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Norepinephrine-Induced Cardiomyopathy in Rabbits

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NOREPINEPHRINE-INDUCED CARDIOMYOPATHY IN RABBITS

by

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Prolonged exposure of the heart to excessive catecholamines induces a cardiac myopathy. Typical lesions include myofibrillar degeneration, leukocyte infiltration and focal necrosis. In addition, cardiac dysfunction has been observed after the administration of high concentrations of catecholamines. However, alterations in diastolic as well as systolic function have not been fully investigated.

In this dissertation it was hypothesized that cardiac function was impaired acutely after a 90 minute, high dose norepinephrine infusion with some functional recovery occurring by 48 hours. To further characterize functional alterations, myocardial systolic and diastolic function was assessed at three levels of norepinephrine-induced injury using the isolated, non-ejecting heart preparation. Diastolic and systolic function was impaired acutely after norepinephrine treatment. Systolic function improved within 48 hours except in hearts treated with the highest dose of norepinephrine. Diastolic function remained depressed. Additionally, myocardial responsiveness to α- and/or β-adrenergic receptor stimulation was not altered acutely or chronically after norepinephrine administration.

Current research indicates that multiple mechanisms are involved in the pathogenesis of catecholamine cardiomyopathy. Toxic metabolites of catecholamine autoxidation, possibly acting as membrane oxidants, may have a role in

catecholamine-induced injury. The myocardial anti-oxidant capacity was increased with the administration of α-tocopherol and selenium. This pretreatment however, did not protect the rabbit myocardium from norepinephrine-induced injury.

Alpha-receptor mediated coronary vasoconstriction with resultant ischemia has also been proposed as a mechanism of catecholamine injury. Coronary blood flow was measured before, during and after a 40 minute, high dose norepinephrine infusion (4 μg/kg/min). At 40 minutes, ventricular blood flow was significantly elevated with little change in the rate-pressure product. Blood flow returned to control by one hour post infusion.

These results suggest that norepinephrine-induced injury is not produced by membrane oxidants or coronary vasoconstriction. The increase in myocardial blood flow coupled with a very small change in rate-pressure product also suggests that the norepinephrine infusion did not result in a hypoxic insult to the myocardium.

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VITA

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
COMT	Catechol O-methyl Transferase
EPI	Epinephrine
G Protein	Guanine Nucleotide Regulatory Protein
KHB	Krebs-Henseleit Buffer
ISO	Isoproterenol
LV	Left Ventricle
LVP	Left Ventricular Pressure
LV dP/dt	First Derivative of Left Ventricular Pressure
MAO	Monoamine Oxidase
NE	Norepinephrine

CHAPTER I

INTRODUCTION

Catecholamines released from sympathetic nerves or adrenal glands are important in the normal regulation of cardiac function. Norepinephrine and epinephrine increase heart rate, contractile force and myocardial metabolism. In pathological situations however, the heart may be exposed to excessive catecholamines which subsequently damage the myocardium. Histological examination of hearts damaged by catecholamines revealed numerous small areas of necrosis throughout the myocardium. This pathology is called catecholamine cardiomyopathy.

Early investigations indicate that cardiac systolic function is impaired forty-eight hours after the administration of high concentrations of norepinephrine. However alterations in cardiac diastolic as well as systolic function have not been fully investigated. In the first study of this dissertation cardiac diastolic and systolic function was evaluated at three levels of catecholamine-induced injury. Additionally, since myocardial function immediately following exposure to high dose catecholamines has not been reported, this study assessed ventricular function acutely after norepinephrine-induced injury and at 48 hours.

Exposure of the heart to catecholamines can reduce myocardial responsiveness

to β-adrenergic receptor agonists. In the second study, the ability of norepinephrine injured hearts to increase ventricular function with α- and β-adrenergic receptor stimulation was assessed. It was hypothesized that norepinephrine administration would desensitize the β-receptor, and β-receptor mediated increases in ventricular function would be depressed. In this case, the α-adrenergic system may be more important in increasing cardiac performance after catecholamine-induced injury.

Current research indicates that multiple mechanisms are involved in the pathogenesis of catecholamine-induced injury. Investigators have suggested that the autoxidation of catecholamines with the production of toxic metabolites is involved in the development of catecholamine cardiomyopathy. These metabolites, which include free radicals, may act as oxidizing agents and initiate peroxidation of the cellular membranes. In the third study, α-tocopherol and selenium were administered to rabbits over a two week period to increase the antioxidant capacity of the heart. Rabbits were then infused with high dose norepinephrine. It was hypothesized that this pretreatment would protect the myocardium from norepinephrine-induced injury.

Alpha-receptor mediated coronary vasoconstriction has also been proposed as a mechanism of catecholamine injury. Vasoconstriction of the coronary vasculature could cause ischemic injury to the myocardium and produce areas of necrosis. Following ischemic injury, myocardial blood flow may not return to normal levels and areas of the myocardium may not be perfused. The purpose of the last study was to determine if there were perfusion abnormalities associated with a forty minute infusion of high dose norepinephrine.

It is hoped that the research presented in this dissertation will provide information regarding the ventricular dysfunction which occurs when a heart is injured by catecholamines and may aid in the treatment of patients with pheochromocytoma or patients who have suffered cerebral trauma. Identifying acute changes in ventricular function of hearts exposed to toxic levels of catecholamines may provide a rational for failure of transplanted hearts donated by trauma patients who had a massive release of catecholamines from the sympathetic nervous system. Additionally, it is hoped that this dissertation gives insight into the mechanisms of norepinephrine-induced injury.

CHAPTER II

LITERATURE REVIEW

Under normal physiologic conditions, catecholamines are involved in the regulation of cardiac function. The administration of high dose catecholamines (256,284,334) or the excessive release of catecholamines from the sympathetic nervous system (76,166,185,269,281) is cardiotoxic and results in the development of catecholamine-induced cardiomyopathy (269,274). Catecholamine cardiomyopathy is defined as an abnormal condition or disease of the heart induced by catecholamines. The research presented in this dissertation focuses on the cardiac dysfunction which occurs in catecholamine cardiomyopathy, specifically, norepinephrine-induced cardiomyopathy and addresses two possible mechanisms of injury. In this chapter, background information on catecholamine cardiomyopathy, disposition of catecholamines, adrenergic receptor desensitization and proposed mechanisms of catecholamine-induced injury will be presented.

A. CATECHOLAMINE-INDUCED CARDIOMYOPATHY

Experimentally, catecholamine cardiomyopathy can be induced *in vivo* or *in vitro* by a variety of catecholamines including epinephrine (EPI) (45,87,88,256),

norepinephrine (NE) (60,277,284,321,334), and isoproterenol (ISO) (60,171,178,269,277,334). Myocardial lesions produced in catecholamine cardiomyopathy are characteristic and similar in frogs (41), rats (64,88), rabbits (178,256,284), hamsters (127), cats (284), pigs (127), dogs (321,334), monkeys (224,228) and humans (269,304,345). Rabbits appear most susceptible to the development of cardiac lesions, while rats are more resistent (171,284). Characteristic cardiac lesions include: edema, contraction bands, myofibrillar degeneration, lipid droplets, hemorrhage, necrosis, leukocyte infiltration, and proliferation of fibrous tissue (88,127,263,264,284,321,335). The severity and extent of these lesions vary directly with the catecholamine, dose and rate of administration (45,127,274). Isoproterenol, particularly in high doses, is most cardiotoxic, able to induce massive infarct-like necrosis of the entire ventricular wall (276). Other catecholamines and low dose ISO generally produce focal or patchy areas of necrosis (15,45).

