic demand because hemodynamic

responses to norepinephrine were unaltered and there was no significant decrease in the P x R product. There are several possible mechanisms by which verapamil may exert its protective effects. These include prevention of norepinephrine-induced coronary vasoconstriction, inhibition of myocardial alpha receptor activation, and prevention of lethal calcium accumulation within the cell. The latter is likely the common pathway of cell death regardless of the initial mechanism of injury. Indeed, verapamil may act at each of these sites. Further studies will be required to elucidate more precisely the individual mechanisms by which verapamil exerts its protective effects against norepinephrine-induced cardiomypathy.

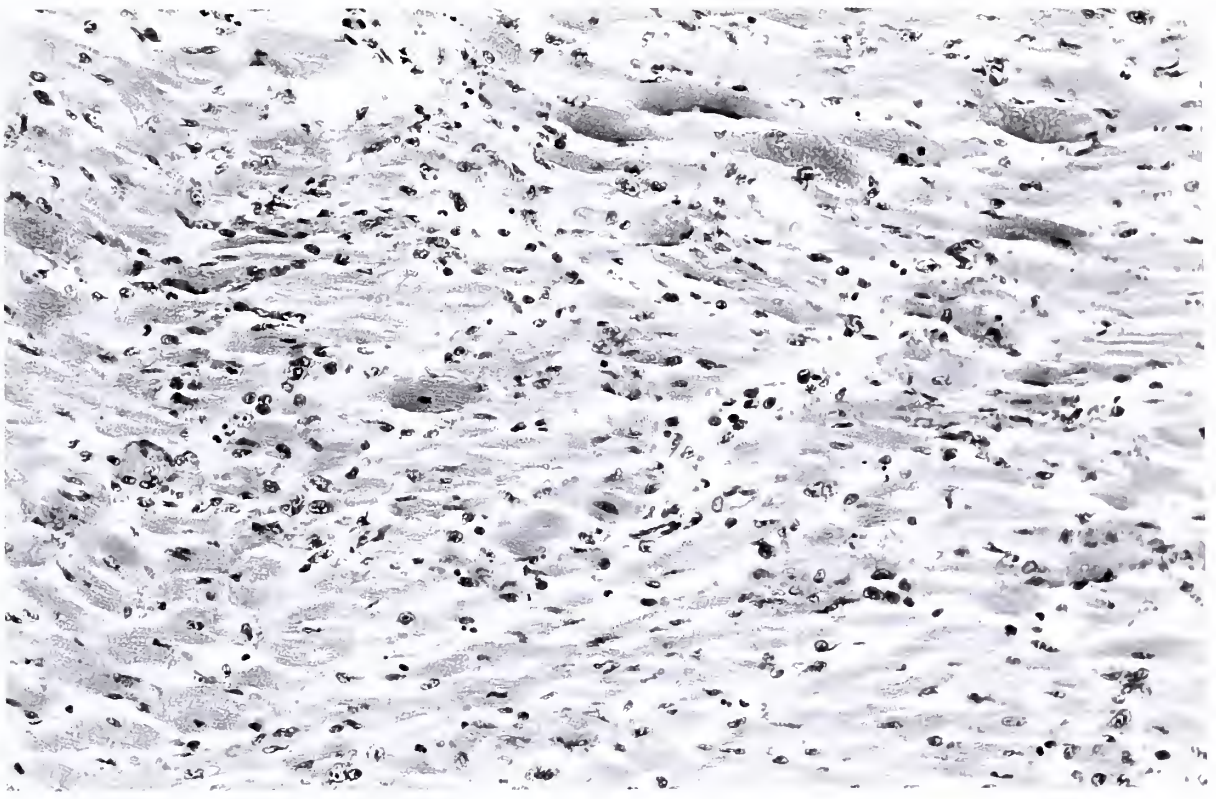

FIGURE 1. Representative histologic (H & E stain) section of left ventricular myocardium from a rabbit sacrificed 48 hours after a ninety minute infusion of norepinephrine at 3 ug/kg/min. There is extensive leukocytic infiltration, contraction band formation, and focal myofiber damage characteristic of lesions scored 2.0. Original magnification 200x.