In humans, trauma (42), subarachnoid hemorrhage (60,121,269), cerebral swelling, brain tumors (60), intracranial infections (269,291) and pheochromocytoma (5,49,182,345) elicit an excessive endogenous release of catecholamines. Upon postmortem examination, myocardial injury similar to that induced in experimental models of catecholamine cardiomyopathy can be identified (265,268). Myocardial damage has also been reported in patients after prolonged infusion of NE for the treatment of shock (127). Severe cardiac lesions induced by catecholamines may impair myocardial function and therefore contribute to organ failure and death.

1. Gross Anatomical Changes

Immediately after catecholamine infusion, gross examination of the heart may show dilation and/or hemorrhage (171,256,284). Cardiac dilation was moderate to extreme depending on the catecholamine and dosage (256,284) and usually was most pronounced in the right atrium and right ventricle (284). In the extreme, the heart lost its conical shape, the apex was rounded and atria were over-distended (256). NE induced necrosis in the canine heart was usually accompanied by hemorrhagic lesions in the subendocardium, papillary muscles, cardiac valves and coronary vessels (221,237,321). Diffuse and local subendocardial congestion and hemorrhage have been observed in rabbit hearts immediately after stopping a 5 hour infusion of 1.2 μg/kg/min NE (284). Patchy and focal subepicardial and subendocardial hemorrhages were found in cats and dogs receiving 2 μg/kg/min NE for 5 hours. Hemorrhages were diffuse and extensive in all animals receiving larger doses (284).

Myocardial edema has been reported following catecholamine injury (87,236,256). In the rat myocardium, capillary dilation and increased permeability were noted within 15 minutes of subcutaneous high dose ISO (236,276). At lower doses, capillary dilation was less frequent and was restricted to areas of injury. In these rats, edema was noted throughout the stroma and in areas of severe injury, the edema separated muscle fibers. Interstitial and intracellular edema of the myocardium has also been reported in other animal models following catecholamine administration (87,236,256).

2. Histological Alterations

Within minutes of exposure to high levels of catecholamines degenerative changes can be observed in myofilaments, mitochondria and the sarcoplasmic reticulum throughout the myocardium (16,63,224,275,335). Some myocardial cells contained thickened Z lines and contraction bands, areas where sarcomeres were hypercontracted (63,86,127,178,275,333,335). Electron microscopic examination revealed swelling of the sarcoplasmic reticulum, transverse tubules and mitochondria (63,87,88,127,311). Mitochondrial cristae were more closely packed and angulated, particularly in EPI-treated animals (41,87,88). Myocardial glycogen was rapidly depleted (41,87,88,351,366) and lipid droplets formed between myofibrils and in the perinuclear cytoplasm (86-88,127,221,284). Investigators have hypothesized that the accumulation of neutral lipids is due to an increased mobilization and uptake secondary to increased catecholamine stimulated lipase activity in adipose tissue (35,86,88,149).

Gradually, some myocardial cells began to recover from the acute insult while the areas of injury became localized to isolated or groups of myocytes. In damaged muscle cells, the number and extent of contraction bands increased (127). Later, characteristic striations and Z lines of cardiac muscle were obliterated (16,276,284). In some cells, myofilaments depolymerized and aggregated in bands of granular myofilament material (269,276,333,335). Between these aggregates, myofilaments torn away from the sarcolemma were occasionally observed (333). By 16 hours, mitochondrial cristae were destroyed and replaced with calcium phosphate deposits (127,224,269,311). The sarcoplasmic reticulum and transverse-tubules were severely

swollen and disrupted (333). Following the appearance of myofilament degeneration, nuclei became irregular, increased their staining, then lost their staining capacity (269,276). By 72 hours, nuclei were not evident in some cells. Activity of lysosomal enzymes and the number of lysosomes in cardiac muscle cells was increased throughout the myocardium following catecholamine infusion (87,273). The increase in lysosome number was most prominent in cells adjoining necrotic areas where lysosomes may play a role in removing dead tissue. In non-necrotic fibers, lipid deposition declined after 24 hours and within 3 to 5 days viable myocardial tissue contained normal amounts of lipid (88,221).

Polymorphonuclear leukocytes are scarce immediately following catecholamine injury (269,284). Ferrans et al. (87) have shown the presence of various mononuclear cells including histiocytes, macrophages, lymphocytes, Anitschkow cells and occasionally polymorphonuclear leukocytes and mast cells, 6 to 10 hours after catecholamine injection in the rat. Muller (236) also reported moderate infiltration of leukocytes and histiocytes in areas of injury in rat hearts after 24 hours with more intense infiltration at 48 hours. Similar results have been reported by Bloom and Cancilla (16) and Rona et al. (276) at 24 and 48 hours post ISO and by Downing and Chen (76) 48 hours after NE infusion. By the third or fourth day, numerous histiocytes, macrophages and occasionally lymphocytes and plasma cells were present in the interstitium surrounding necrotic myofibers (16,284). Seven days after catecholamine administration the majority of necrotic cells had been removed by phagocytosis and only a few leukocytes remained (171,269,284).

Proliferation of fibroblasts and increased interstitial fibrosis follows necrotic injury (321). In rats, fibroblast proliferation was noted 24 and 48 hours after ISO treatment (236,276) and a network of thin collagen fibers was formed within 48 hours (260). Thicker filaments became entwined in the network and by the eight day following catecholamine administration, a dense mesh of collagen fibers had formed to encircle muscle and fill intermuscular spaces. Myocardial fibrosis appeared to be stimulated by interstitial edema and disruption of fibrillar collagen. Three days after NE infusion, connective tissue deposition became apparent in dogs, cats and rabbits (284). Within two weeks of catecholamine treatment, fibrous scars were well developed and were the only evidence of earlier necrosis and inflammation (16,284).

B. CARDIAC DYSFUNCTION IN CATECHOLAMINE CARDIOMYOPATHY

Cardiac dysfunction may result from catecholamine-induced lesions. In a rabbit model of NE-induced cardiomyopathy, ventricular systolic function was depressed *in vivo* 48 hours after NE infusion (32,198,355). Downing and his co-investigators reported that contractility, as measured by LV function curves, was reduced (198,355). Additionally, the ability of cardiomyopathic hearts to change systolic function in response to afterload stress (355) or calcium infusion (98) was impaired. Positive inotropic and chronotropic responses were elicited in cardiomyopathic hearts (98,198), however, maximal LV stroke volume, LV +dP/dt and maximal heart rate were attenuated (32,79,98,198). In these *in vivo* studies, it

is unknown if the endocrine and/or nervous systems are modifying ventricular function. Hearts isolated from rats injected with 40 mg/kg ISO showed no change in contractile force at 3 hours. Depression in contractile function was noted at 9 and 24 hours (66). Recently, Chen and Downing (47) have reported that hearts isolated from rabbits at the completion of a NE infusion have a decreased ability to increase stroke work. Left ventricular diastolic function and acute alterations in other parameters of systolic function have not been evaluated.

In this dissertation it is hypothesized that cardiac function is depressed acutely after the administration of catecholamines and significant functional recovery occurs within 48 hours. To test this theory, left ventricular diastolic and systolic function will be evaluated immediately after a NE infusion and at 48 hours. The isolated perfused rabbit heart preparation will be used to measuring ventricular function so neural and humoral influences are eliminated.

C. DISPOSITION OF CATECHOLAMINES

1. Enzyme Systems

Catecholamines are metabolized by monoamine oxidase (MAO) and catechol O-methyltransferase (COMT) (7,113). MAO is a flavin-containing enzyme tightly bound to the outer mitochondrial membrane (339). This oxidase has been found in most body organs but it is virtually absent in skeletal muscle, blood plasma and erythrocytes (293,331). MAO maintains very low levels of free intracellular catecholamines by catalyzing the oxidative deamination of amines to aldehydes

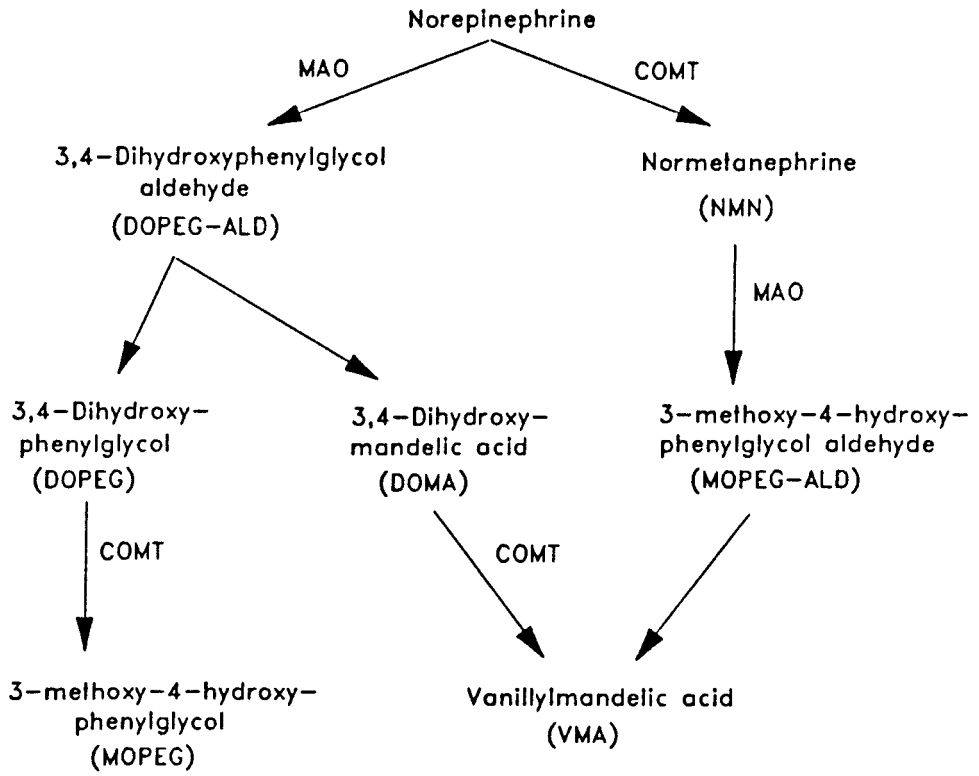
(331,332). Two forms of MAO have been identified based on substrate and inhibitor specificity (61). MAO-A preferentially deaminates NE and serotonin and has been located in adrenergic nerve endings (347) and at extraneuronal sites. MAO-B, found only at extraneuronal sites (347), acts on a broader range of amines. The cytosolic enzyme COMT is widely distributed in all mammalian tissue (7,9). COMT catalyzes the transfer of a methyl group from S-adenosylmethionine to catechols in the presence of magnesium (9). Substrates for COMT include EPI, NE, and dihydroxyphenylalanine (DOPA) (9).

2. Pathways for Catecholamine Metabolism

After transport into the cytosol, the pathway for NE metabolism is dependent on enzyme availability. In tissue rich in MAO, NE is metabolized to 3,4-dihydroxyphenolglycol aldehyde (DOPEG-ALD) (Figure 2-1). The aldehyde is rapidly metabolized by aldehyde oxidase to 3,4-dihydroxymandelic acid (DOMA) (292). COMT converts DOMA to vanillylmandelic acid (VMA). In some tissues, the oxidative intermediate DOPEG-ALD is converted to 3,4-dihydroxyphenolglycol (DOPEG) then to 3-methoxy-4-hydroxyphenylglycol (MOPEG) by COMT. DOPEG is the main deaminated metabolite formed within sympathetic nerves (109). This metabolite can readily diffuse out of the neuron into the circulation or be converted to MOPEG at extraneuronal sites (8). NE transported into extraneuronal tissues with an abundance of COMT, can be converted to its O-methyl metabolite, normetanephrine (NMN) (7). NMN is deaminated to 3-methoxy-4-hydroxyphenylglycol aldehyde (MOPEG-ALD) (7), then further metabolized to VMA.

FIGURE 2-1

METABOLISM OF NOREPINEPHRINE



Pathway for norepinephrine metabolism by monoamine oxidase (MAO) and catechol O-methyltransferase (COMT).

3. Uptake Mechanisms

In 1965, Iverson (157) identified two distinct mechanisms used for the translocation of catecholamines across a cell membrane, $uptake_1$ and $uptake_2$. These uptake mechanisms were saturable, temperature sensitive and obeyed Michaelis-Menten kinetics (118,158,159). $Uptake_1$ defined the neuronal membrane carrier system which transports catecholamines from the extracellular space into neuronal tissue (158).

$Uptake_1$ is inhibited by cocaine (118) and driven by sodium concentration gradient across the neuronal membrane (20,106). Once NE is transported into nerve terminals by $uptake_1$, it is stored in intracellular vesicles or rapidly degraded by MAO (110). $Uptake_2$, uniquely different from $uptake_1$, transports catecholamines across extraneuronal membranes. $Uptake_2$ is corticoid sensitive and depends on the potassium concentration gradient and/or resting membrane potential for its function (20). Originally $uptake_2$ was thought to operate only at very high extracellular concentrations of noradrenaline (157). However, Lightman and Iversen (210) and Fiebig and Trendelenburg (90) have clearly demonstrated that extraneuronal $uptake_2$ operated effectively at low concentrations of noradrenaline. Within extraneuronal tissues, catecholamines are metabolized or bound to intracellular proteins (106).