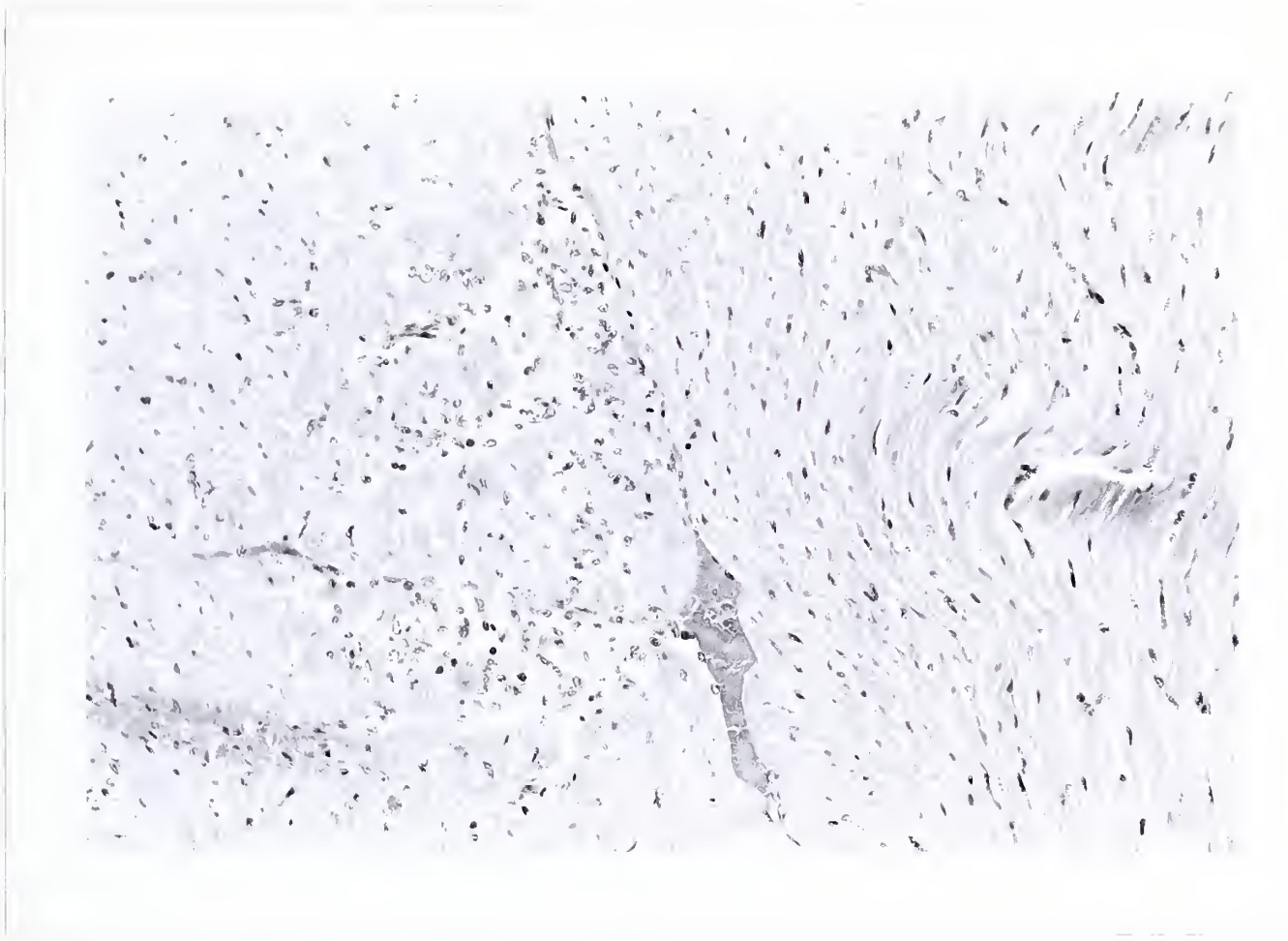

FIGURE 2. Representative histologic (H & E stain) section of left ventricular myocardium from rabbit sacrificed 48 hours after simultaneous infusion with verapamil at 1 ug/kg/min and norepinephrine at 2 ug/kg/min for ninety minutes. There are definite but sparse lesions with damage limited to the subendocardium and less extensive necrosis, cellular infiltrate, and vacuolization characteristic of lesions scored 1.0. Original magnification 200X.

FIGURE 3. Representative histologic (H & E stain) section of left ventricular myocardium from a rabbit sacrificed 48 hours after saline infusion for ninety minutes which illustrates the normal histologic appearance of myocardium. Original magnification 200X.

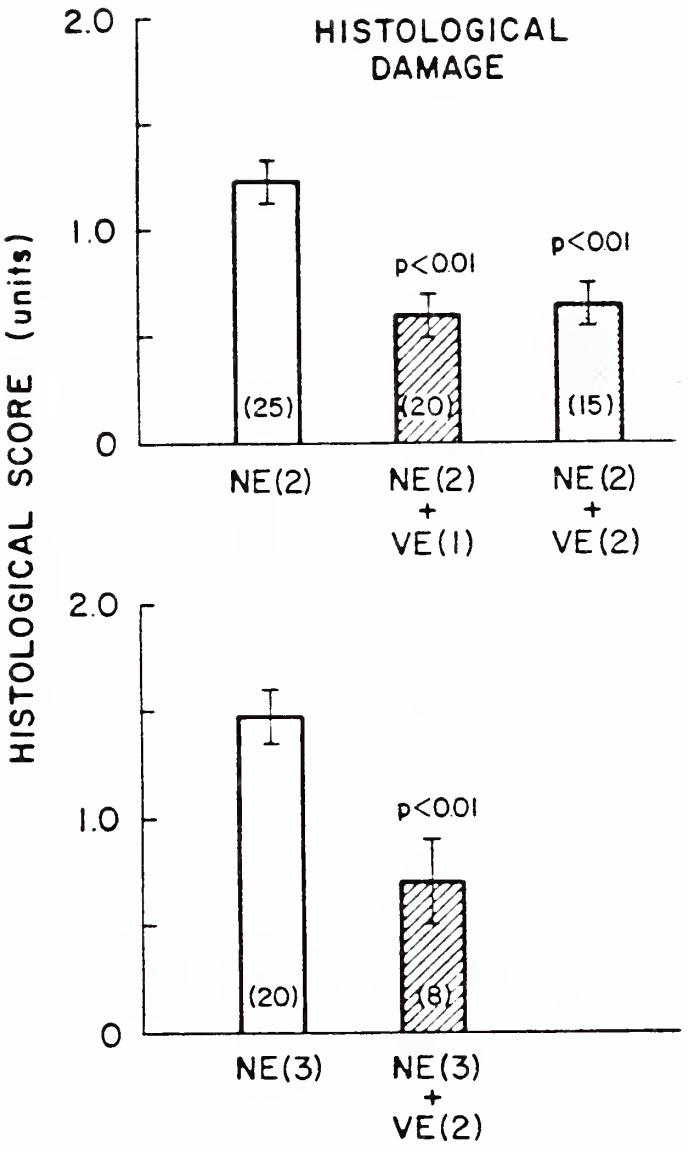

FIGURE 4. Histological scores from hearts in various groups of rabbits infused with norepinephrine only (NE) or NE simultaneously with verapamil (VE). Numbers in parentheses indicate ug/kg/min for NE and VE. Numbers within columns represent the number of animals in each group. Values are expressed as mean histological scores ± SEM.

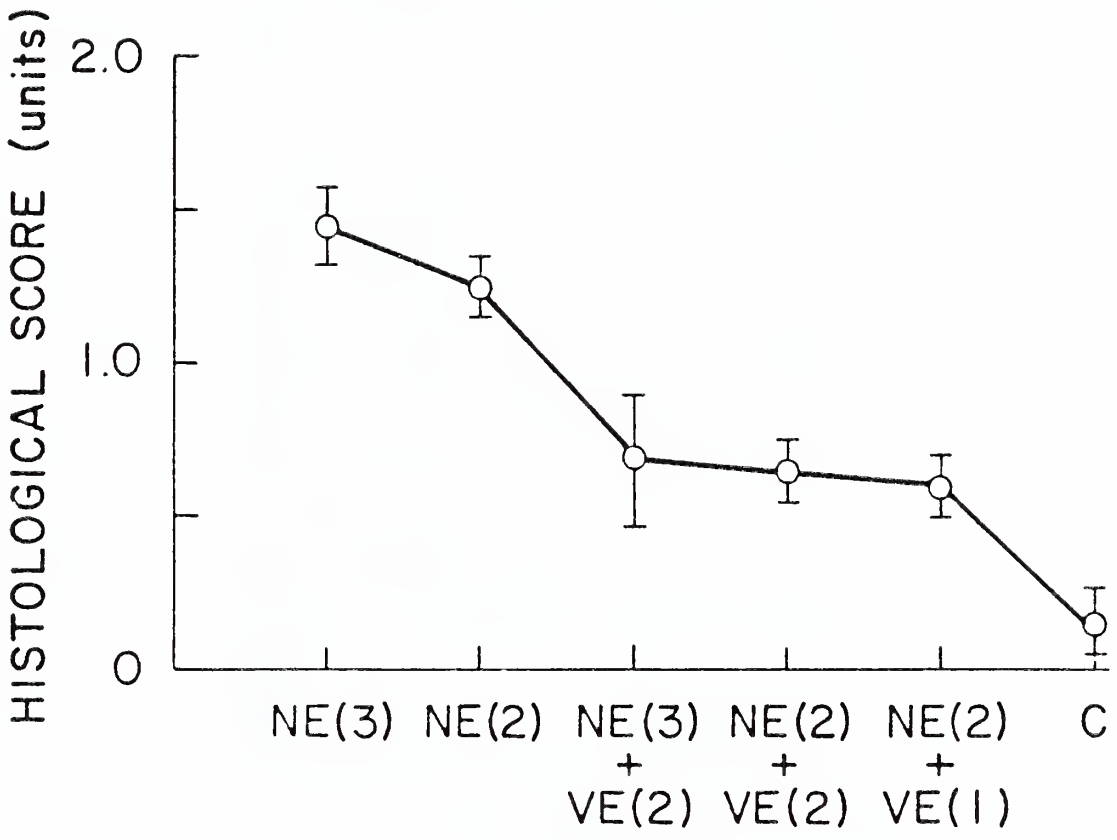

FIGURE 5. Relationship of norepinephrine (NE) and verapamil (VE) dosages to histological score. Numbers in parentheses indicate ug/kg/min for NE and VE. C = control group of saline infused rabbits. Values are expressed as mean histological scores ± SEM.