4. Tissue and Species Specificity of Inactivation Mechanisms

After an intravenous catecholamine injection, two phases of removal have been demonstrated (357). There is an initial fast phase of tissue uptake, metabolism and storage followed by a slow exponential removal. During the slow phase,

catecholamines which had been taken up by tissues and not metabolized are slowly released back into the circulation (118). The removal of circulating catecholamines involves numerous tissues (159,357). Skeletal muscle, vascular smooth muscle, erythrocytes and platelets efficiently remove and metabolize circulating catecholamines (106,124,206,250,252,293,347). Whitby et al. (357) demonstrated a predominant uptake and retention of H^3-NE by the heart, spleen and adrenal gland in the cat after a 30 minute intravenous infusion. However, H^3-normetanephrine in varying concentrations was found in all tissues analyzed including the kidneys, salivary glands and pancreas. Using an isolated preparation, investigators found the kidney metabolized catecholamines and filtered unmetabolized catechols in proportion to the glomerular filtration rate (143,300). The lung of the dog, cat and rat efficiently removed 30 to 40% of the NE passing through the pulmonary circulation (4,107). Significant amounts of NE were also removed by the intestines, spleen and kidneys in these animal models (4,107,196). A single pass through the liver almost completely removed (80%) circulating catechols (187). Similarly, the human lung and hepatomesentaric circulation have been shown to clear about 70% of total plasma NE (81). The relative importance of $uptake_1$ and $uptake_2$ for removing catechols varies with different tissues and depends on the density of sympathetic innervation and the availability of extraneuronal transport mechanism (109,111,159,176,187,210,313).

The major metabolic route for the inactivation of circulating amines appears to be O-methylation (7,33,143,144,188,195,300,332,357). Five minutes after adult male mice were injected with H^3-NE, 45% had been metabolized to NMN (357). In

humans, urinary analysis after a 30 minute intravenous infusion of radioactive EPI showed 65% O-methylation of the catecholamine and 25% deamination with the major excretory products being VMA and metanephrine (195). Maas and Landis (217) have shown that metabolism of circulating NE by human subjects resulted in the urinary excretion of free NE, conjugated NE and normetanephrine. Similarly, Goodall and Rosen (114) have reported that normetanephrine is the initial metabolite excreted during an infusion of NE. Conjugated NE and free NE were also excreted in large amounts. After 30 minutes, the major metabolite was VMA.

The pattern of cardiac uptake and metabolism of plasma catecholamines is species specific. Kopin et al. (189) reported the predominant route of non-metabolic inactivation of NE in the isolated perfused rat heart was tissue binding or uptake while O-methylation was the primary metabolic pathway. In this model, neuronal and extraneuronal uptake and metabolism contributed equally to total steady state removal of NE (90). NE transported into sympathetic neurons was stored or metabolized. There was no extraneuronal accumulation of NE during perfusion with low concentrations since metabolism was very pronounced (91,210,338). With exposure to high NE concentrations, intracellular COMT became saturated so the unchanged amine accumulated within extraneuronal cells (194,210,338). Isolated feline, mouse and pigeon hearts formed significant amounts of DOPEG and NMN suggesting increased neuronal and extraneuronal uptake and metabolism in response to increasing concentrations of noradrenaline (117,165). High concentrations of exogenous NE increased the production of DOPEG and DOMA in guinea pig atria (313) and isolated

rabbit hearts (117,219). DOPEG, the major oxidized metabolite, was formed primarily within the nerve terminals (219,312,313). In guinea pig atria significant amounts of DOPEG were also formed in extraneuronal cells (313). DOMA was only produced intraneuronally (219,313) and amounted to 25-33% of the oxidized metabolites (313). Very little NMN was formed within the rabbit myocardium (117). Graefe, Bonisch and Keller have reported that the $uptake_2$ mechanism within the rabbit heart is poorly developed and suggested that neuronal uptake is the major mechanism for catechol removal in this preparation (118).

5. Conjugation

Although catecholamines are primarily inactivated by uptake and catabolic enzyme systems, inactivation by conjugation with sulfate and glucuronide may be physiologically important (7,191,292,353). Kuchel and Buu (191) have suggested that the clearance of free NE unaccounted for by neuronal storage, *O*-methylation or oxidation may be due to conjugation. Phenolsulfotransferase, an enzyme which transfers a sulfate group from 3'-phosphoadenosine-5'-phosphosulfate to a phenolic substrate, sulfoconjugates the 3-*O*- or 4-*O*-hydroxyl group of NE, NMN, MOPEG, and VMA (2). This cytoplasmic transferase has been found in the liver, adrenals, and small intestine. Yet, the presence of NE sulfate within these tissues and the release of this conjugate have not been demonstrated (191). Significant conjugation of catecholamines has been shown to occur in platelets (2,138,293) and the kidney (143,270). Catecholamine conjugates appear to be biologically inactive and protected from metabolism by MAO and COMT (271) but they are not inert (229). Merits has

reported the urinary excretion of catecholamine metabolites after dopamine sulfate was administered to dogs, rats and guinea pigs (229). It may be that sulfoconjugation is a reversible process allowing catecholamine sulfates to be hydrolysed to free catecholamines. Clinical data suggests that sulfoconjugating systems buffer biologically active catecholamines within the circulation (192,193). In some pheochromocytoma patients, baseline conjugated catecholamine levels are very high (192). During periods of excessive catecholamine release or a hypertensive crises conjugated NE and EPI levels increase (192). It appears that the excess circulating catecholamines were buffered by enhanced sulfoconjugation. In NE-induced cardiomyopathy, high levels of circulating catecholamines may also be buffered by sulfoconjugation.

6. Autoxidation

Saturation of transport and enzyme systems by high levels of circulating catecholamines, allow more catechols to undergo spontaneous (21,119,302) or enzymatic (227,308,342) autoxidation. Ceruloplasmin normally catalyzes the cyclization of a small portion of circulating catecholamines to adrenochrome (293). During prolonged infusion of high dose NE, ceruloplasmin interacts with more NE and may produce abnormally high levels of adrenochrome. Ferritin (Fe^{+2}), an iron storage protein, accelerates the non-enzymatic oxidation of catecholamines (120,133,231,359). Catecholamine autoxidation produces physiologically active metabolites such as adrenochrome, hydrogen peroxide (H_2O_2)and free radicals (21,156,262). If produced in large quantities, these metabolites can impair normal

cell function and contribute to the formation of myocardial lesions.

D. ADRENERGIC RECEPTOR MECHANISMS

Adrenergic receptors do not directly influence effector enzyme systems or ion channels but require coupling proteins, specifically, guanine nucleotide regulatory (G) proteins (202,297). G proteins consist of three subunits, α, β and γ (83,245). The β- and γ-subunits appear to be structurally similar in all G proteins while the α-subunit shows heterogeneity. The α-subunits binds guanine nucleotides, contains guanosine triphosphatase (GTPase) activity and interacts with effector systems when guanosine triphosphate (GTP) is bound (83,245).

Agonist-receptor binding promotes the interaction of the occupied receptor with a G protein. The agonist, receptor and G protein complex triggers a conformational change in the α-subunit that facilitates the exchange of GTP for guanosine 5'-diphosphate (GDP) bound to the G protein (136,139). The activated α-GTP subunit dissociates from the G protein (83,244) and interacts with effector systems (11,244). With GTP hydrolysis, the α-subunit is inactivated and separates from the effector enzyme or ion channel (11,136). The inactive α-GDP subunit reassociates with β- and γ-subunits to restore the G protein.

In the adenylate cyclase system, β-adrenergic receptors are coupled to G proteins which stimulate adenylate cyclase, G_s (82). The resultant increase in intracellular 3'-5'cyclic adenosine monophosphate (cAMP) initiates a cascade reaction and activates specific cytosolic enzymes including protein kinase A. Protein kinase

A phosphorylates the calcium channel in cardiac muscle and increases the slow inward calcium current (29). The activated α-subunit of G_s also directly modulates this calcium channel (29). The greater influx of calcium through the calcium channel results in an increased rate and force of contraction of cardiac muscle. β-Adrenergic agonists also augment the pacemaker current increasing the frequency of contraction (139). Phosphorylation of phospholamban increases the rate of calcium uptake and sequestration into the sarcoplasmic reticulum and increases the rate of relaxation (139). The inotropic and chronotropic effects of β-receptor stimulation have been studied in humans (30,167,208), many other animals models including rabbits (27,79), guinea pig (30), rats (306,307) and dogs (213).

Another G protein couples the α_1-receptor to phospholipase C and regulates hydrolysis of phosphoinositol lipids (297). Agonist occupancy of the α_1-receptor results in the hydrolysis of phosphatidyl inositol-4,5-bisphosphate to inositol triphosphate and diacylglycerol (14,83). Inositol triphosphate mobilizes calcium from intracellular stores while diacylglycerol in the presence of calcium activates protein kinase C (11,14,139,242). Stimulation of the α_1-adrenoceptor increases the slow inward calcium current (83). In 1967, Govier identified α-receptors on the guinea-pig atria and demonstrated that stimulation produced a positive inotropic effect (116). Since then, positive inotropic responses to α_1-adrenoceptor stimulation have been demonstrated in atria, papillary muscles and ventricles of various mammalian species including rats (6,19,77,79,239,295), mice (115), guinea pigs (116,197,233,350), rabbits (1,10,28,151,255,287,288,349,350), lambs (199), cats (34,266,348) and dogs

(78).

E. RECEPTOR DESENSITIZATION

Within minutes of exposure to an agonist, cells adapt by decreasing receptor responsiveness. This agonist-induced process is called desensitization or tachyphylaxis. In the adrenergic receptor system, desensitization results in reduced stimulation of adenylate cyclase by β-adrenergic agonists (136,294,299,352) or decreased turnover of inositol phospholipids with α_1-adrenergic receptor stimulation (200). Two major patterns of desensitization of adrenergic receptor-coupled second messenger systems have been distinguished (136,316). Homologous desensitization is characterized by a diminished responsiveness to the desensitizing hormone. If exposure to an agonist attenuates the responsiveness of hormones utilizing other receptors or non-hormonal activators, the desensitization is heterologous (136). The mechanisms of desensitization include receptor phosphorylation, internalization or sequestration, and downregulation of membrane receptors (200,202,214).

1. Receptor Phosphorylation and Uncoupling

Phosphorylation of cytosolic serine and/or threonine residues on α_1- and β-adrenergic receptors (24,200,202,214,297) decreases the coupling of the receptors to G proteins thereby diminishing the activation of effector enzyme systems (13,23,297). Exposure of smooth muscle cells to NE rapidly attenuated the agonist stimulated hydrolysis of phosphatidylinositol phosphates via the α_1-receptor and decreased receptor affinity (200). These functional alterations were closely correlated with a

two-fold increase in the phosphate content of the protein receptor. Further investigation indicated that protein kinase C, activated after α_1-receptor stimulation, phosphorylated the receptor (24,201). Leeb-Lundberg et al. (24,200) suggested that α-receptor phosphorylation was responsible for the desensitization and functional uncoupling. β-Adrenergic desensitization studies have demonstrated selective activation of protein kinase A at low concentrations of β-adrenergic agonists which was followed by phosphorylation of β-receptors (214,297). Resultant phosphorylation of the β-receptors correlated with a decreased sensitivity of the adenylyl cyclase response to agonist stimulation (141). At high agonist concentrations, both protein kinase A and β-adrenergic receptor kinase were activated. These kinases phosphorylated the β-receptor (11,203,214,299) and effectively decreased the maximal responsiveness of adenylate cyclase to an agonist (141). Furthermore, β-adrenergic receptor kinase, probably working in concert with an "arrestin-like protein" (11), inhibited up to 80% of receptor stimulated enzyme activity (12). Benovic et al. (12) hypothesized that β-receptor phosphorylation impaired receptor coupling to G_s.

2. Internalization

Chuang and Costa provided the first direct evidence that adrenergic receptors are sequestered into an intracellular compartment devoid of adenylyl cyclase and G proteins (51,52). These investigators observed a decrease in plasma membrane β-receptors number which paralleled an increase in cytosolic receptors after exposure of erythrocytes to ISO (51,52). Harden et al. (137) later demonstrated that the receptors were sequestered into light vesicles. These results have been confirmed by

several laboratories (51,52,145,200,310). Quantitative studies (230,234,294,310,317) have reported that 50-95% of plasma membrane β-receptors were internalized with exposure to catecholamines. The mechanism of sequestration remains to be elucidated but may involve the endocytosis of receptors (52,112,145,257). Phosphorylation appears to promote the internalization of β-receptors (52,297) but does not appear to be essential (48,214,317). In contrast, Leeb-Lundberg et al. (200) reported that internalization of the α_1-adrenoceptor required both receptor phosphorylation and agonist occupancy.

Within the light vesicles, receptors are dephosphorylated (13,299) and retain their structural and functional integrity (54,318). Upon removal of the agonist, sequestered receptors are recycled back to the plasma membrane where they can interact with G proteins and activate their second messenger system (299,318). Mukherjee et al. (235) have demonstrated the complete recovery of plasma membrane β-adrenergic receptors in frog erythrocytes after the agonist was removed. Similarly, Doss et al. (75) have also shown that a 95% loss in plasma membrane receptor number of preconfluent cultures of astrocytoma cells was completely reversible within 48-72 hours after removal of the agonist. In both studies (75,235), receptors were recovered even when protein synthesis was blocked.