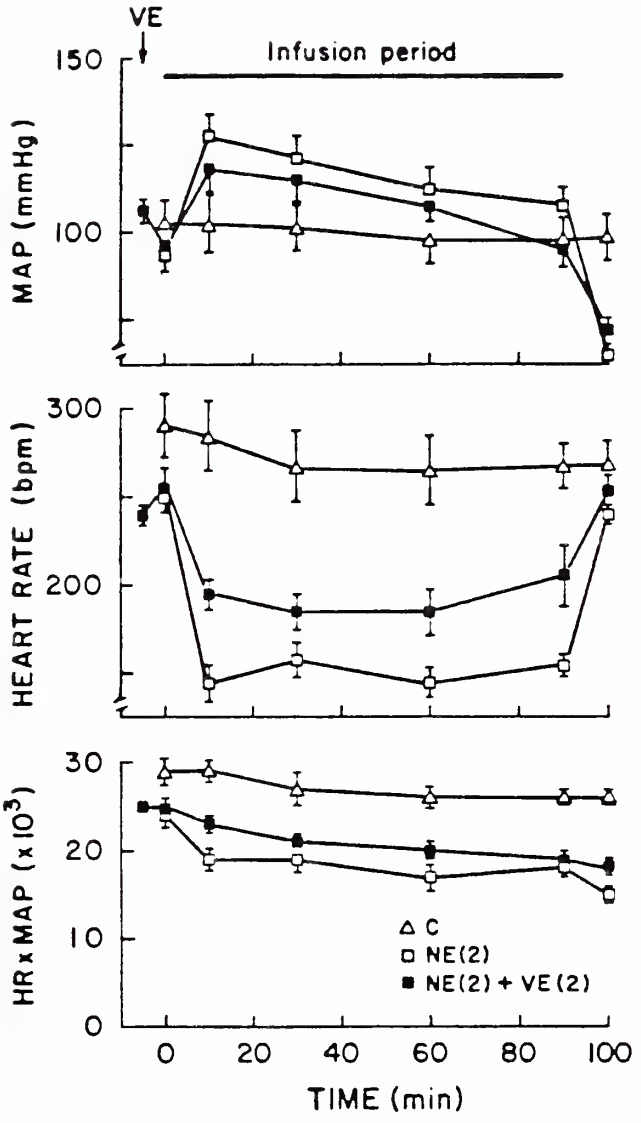

FIGURE 6. Values of the mean arterial pressure (MAP), heart rate (HR), and pressure-rate product (MAP X HR) at designated intervals before, during, and following norepinephrine (NE), NE + verapamil (VE), or saline (C) infusion. Numbers in parentheses indicate ug/kg/min for NE and VE. A 50 ug loading dose of VE was given five minutes prior to beginning infusion with NE and VE as indicated. Values are expressed as means $\pm$ SEM.

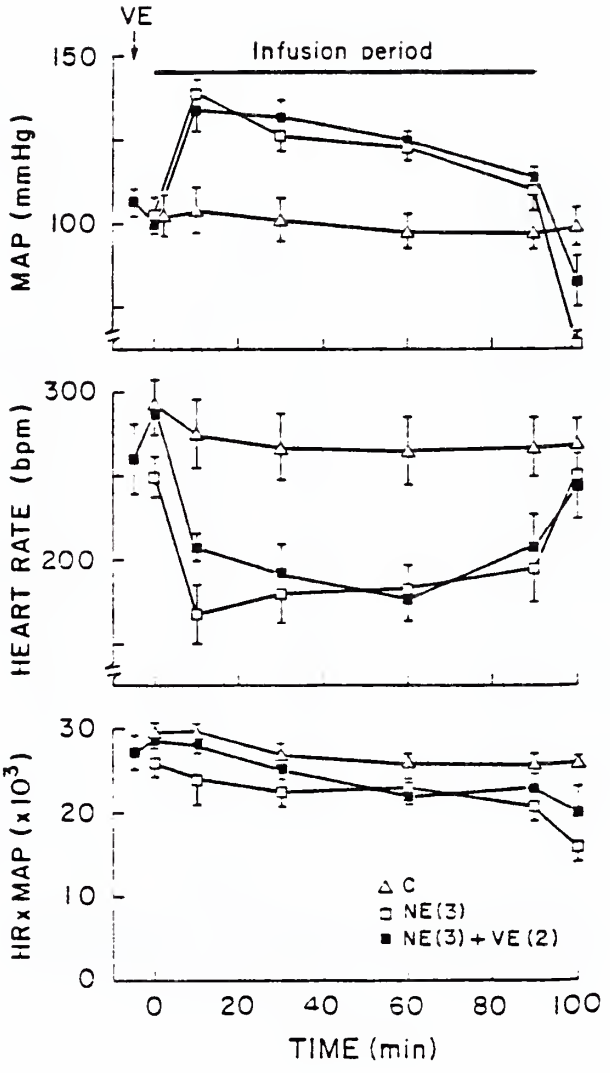

FIGURE 7. Values of the mean arterial pressure (MAP), heart rate (HR), and pressure-rate product (MAP X HR) at designated intervals before, during, and following norepinephrine (NE), NE + verapamil (VE), or saline (C) infusion. Numbers in parentheses indicate ug/kg/min for NE and VE. A 50 ug loading dose of VE was given five minutes prior to beginning infusion with NE and VE as indicated. Values are expressed as means $\pm$ SEM.