The contribution of receptor internalization in the desensitization process is still being investigated. Some investigators have proposed that receptor phosphorylation is the mechanism of desensitization (23,25,200,298,299,319,352) while receptor internalization is a secondary process (25,200,310,319). Waldo et al. (352) have

shown that agonist induced uncoupling of human astrocytoma β-receptors from adenylate cyclase preceded internalization. When sequestration was inhibited, uncoupling still occurred (352). Similar results were reported by Homburger et al. (152) in cultured glioma cells and by Bobik et al. (18) in chick embryo cardiac cells. In contrast, it has been reported that internalization is not dependent on receptor phosphorylation and contributes significantly to cellular desensitization (51,214,317). The sequestration response to ISO is not altered when phosphorylation sites on the carboxyl terminus of hamster β-adrenergic receptors are removed (317). Lohse et al. (214) have reported that sequestration resulted in a 20-30% decrease in the β_2-receptor-stimulated adenylyl cyclase activity in human epidermoid carcinoma A431 cells exposed to high agonist concentrations while kinase activities were inhibited. The apparently conflicting results may indicate that receptor sequestration as a mechanism of desensitization is species and/or cell line specific. Alternately, it may be that sequestration normally removes phosphorylated receptors from the cell surface for dephosphorylation and recycling but at high agonist concentrations becomes a mechanism of desensitization.

3. Long Term Regulation

Longer term desensitization may involve additional mechanisms. Hausdorff et al. (141) suggested that modification of G proteins or adenylyl cyclase may occur with prolonged exposure to an agonist. Katada et al. (175) reported that the dissociated α-subunit of purified G_i protein is a substrate for protein kinase C and its phosphorylation altered the function of the α-subunit. Phosphorylation of the purified

catalytic unit of adenylyl cyclase by protein kinase A and C has also been reported (11). However, in vivo studies are needed to elucidate the physiologic significance of G protein or cyclase modification. Down-regulation of receptors, ie, the loss of total receptor binding sites from a cell, has been demonstrated in some models of desensitization (136,152,319). The total number of β-adrenergic receptors was reduced 45.4% in glioma cells after a 3 hour incubation with ISO (152). Confluent cultures of astrocytoma cells recovered only 60 to 70% of the β-receptors following the removal of ISO (75). Su et al. (319) also reported β-receptor loss in 1321N1 astrocytoma cells, S49 lymphoma cells and HC-1 hepatoma cells with long term incubation with ISO. In these cell lines, complete recovery of the plasma membrane receptors required protein synthesis (11,75). The data suggests that chronic exposure to an agonist results in receptor degradation and loss from the cellular pool.

4. Desensitization of Cardiac Adrenergic Receptors

Cardiac desensitization of adrenergic receptors has been demonstrated in a number of experimental models (94,177,222,223,337,340). Kirkendol and Woodbury (180) demonstrated a tolerance in dogs within 15 minutes of a 60 minute intravenous infusion of NE (2.5 μg/kg/min). A failure of NE to maintain the increased blood pressure and contractile force was noted. With further investigation it was concluded that the heart was the primary site of tachyphylaxis (180). Kaumann and Birnbaumer (177) have reported decreased inotropic and chronotropic responsiveness of kitten atria to isoprenaline after 3 hour exposure to the agonist. Chick ventricular tissue preincubated with ISO for 30 minutes also showed diminished the inotropic response

to the agonist while the responsiveness to extracellular calcium was not altered (222). β-Adrenergic receptor number and affinity was not changed but the ability of ISO to stimulate adenylate cyclase was reduced by 29%. Further investigation showed no abnormality in the cyclase (222). Cultured chick embryo ventricular cells exhibited a similar concentration and time dependent β-receptor desensitization with a 30 minute exposure to ISO (223).

Chang et al. have reported a selective desensitization of rat cardiac β-receptors following prolonged in vivo infusion of 400 μg/kg/hr ISO or 200 μg/kg/hr NE (44). Decreased inotropic and chronotropic responsiveness of the right and left atria to ISO was apparent by 2 hours of catecholamine infusion. β-Receptor density was not significantly decreased until seven days of catecholamine infusion. In this model, the cardiac α_1-receptor was not desensitized by the mixed α/β agonist NE. In contrast Corder et al. (62) demonstrated a reduction in β-receptors at 9 hours post ISO injection (40 mg/kg) and a reduction in α-receptors by 24 hours. The disparity of these studies may be related to the specific catecholamine administered or the dosage.

Isoproterenol in doses high enough to produce necrosis did not change basal activity of adenylate cyclase in the rat myocardium but depressed adenylate cyclase response to ISO stimulation in the necrotic left ventricular tissue (286). Schulze et al. suggested the damage in the necrotic regions destroyed the coupling of the receptor, G protein and the enzyme (286). However, additional research suggested that the G protein may be altered by high dose catecholamines. Tse et al. (340) reported a decreased ventricular basal and NaF-stimulated adenylate cyclase activity

after chronic ISO treatment. Corder et al. (62) showed a decreased EPI-, NaF- and Gpp(NH)p-stimulated adenylate cyclase activity within 3 hours of a high dose ISO injection while basal activity was not altered. The attenuated responses to Gpp(NH)p and NaF-stimulation indicated a defect in the G protein. Adenylate cyclase response to forskolin, a direct stimulator of the enzyme, needs to be measured to rule out changes in the enzyme itself (62).

If high levels of circulating catecholamines uncouple the β-receptor from its G protein or alter the function of G_s, β-receptor mediated increases in ventricular function should be depressed acutely with NE-induced injury. Chang et al. (44) found that the rat cardiac α-adrenergic receptor was not desensitized by a prolonged infusion of high dose NE. In this case, the α-adrenergic system may be more important in increasing cardiac performance in norepinephrine cardiomyopathy. To test this hypothesis, the functional responsiveness of the left ventricle to α- and β-adrenoceptor stimulation will be assessed immediately after a 90 minute NE infusion and at 48 hours post-infusion.

F. SUGGESTED MECHANISMS OF CATECHOLAMINE-INDUCED INJURY

Experimental research indicates that multiple mechanisms are involved in the pathogenesis of catecholamine-induced cardiac injury.

1. Calcium Overload Theory

In 1971, Fleckenstein demonstrated an increase in calcium influx into ISO-treated hearts and suggested that calcium overload was a mechanism of catecholamine-

induced injury (93). It was hypothesized that excessive calcium influx into myocardial cells by β-adrenergic receptor stimulation induced calcium dependent ATPases and increased the utilization of high energy phosphates stores. High intracellular calcium impaired mitochondria function and decreased oxidative phosphorylation and ATP production. The subsequent deficiency in high energy phosphates resulted in the development of myocardial lesions. Further investigation did not totally support this hypothesis. Opie et al. (153,249) found epinephrine induced the release of myocardial lactate dehydrogenase without a significant change in tissue ATP. Simply, myocardial damage occurred without a deficiency in ATP. Other investigators (204) reported a decrease in high energy phosphates 2 hours post ISO injection. At 2 hours, significant cellular injury had already occurred. These studies indicate that ATP deficiency is not a primary mechanism of catecholamine injury. It was still apparent however, that increased intracellular calcium was intricately involved in the etiology of catecholamine injury (17,249) since calcium overload of myocardial cells had been demonstrated repeatedly with the administration of high dose catecholamines (17,68,178,249) and calcium channel blockade reduced the deleterious effects of catecholamines on the heart (53,178,314).

During normal cell functioning, calcium is extruded from the cell by the sarcolemmal sodium-calcium exchange and calcium pump or removed by uptake into the sarcoplasmic reticulum and mitochondria (68,140). In catecholamine cardiomyopathy, removal of calcium from the cytosol is impaired. Makino et al. (220) showed a depression in the activity of the sarcolemmal sodium-calcium exchange

in rat hearts 3, 9 and 24 hours after ISO treatment. Calcium pump activity was reduced at 24 hours (67) but sodium-potassium ATPase activity was not affected (220). With these experimental results, Makino et al. (67,220) suggested that the reduced ability to remove calcium through the sarcolemmal sodium-calcium exchange and calcium pump contributed to the intracellular calcium overload seen in catecholamine-induced injury. Calcium uptake by the sarcoplasmic reticulum was also altered. Microsomal uptake of calcium was elevated 3 hours after ISO injection in rats but depressed at 24 hours (253). Calcium pump activity of rabbit microsomes was also decreased with ISO treatment (346). This decrease in sarcoplasmic calcium uptake appears to be associated with the loss of the calcium ATPase transport protein from the sarcoplasmic reticulum membrane (253). These studies indicate that impaired removal of calcium from the cytosol by sarcolemmal and sarcoplasmic reticulum mechanisms coupled with increased influx of calcium may contribute to the intracellular calcium overload seen after the administration of high dose catecholamines.

Elevated intracellular calcium activates hydrolytic enzymes which may contribute to membrane defects seen in catecholamine injury (68). Calcium overload can activate intracellular proteases and release lysosomal enzymes (68,248). Phospholipases which release free fatty acids from the membrane and stimulate the generation of toxic intermediates of arachidonic acid metabolism are also activated (26,186,282). Arachidonic acid intermediates initiate lipid peroxidation of intracellular and sarcolemmal membranes which can further increase intracellular

calcium (282). Several investigators have also shown that the rate of lipoperoxidation was enhanced in the presence of calcium (161,168,170).

Uncoupling of oxidative phosphorylation has been observed in mitochondria from rats treated with EPI, NE or ISO (309). The decrease in ATP formation was not a direct effect of these amines since catecholamines applied to normal mitochondria in vitro did not affect oxidative phosphorylation. At 9 and 24 hours post ISO treatment, a time when myocardial injury has already been identified, mitochondrial uptake of calcium increased (253). Elevated mitochondrial calcium was associated with depressed respiratory activity and decreased ATP production (65,68). Accumulation of long chain fatty acids within the cell and products of catecholamine autoxidation also uncouple oxidative phosphorylation and reduce ATP production (149). The subsequent depletion of high energy phosphates may then contribute to cellular necrosis.

2. Relative Hypoxia

Rona et al. (276) proposed that relative hypoxia, due to imbalance between oxygen supply, and demand caused ISO-induced myocardial necrosis. In most tissue oxygen supply can be augmented by increasing oxygen extraction from arterial blood and/or increasing blood flow. In the heart, extraction is near maximal at rest so increased oxygen supply must come through an augmented blood flow. Rona and his co-investigators observed an increase in systolic pressure then a decrease toward control during sustained catecholamine infusion while coronary vascular resistance changed very little. Since coronary flow was dependent on systolic pressure, it was

concluded that the oxygen delivery was not adequate resulting in hypoxia and myocardial necrosis. Subsequent research did not support this hypothesis since catecholamine injury occurred where oxygen supply and/or coronary flow was adequate (153,178).

3. Microcirculatory Changes

In the early 1960's, Handforth (135) observed a reduction in flow to the inner areas of the hamster left ventricular wall after ISO, even prior to the development of edema or necrosis. He suggested that impaired perfusion was due to local vasoconstriction or shunting of blood to bypass the endocardium. Later, Collins and Billings (56) reported that ISO reduced perfusion of subendocardium in rat left ventricle. Similar changes in flow have been reported in rabbits and dogs in response to NE (301,354). Simons and Downing (301) demonstrated augmented coronary blood flow after 3 minutes of NE infusion in the rabbit and a return to control at 10 minutes. After 40 minutes of NE infusion, coronary vascular resistance was increased and myocardial blood flow sharply declined. α-Adrenergic receptor blockade prevented the decrease in blood flow and increase in vascular resistance and minimized histological damage. These studies suggest that coronary vasoconstriction during catecholamine infusion may decrease endocardial blood flow and contribute to catecholamine-induced injury.

Haft et al. (129) reported that aspirin and dipyridamole, inhibitors of platelet aggregation, protected the canine myocardium from EPI-induced necrosis. Thrombocytopenia or inhibition of platelet aggregation strongly reduced the number

and area of cardiac necrosis induced by ISO in the rat (173). Electron micrograph analysis revealed platelet aggregates within small vessels of the myocardium immediately following a prolonged NE infusion in the dog (130) or ISO injection in the rat (128). These investigators suggested that intravascular platelet aggregation had a role in the etiology of catecholamine-induced cardiac necrosis. In contrast, other investigators have not observed platelet aggregates in the myocardium after catecholamine administration. Moschos et al. (232) did not find ^{51}Cr-prelabelled platelets in the canine heart after a systemic infusion of toxic levels of EPI. Similarly, platelet thrombi were not found in rabbit hearts after high dose NE infusion (76).

4. Metabolites of Catecholamine Autoxidation

It has been proposed that products of catecholamines oxidation are responsible, in part, for catecholamine cardiotoxicity. Yates and Dhalla have shown decreased cardiac function and ultrastructural damage in isolated rat hearts perfused 40-50 minutes with spontaneously oxidized ISO (69,362). Similar concentrations of fresh ISO increased left ventricular function and produced no structural damage. Perfusion of rat hearts with purified adrenochrome induced ultrastructural changes and contractile failure (363). Mitochondrial and sarcotubular swelling, intracellular edema, hypercontraction of myofibrils and partial separation of intercalated discs became apparent within 5-10 minutes after the administration of intravenous adrenochrome (4-32 mg/kg) to rats (303). These ultrastructural lesions in hearts subjected to oxidized ISO or adrenochrome were qualitatively identical to those seen after the administration of high dose catecholamines in vivo (334). Further

investigation indicated that oxidation products of catecholamines, in addition to adrenochrome, damage the myocardium and may be involved in the genesis of catecholamine cardiotoxicity (162,305).

Decreased calcium uptake activity has been reported for microsomal membranes from hearts damaged by oxidized ISO while microsomal ATPase activity was not modified (69). Changes in membrane integrity, indicated by sarcoplasmic reticular swelling, may have uncoupled calcium transport from ATP hydrolysis (69,146). Mitochondrial calcium transport and ATPase activity were not effected by oxidized ISO but oxidative phosphorylation was depressed (69). Exposure to adrenochrome alters membrane ATPases and calcium transport of the sarcolemma, sarcoplasmic reticulum and mitochondria. Takeo et al. (322) reported a reduction in sarcolemmal sodium-potassium ATPase activity after hearts were perfused with adrenochrome. Additionally, calcium uptake by microsomal and mitochondrial membranes was depressed (323,324). Calcium ATPase activity was not affected by low dose adrenochrome. At higher concentrations, this ATPase activity was inhibited in microsomes, the sarcolemma and mitochondria (322-324). Adrenochrome also has been shown to be a potent inhibitor of oxidative phosphorylation (69,254).