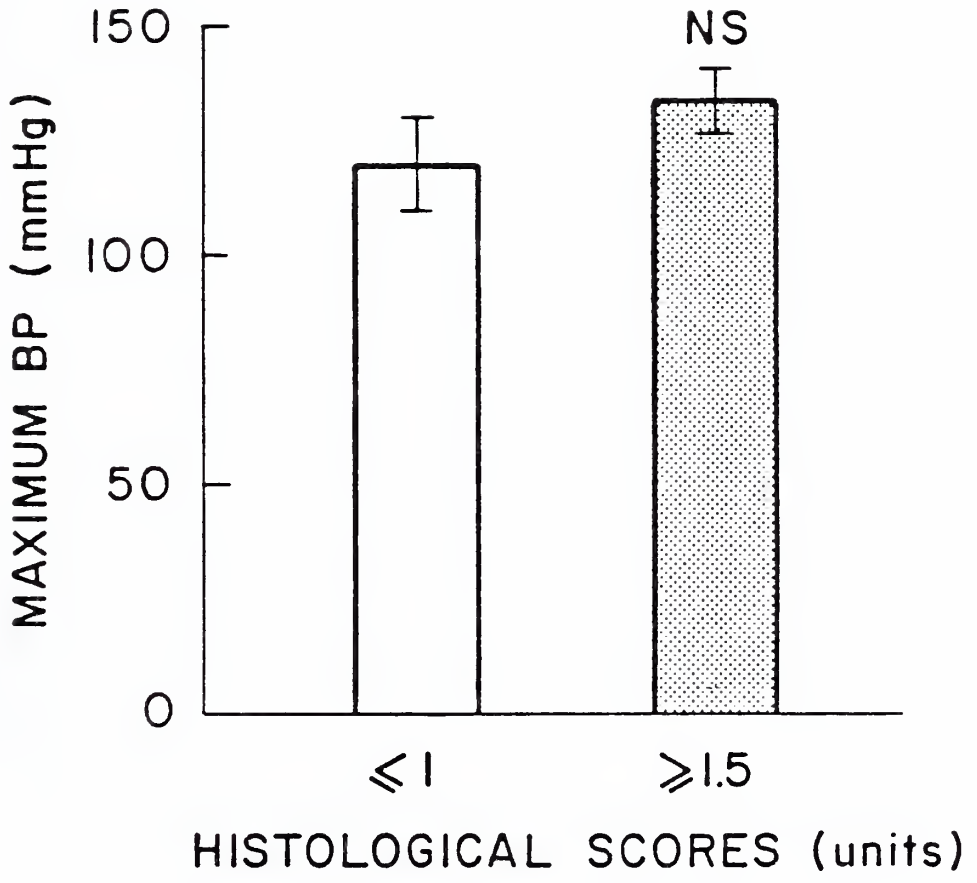

FIGURE 8. Comparison of the average maximum blood pressure (BP) attained during infusion with norepinephrine alone at 2 ug/kg/min for ninety minutes by rabbits with histological scores less than or equal to 1.0 and those with scores greater than or equal to 1.5. NS indicates non-significance. Values are expressed as means $\pm$ SEM.

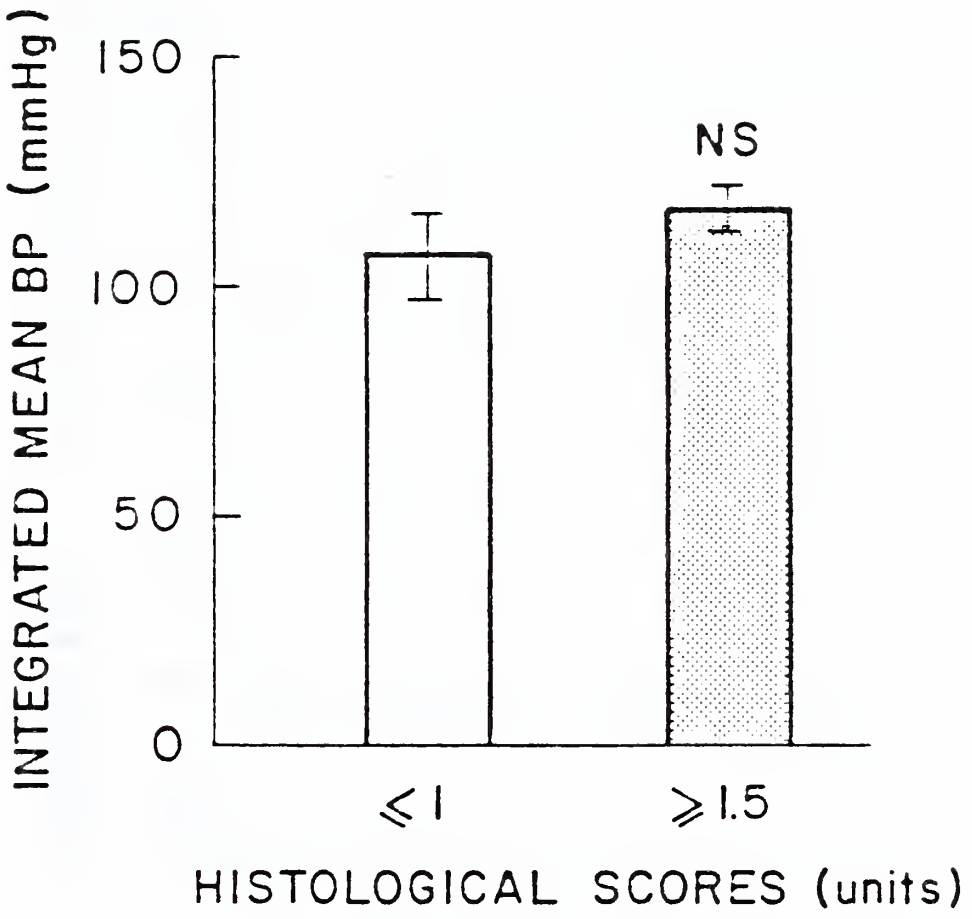

FIGURE 9. Comparison of the average integrated mean blood pressure (BP) over the ninety minute infusion period with norepinephrine at 2 ug/kg/min of rabbits with histologic scores less than or equal to 1.0 and those with scores greater than or equal to 1.5. NS indicates non-significance. Values are expressed as means ± SEM.

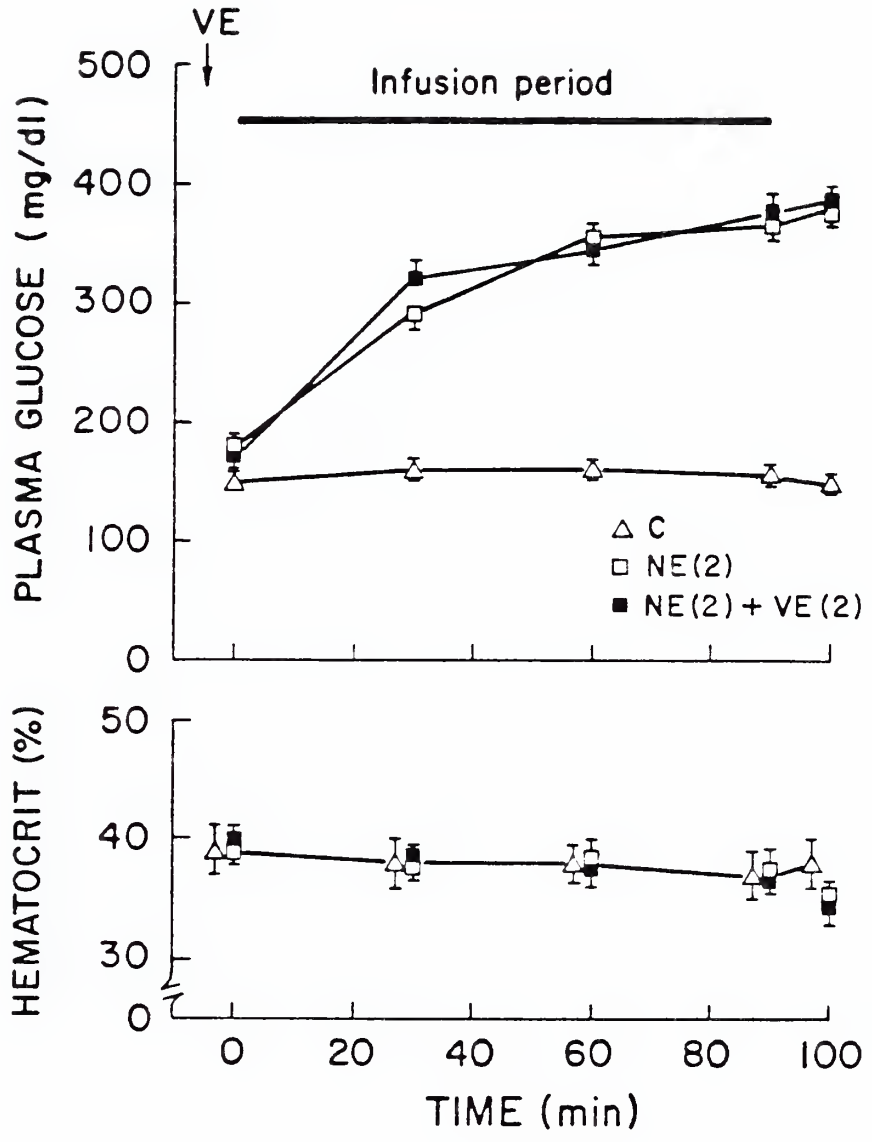

FIGURE 10. Mean values for plasma glucose and hematocrit at designated intervals before, during, and following norepinephrine (NE), NE + verapamil (VE), or saline (C) infusion. Numbers in parentheses indicate ug/kg/min for NE and VE. A 50 ug loading dose of VE was given five minutes prior to beginning infusion with NE and VE as indicated. Values are expressed as means $\pm$ SEM.

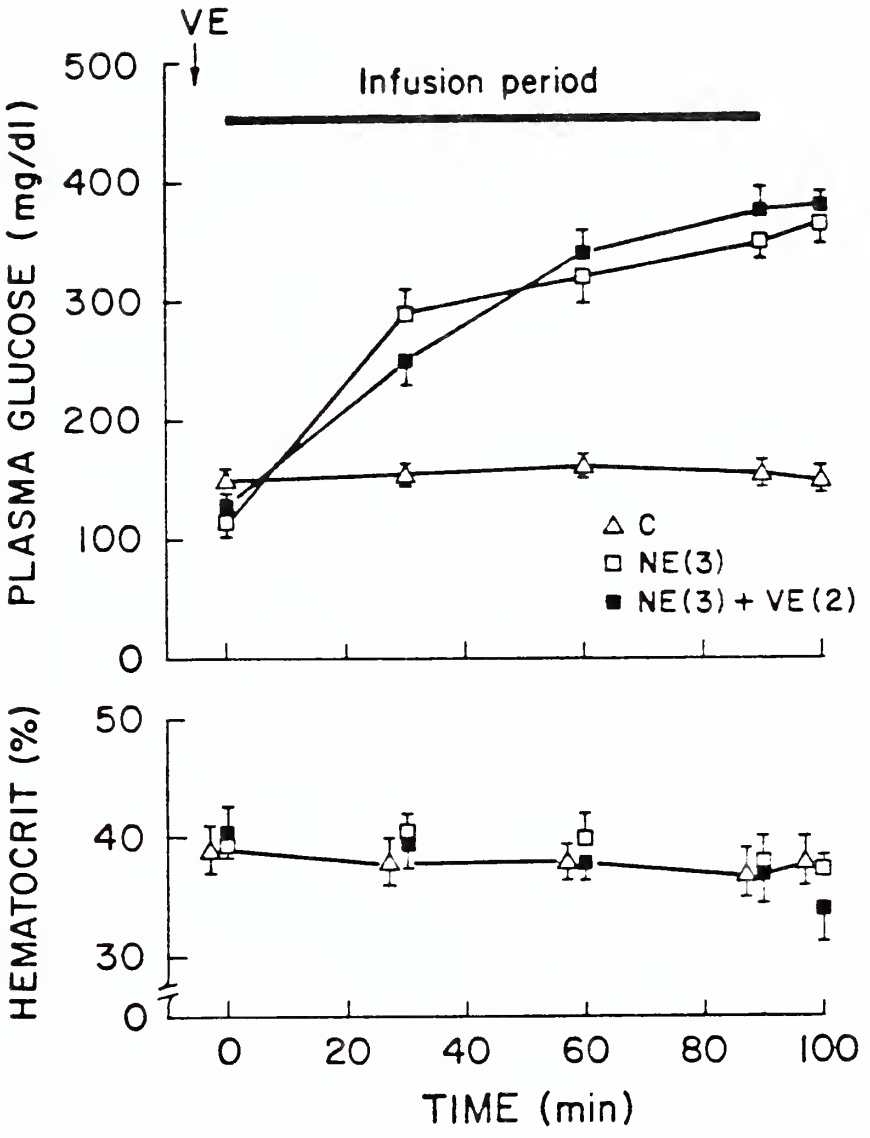

FIGURE 11. Mean values for plasma glucose and hematocrit at designated intervals before, during, and following norepinephrine (NE), NE + verapamil (VE), or saline (C) infusion. Numbers in parentheses indicate ug/kg/min for NE and VE. A 50 ug loading dose of VE was given five minutes prior to beginning infusion with NE and VE as indicated. Values are expressed as means ± SEM.

TABLE 1

BLOOD PRESSURE CHANGES IN RESPONSE TO VARIOUS AGENTS INDICATED

	MEAN ARTERIAL PRESSURE (mmHg ± S.E.)				
	CONTROL BP	BP(10)	BP(10)-BP(C)	INT.BP-BP(C)	n
NE-2	94±5.0	128±5.6	34±2.2	18±2.1	13
NE-2VE-1	80±3.2[a]	101±5.6[c]	21±5.9[b]	16±3.8	10
NE-2VE-2	95±4.3[a]	118±7.4	23±4.0	14±2.6	8
NE-3	102±4.8	139±4.9	37±2.6	22±2.2	10
NE-3VE-2	100±3.3[a]	134±5.8	34±3.6	25±2.4	4
SALINE	102±6.0	103±7.9	0.6±2.1	1±1.6	5

[a] five minutes post 50 ug loading dose of verapamil
[b] $P < 0.05$
[c] $P < 0.01$

TABLE 1. Mean arterial pressure changes during ninety minute infusion of the treatment combinations of norepinephrine and verapamil indicated. Control BP = control blood pressure (prior to initiation of infusion). BP(10) = mean arterial pressure ten minutes after beginning infusion of drugs indicated. BP(10)-BP(C) = the difference between the blood pressure at ten minutes and control blood pressure. INT. BP - BP(C) = the difference between the integrated blood pressure over the ninety minute infusion period and control blood pressure. n = number of rabbits. NE-2, NE-3 = infusion of norepinephrine at 2 ug/kg/mg and 3 ug/kg/min, respectively. VE-1, VE-2 = infusion of verapamil at 1 ug/kg/min and 2 ug/kg/min, respectively.