In contrast, administration of adrenochrome (10 mg/kg) had no effect on the myocardium of embryonic chicks while oxidized isoprenaline caused severe damage (162). Wheatley et al. (356) have reported that rat hearts perfused with 10^{-6} to 10^{-4} M adrenochrome for 45 minutes had no myocyte damage. However, perfusion with 10^{-4} M adrenochrome for 120 minutes induced myocardial damage and contractile

failure. These investigators proposed that 10^{-4} M adrenochrome was not a physiologic concentration and therefore irrelevant to the pathogenesis of catecholamine cardiomyopathy (356). However, concentrations of oxidized metabolites within the myocardium have not been reported. The disparity of results may be due to the difference in composition of the oxidized agent. Chromatographic analysis revealed a significant differences in the composition of spontaneously oxidized isoprenaline, purified adrenochrome and commercially obtained adrenochrome (162).

Catecholamine autoxidation also creates superoxide anion radicals ($O_2^{\cdot-}$) (21,92,119,142,227,262,302,308,342) hydrogen peroxide (119,231). Superoxide anion, as well as subsequently formed free radicals, are highly reactive molecules containing a single unpaired electron in its outer shell (74,218). These radicals cause tissue damage by reacting with polyunsaturated lipids within membranes (40,267), nucleotides in DNA, or sulfhydryl bonds in proteins (218). Superoxide anion and hydrogen peroxide directly damage cells but the primary mechanism of toxicity appears to be the generation of more reactive species (43,133,163). In reactions secondary to superoxide generation, singlet oxygen, a spin altered form of oxygen (92), is produced (58,179,238). Singlet oxygen reacts very rapidly with unsaturated fatty acids within membranes to yield lipid peroxides (55,58,179). Lipid peroxides within biological membranes can change membrane fluidity, permeability and function. Alternately, superoxide may dismutate to hydrogen peroxide (100) and generate hydroxyl radicals via Fenton chemistry (57,132). The highly reactive hydroxyl radical usually initiates a radical chain reaction. If produced in the

immediate vicinity of phospholipid membranes, hydroxyl radicals initiate peroxidation by abstracting hydrogen atoms from unsaturated bonds of membrane lipids (43,95,133,174,261,267,302,330). Normally, free radicals are scavenged or instantly trapped by antioxidants (74). Accumulation of free radicals is dependant on the capacity of enzymatic and non-enzymatic antioxidant systems within the tissue (285). In conditions where the antioxidant capacity is overwhelmed, free radical production may result in lipid peroxidation and subsequent alterations in membrane structure, enzyme inactivation, and decreased energy production.

5. Changes in Membrane Permeability

Rona et al. (275) reported an increased sarcolemmal permeability 90 minutes post ISO injection in rats. Horseradish peroxidase reaction products were deposited on myofilaments of damaged cells and cells which appeared ultrastructurally intact. Later peroxidase reaction products were identified within mitochondria. Subsequently, Boutet et al. (22) demonstrated horseradish peroxidase within rat cardiac myocytes as early as 10 minutes after NE infusion and at 60 and 90 minutes post ISO. Increased membrane permeability was also noted in isolated rabbit hearts after a 60 minute perfusion with ISO (336). More recently, Yunge et al. (365) reported horseradish peroxidase within cardiac myocytes and irregular tears in sarcolemmal membranes of some myocytes 10 and 60 minutes after subcutaneous ISO. Similar results were obtained in isolated rat hearts perfused with ISO or oxidized ISO (365) indicating a role for oxidized metabolites in ISO-induced injury. In agreement with these histological studies, Schenk et al. (283) demonstrated a progressive dose related

increase of the cardiac isoenzyme of lactate dehydrogenase the plasma of dogs within 15 minutes of a NE infusion. The presence of the heart isoenzyme in plasma indicated damage to the cardiac sarcolemma with subsequent release of this intracellular enzyme. These studies suggest that alterations in cardiac sarcolemmal permeability is an early event in catecholamine-induced injury.

The increased membrane permeability seen with high dose catecholamines may change electrolyte distribution in the myocardium. Lehr et al. (205) and Nirdlinger and Bramante (241) found significant alterations in electrolyte content and distribution in rat hearts after catecholamine injection. Magnesium and inorganic phosphate were depleted, sodium and calcium content increased. Intracellular calcium was sequestered by mitochondria at the expense of energy production (68). Other investigators found ISO treatment increased cardiac sodium, water and chloride but decreased potassium (311). In fact, catecholamines stimulated a rapid outward movement of intracellular potassium and a high potassium diet afforded some protection to ISO-induced injury (171). These electrolyte shifts were correlated with swelling and disintegration of mitochondria and enlargement of the sarcotubular system in cardiac myocytes.

6. Lipid Peroxidation

Lipid peroxidation is initiated by the abstraction of a hydrogen ion from an unsaturated lipid and the formation of a lipid free radical, $L^{\cdot}$ (Reaction [1]) (38,174). With the rich supply of oxygen within membranes, the lipid radical is quickly oxidized to a peroxyl radical ($LOO^{\cdot}$) (Reaction [2]). This peroxyl radical can attack another

unsaturated lipid producing a hydroperoxide (LOOH) and a new lipid free radical (Reaction [3]), thus establishing a chain reaction. Hydroperoxides can later decompose to peroxy and alcoxy (LO·) radicals (Reaction [4]) which initiate other chain reactions (Reactions [3] and [5]). Without an antioxidant within the membrane, lipid peroxidation is propagated until two lipid radicals react together to form nonreactive molecular products (125). By a chain reaction, many unsaturated lipids can be oxidized for each lipid radical formed (38,226,360).

[1] $$LH \rightarrow L\cdot$$

[2] $$L\cdot + O_2 \rightarrow LOO\cdot$$

[3] $$LOO\cdot + LH \rightarrow LOOH + L\cdot$$

[4] $$LOOH \rightarrow LOO\cdot \text{ or } LO\cdot$$

[5] $$LO\cdot + LH \rightarrow LOH + L\cdot$$

Lipid peroxidation changes membrane characteristics. Membrane permeability is increased. Rat brain synaptosomes rapidly accumulated calcium after peroxidation was stimulated (26). Increased calcium uptake was highly correlated with the formation of products of peroxidation. Deferoxamine, a lipid peroxidation inhibitor, blocked the formation of products of lipoperoxidation and uptake of calcium into synaptosomes after exposure to peroxidizing agents. Calcium channel blockers did not affect calcium uptake by synaptosomes with peroxidation. Using oleic and linoleic acid ufasomes, Hicks and Gebicki (147) demonstrated a significant increase in glucose leakage after peroxidation of approximately 4-5% of membrane lipids. Lipid

peroxidation in cardiac sarcoplasmic reticulum vesicles induce the complete release of calcium (209). Calcium leakage was mediated by the formation of lipid hydroperoxide channels (360) in the lipid phase of the sarcoplasmic membranes and in the lipoprotein complex of calcium ATPase (169). Calcium leakage from the