TABLE 2

EFFECT OF THE VARIOUS TREATMENT COMBINATIONS OF NOREPINEPHRINE AND VERAPAMIL ON CARDIAC METABOLIC DEMAND

	P X R PRODUCT (X 10^3 ± S.E.)					
	CONTROL	10 MIN	30 MIN	60 MIN	90 MIN	n
SALINE	29.3±1.8	28.7±1.9	26.7±1.9	25.6±1.5	25.9±0.9	5
NE-2	23.7±1.8	19.0±2.1	19.3±1.8	16.7±1.6[b]	16.7±1.4[b]	13
NE-3	25.7±1.8	23.7±3.5	23.0±2.5	22.6±2.1	20.9±1.7	10
NE-2	23.7±1.8	19.0±2.1	19.3±1.8	16.7±1.6	16.7±1.4	13
NE-2VE-1	19.5±1.0[a]	18.6±0.9	16.8±1.0	16.6±0.8	16.3±0.8	8
NE-2VE-2	24.1±1.5	22.6±1.2	20.9±0.8	19.7±0.8	19.3±0.9	10
NE-3	25.7±1.8	23.7±3.5	23.0±2.5	22.6±2.1	20.9±1.7	10
NE-3VE-2	28.6±0.9	27.7±0.8	25.1±1.5	22.1±1.2	23.4±2.0	4

[a] $P < 0.05$
[b] $P < 0.01$

TABLE 2. The effect of the various treatment combinations of norepinephrine and verapamil on cardiac metabolic demand. Cardiac metabolic demand is represented by the P X R product (X 10^3 + S.E.) of each treatment group at 0 minutes (control), 10 minutes, 30 minutes, 60 minutes, and 90 minutes after beginning infusion of the agent(s) indicated. P X R = the product of mean arterial pressure and heart rate. n = number of rabbits. NE-2, NE-3 = infusion of norepinephrine at 2 ug/kg/min and 3 ug/kg/min, respectively. VE-1, VE-2 = infusion of verapamil at 1 ug/kg/min and 2 ug/kg/min, respectively.

REFERENCES

1. Haft, J.I.: Cardiovascular injury induced by catecholamines. Progress in Cardiovascular Diseases. 17(1):73-86, 1974.

2. Ziegler, K.: Uber die wirkung intravenoser adrenalininjektion auf das gefasssystem und ihre beziehung zur Arteriosklerose. Ziegler's Beitrage. 38:229-254, 1905.

3. Fleisher, M.S., and L. Loeb: Experimental myocarditis. Arch. Intern. Med. 3:78-91, 1909.

4. Christian, H.A., R.M. Smith, and I.C. Walker: Experimental cardiorenal disease. Arch. Intern. Med. 8:468-551, 1911.

5. Waters, I.L., and G.I. de Suto-Nagy: Lesions of the coronary arteries and great vessels of the dog following the injection of adrenaline. Science. 111:634-635, 1950.

6. Nahas, G.G., J.G. Brunson, et al: Functional and morpholo-gical changes in heart-lung preparations following administration of adrenal hormones. American Journal of Pathology. 34:717-729, 1958.

7. Maling, H.M., and B. Highman: Exaggerated ventricular arrhythmias and myocardial fatty changes after large doses of norepinephrine and epinephrine in unanesthetized dogs. Am. J. Physiol. 194:590-596, 1958.

8. Chappel, C.I., G. Rona et al: Comparison of cardiotoxic actions of certain sympathomimetic amines. Can. J. Biochem. Physiol. 37:35-42, 1959.

9. Rosenblum, I., A. Wohl, A.A. Stein: Studies in cardiac necrosis: I. Production of cardiac lesions with sympathomimetic amines. Toxicology and Applied Pharmacology. 7:1-8, 1965.

10. Ferrans, V.J., R.G. Hibbs, et al: Histochemical and electron microscopical studies on the cardiac necroses produced by sympathomimetic agents. Ann. N.Y. Acad. Sci. 156:309-332, 1962.

11. Barger, G., H.H. Dale: Chemical structure and sympathomimetic action of amines. J. Physiol. 41:19, 1910.

12. von Euler, U.S.: A specific sympathomimetic ergone in adrenergic nerve fibers (sympathin) and its relation to adrenaline and noradrenaline. Acta Physiol. Scandinavia. 12:73, 1946.

13. Szakacs, J.E., and A. Cannon: l-norepinephrine myocarditis. Am. J. Clin. Path. 30:425-434, 1958.

14. Maling, H.M., B. Highman, E.C. Thompson: Some similar effects after large doses of catecholamines and myocardial infarction in dogs. Am. J. Cardiol. 5:628-633, 1960.

15. Schenk, E.A., and A.J. Moss: Cardiovascular effects of sustained norepinephrine infusions: II. Morphology. Circulation Research. 28:605-615, 1966.

16. Hoak, J.C., E.D. Warner, and W.E. Conner: New concept of levarterenol-induced acute myocardial necrosis. Archives of Pathology. 87:332-338, 1969.

17. Rona, G., et al: An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. Arch. Pathol. 67:443-455, 1959.

18. Handforth, C.P., and N.S. Halifax: Isoproterenol-induced myocardial infarctions in animals. Arch. Path. 73:161-165, 1962.

19. Rona, G., D.S. Kahna, and C.I. Chappel: Studies on infarct-like myocardial necrosis produced by isoproterenol: A review. Rev. Can. Biol. 22:241-255, 1963.

20. Kahn, D.S., G. Rona, and C.I. Chappel: Isoproterenol-induced cardiac necrosis. Ann. N.Y. Acad. Sci. 156:285-293, 1969.

21. Wexler, B.C.: Protective effects of propranolol on isoproterenol-induced myocardial infarction in arteriosclerotic and nonarteriosclerotic rats. Atherosclerosis. 18:11-42, 1973.

22. Todd, G.L., G.E. Cullan, and G.M. Cullan: Isoproterenol-induced myocardial necrosis and membrane permeability alterations in isolated perfused rabbit heart. Exp. Mol. Pathol. 33:43-54, 1980.

23. Singh, K.P., K.C. Joshi, K.C. Somani, and M. Tarig: Effect of phenelzine, a monoamine oxidase inhibitor, on isoproterenol-induced myocardial damage. Adv. in Myocardial. 2:165-170, 1980.

24. Singal, P.K., K.S. Dhillon, R.E., Beamish, and N.S. Dhalla: Protective effect of zinc against catecholamine-induced myocardial changes. Electrocardiographic and ultrastructural studies. Lab. Invest. 44:426-433, 1981.

25. Singal, P.K., N. Kapur, et al: Role of free radicals in catecholamine-induced cardiomyopathy. Can. J. Physiol. Pharmacol. 60(11):1390-1397, 1982.

26. Kline, I.K.: Myocardial alterations associated with pheochromocytomas. Am. J. Pathol. 38:539-557, 1961.

27. Van Vliet, P.D., H.B. Burchell, and J.L. Titus: Focal myocarditis associated with pheochromocytoma. New England Journal of Medicine. 274:1102-1108, 1966.

28. Raab, W., E. Stark, W.H. MacMilan, and W.R. Gigee: Sympathogenic origin and antiadrenergic prevention of stress-induced myocardial lesions. Am. J. Cardiol. 8:203-211, 1961.

29. Selye, H. The Chemical Prevention of Cardiac Necroses. New York: Ronald Press Co., 1958.

30. Raab, W.: Neurogenic multifocal destruction of myocardial tissue. Rev. Canad. Biol. 22:217-239, 1963.

31. Raab, W., J.P. Chaplin, and E. Bajusz: Myocardial necroses produced in domesticated rats and wild rats by sensory and emotional stresses. Proc. Soc. Exp. Biol. Med. 116:665-669, 1964.

32. Howe, B.B., P.A. Fehn, and R.R. Pensinger: Comparative anatomical studies of the coronary arteries of canine and porcine hearts. I. Free ventricular walls. Acta Anat. 71:13, 1968.

33. Ratcliffe, H.L., and H. Luginbuhl: The domestic pig: A model for experimental atherosclerosis. Atherosclerosis. 13:133, 1971.

34. Johansson, G., J. Jonsson, N. Lannek, et al: Severe stress-cardiomyopathy in pigs. Am. Heart J. 87:451-457, 1974.

35. Mackenzie, W.F., R.R. Burton, and W.I. Butcher: Cardiac pathology associated with high sustained positive Gz: II. Stress cardiomyopathy. Aviat. Space Environ. Med. 47:718-725, 1976.

36. Meerson, F.Z.: Pathogenesis and prophylaxis of cardiac lesions in stress. Adv. Myocardial. 4:3-21, 1983.

37. Reichenbach, D.D., and E.P. Benditt: Catecholamines and cardiomyopathy: The pathogenesis and potential importance of myofibrillar degeneration. Hum. Path. 1:125-150, 1970.

38. Cebelin, M.S., and C.S. Hirsch: Human stress cardiomyopathy. Hum. Path. 11:123-132, 1980.

39. Fleckenstein, A., J. Janke, et al. "Calcium overload as the determinant factor in the production of catecholamine-induced myocardial lesins." in Recent Advances in Studies on Cardiac Structure and Metabolism.

Vol. II. Cardiomyopathies. Edited by E. Bajusz and G. Rona. Baltimore: University Park Press, 1973. pp. 455-466.

40. Fleckenstein, A., J. Janke, et al. "Myocardial fiber necrosis due to intracellular calcium overload - a new principle in cardiac pathophysiology." in Recent Advances in Studies on Cardiac Structure and Metabolism. Vol IV. Myocardial Biology. Edited by N. Dhalla. Baltimore: University Park Press, 1974. pp. 563-580.

41. Huxley, H.E.: The mechanism of muscular contraction. Science. 164:1356-1366, 1969.

42. Downing, S.E., and J.C. Lee: Effects of insulin on experimental catecholamine cardiomyopathy. American Journal of Pathology. 93(2):339-350, 1978.

43. Lee, J.C., and S.E. Downing: Contribution of alpha-adrenoceptor activation to the pathogenesis of norepinephrine cardiomyopathy. Circulation Research. 52:471-478, 1983.

44. Szakacs, J.E., and B. Mehlman: Pathologic changes induced by l-norepinephrine. Am. J. Cardiol. 5:619-627, 1960.

45. Ferrans, V.J. "Overview of morphologic reactions of the heart to toxic injury." in Cardiac Toxicology, Vol. III. Edited by Tibor Balazs. Boca Raton: CRC Press, Inc., 1981. pp. 83-109.

46. Ferrans, V.J., R.G. Hibbs, et al: Isoproterenol-induced myocardial necrosis: a histochemical and electron microscopic study. American Heart Journal. 68:71-90, 1964.

47. Lehr, D., M. Krukowski, and R. Chan: Acute myocardial injury produced by sympathomimetic amines. Isr. J. Med. Sci. 5:519-524, 1969.

48. Lehr, D. "Studies on the cardiotoxicity of alpha and beta adrenergic amines." in Cardiac Toxicology, Vol. II. edited by Tibor Balazs. Boca Raton: CRC Press, Inc., 1981. pp. 75-112.

49. Haft, J.I., K. Gershengorn, P.D. Kranz, et al: Protection against epinephrine-induced myocardial necrosis by drugs that inhibit platelet aggregation. Am. J. Cardiol. 30:838-843, 1972.

50. Haft, J.I., P.D. Kranz, et al: Intravascular platelet aggregation in the heart induced by norepinephrine. Circulation. 46:698-708, 1972.

51. Haft, J.I., K. Fani, et al: Effect of propanolol on stress-induced intravascular platelet aggregation in

the heart. Circulation. 48(Supplement IV):57, 1973.

52. Ostadel, B., V. Rychterova, and O. Poupa: Isoproterenol-induced acute experimental cardiac necrosis in the turtle. Am. Heart J. 76:645-649, 1968.

53. Durrett, L.R., and H.R. Adams. "Myocardial function and drug actions." in Cardiac Toxicology. Vol. I. edited by Tibor Balazs. Boca Raton: CRC Press, Inc., 1981.

54. Bloom, S. "Reversible and irreversible injury: calcium as a major determinant." in Cardiac Toxicology. Vol. I. edited by Tibor Balazs. Boca Raton: CRC Press, Inc., 1981. pp. 179-199.

55. Fleckenstein, A., J. Janke, et al. "Key role of calcium in the production of noncoronagenic myocardial necroses." in Recent Advances in Cardiac Structures and Metabolism. Vol. VI. Pathophysiology and Morphology of Myocardial Cell Alterations. Edited by A. Fleckenstein and G. Rona. Baltimore: University Park Press, 1975. pp. 21-32.

56. Fleckenstein, A. "Prevention by calcium antagonists of deleterious calcium overload: a new principle of cardioprotection." in Calcium Antagonism in Heart and Smooth Muscle. by A. Fleckenstein. New York: John Wiley and Sons, Inc., 1983. pp. 109-320.

57. Rona, G., M. Boutet, et al. "Pathogenesis of isoproterenol-induced myocardial alterations: functional and morphological correlates." in Recent Advances in Studies of Cardiac Structure and Metabolism. Vol. III. Edited by N.S. Dhalla. Baltimore: University Park Press, 1974. pp. 507-525.

58. Boutet, M., et al: Permeability alteration of sarcolemmal membranes in catecholamine-induced cardiac muscle cell injury. Lab. Invest. 34:482-488, 1976.

59. Rona, G., and C. Bier. "The role of coronary no-flow, reflow phenomena in myocardial injury." in Cardiac Toxicology. Vol. I. edited by Tibor Balazs. Boca Raton: CRC Press, Inc., 1981. pp. 159-178.

60. Rona, G., M. Boutet, and I. Huttner: Reperfusion injury: a possible link between catecholamine-induced and ischemic myocardial alterations. Adv. Myocardial. 4:427-439, 1983.

61. Mallov, S.: Role of calcium and free fatty acids in epinephrine-induced myocardial necrosis. Toxicology and Applied Pharmacology. 71:280-287, 1983.

62. Yates, J.C., et al: Ventricular dysfunction and necrosis produced by adrenochrome metabolites of epineph-

rine: relation to pathogenesis of catecholamine cardiomyopathy. Am. Heart J. 102:210-221, 1981.

63. Yates, J.C., N.S. Dhalla, et al: Induction of necrosis and failure in the isolated perfused rat heart with oxidized isoproterenol. J. Mol. Cell. Cardiol. 7:807, 1975.

64. Dhalla, N.S., J.C. Yates, et al: Functional and subcellular changes in the isolated rat heart perfused with oxidized isoproterenol. J. Mol. Cell. Cardiol. 10:31, 1978.

65. Werner, J.C., J.C. Lee, and S.E. Downing: Preservation of left ventricular function by insulin in experimental catecholamine cardiomyopathy. Am. J. Physio. 238(Heart Circulation Physiology 7):H257-H262, 1980.

66. Lee, J.C., and S.E. Downing: Ventricular function in norepinephrine-induced cardiomyopathic rabbits. Am. J. Physio. 242 (Heart Circulation Physiology 11):H191-H196, 1982.

67. Lee, J.C., and S.E. Downing: Effects of insulin on cardiac muscle contraction and responsiveness to norepinephrine. Am. J. Physiol. 230:1360-1365, 1976.

68. Nudel, D.B., J.C. Lee, and S.E. Downing: Reciprocal inhibition of cardiac responses to norepinephrine and insulin. Am. J. Physiol. 233:H665-H669, 1978.

69. Fleckenstein, A. "Specific inhibitors and promotors of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention or production of myocardial lesions." in Calcium and the Heart. Edited by P. Harris and L. Opie. New York: Academic Press, Inc., 1971. pp. 135-188.

70. Nelson, A., et al: Hemodynamic predictors of myocardial oxygen consumption during static and dynamic exercise. Circulation. 50:1179-1189, 1974.

71. Simonsen, S.: Pharmacological effects of coronary hemodynamics: a comparative study between atenolol, verapamil, nifedipine, and carbocromen. Acta Med. Scand. 645(Supplement):97-104, 1981.

72. Simonsen, S.: Effect of verapamil on coronary hemodynamics in patients with coronary artery disease. Eur. J. Cardiol. 8:9-18, 1978.

73. Simons, M.: Coronary blood flow and the pathogenesis of catecholamine cardiomyopathy. Am. Heart J., 1984, in press.

74. Braunwald, E.: Mechanism of action of calcium-channel blocking agents. New England Journal of Medicine.

307(26):1618-1627, 1982.

75. Fleckenstein, A.: History of calcium antagonists. Circ. Res. 52(2Pt2):I3-I16, 1983 Feb.

76. Melville, K.I., H.E. Shister, and S. Huq: Iproveratril: experimental data on coronary dilatation and antiarrhythmic action. Canad. Med. Assoc. J. 90:761-770,1964.

77. Rowe, G.G., et al: The systemic and coronary hemodynamic effects of Iproveratril. Arch. Int. Pharmacocyn. Ther. 193:381-390, 1971.

78. Benfey, B.G.: Function of myocardial alpha-adrenoceptors. Life Sciences. 31(2):101-112, 1982, July.

79. Schumann, H.J., et al: Demonstration in human atrial preparations of alpha-adrenoceptors mediating positive inotropic effects. Nauyn Schmiedeberg's Arch. Pharmacol. 302:333-336, 1978.

80. Wenzel, D.G., and J.L. Su: Interactions between sympathomimetic amines and blocking agents on the rat ventricle strip. Arch. Int. Pharmacodyn. 160:379-389, 1966.

81. Benfey, B.G., and D.R. Varma: Interactions of sympathomimetic drugs, propranolol and phentolamine on atrial refractory period and contractility. Br. J. Pharmacol. Chemother. 30:603-611, 1967.

82. Rabinowitz, B. W.W. Parmley, et al: Interaction of phentolamine and noradrenaline on myocardial contractility and adenyl cyclase activity. Cardiovasc. Res. 8:243-248, 1974.

83. Rabinowitz, B. L. Chuck, et al: Positive inotropic effects of methoxamine: evidence for alpha adrenergic receptors in ventricular myocardium. Am. J. Phys. 299:582-585, 1975.

84. Lee, J.C., R.R. Fripp, and S.E. Downing. Myocardial responses to alpha adrenoceptor stimulation with methoxamine in lambs. Am. J. Phys. 11:H405-H410, 1982.

85. Aass, H., et al: Demonstration of an alpha adrenoceptor-mediated inotropic effect of norepinephrine in rabbit papillary muscle. J. Pharmacol. Exp. Ther. 226(2):572-578, 1983 August.

86. Endoh, M., J. Wagner, and H.J. Schumann: Influence of temperature on the positive inotropic effects mediated by alpha and beta adrenoceptors in the isolated rabbit papillary muscle. Naunyn Schmiedebergs Arch. Pharmacol. 287:61-72, 1975.

87. Glossman, H., and R. Hornung: Calcium and potassium channel blockers interact with alpha adrenoceptors. Mol. Cell. Endocrin. 19:243-251, 1980.

88. Karliner, J.S., H.J. Motulsky, et al: Verapamil competitively inhibits alpha-1 adrenergic and muscarinic but not beta adrenoceptors in rat myocardium. J. Cardiovasc. Pharmacol. 4:515-520, 1982.

89. Motulsky, H.J., et al: Interaction of verapamil and other calcium channel blockers with alpha-1 and alpha-2 adrenoceptors. Circ. Research. 52(2):226-231, 1983 Feb.

90. Mukherjee, A., et al: Differences in myocardial alpha and beta adrenergic receptor numbers in different species. Am. J. Physiol. 245(Heart Circulation Physiology 14):H957-H961, 1983.

91. Siegl, P.K., and J.H. McNeill: Antagonism with dibenamine, D-600, and Ro 3-7894 to estimate dissociation constants and receptor reserves for cardiac adrenoceptors in isolated rabbit papillary muscles. Can. J. Physiol. Pharmacol. 60(8):1131-1137, 1982 August.

92. Farber, J.L., K.R. Chien, and S. Mittnacht: The pathogenesis of irreversible cell injury in ischemia. Am. J. of Path. 102:271-281, 1981.

93. Schanne, F.A., A.B. Kane, et al: Calcium dependence of toxic cell death: a final common pathway. Science. 206:700-702, 1979.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by . has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE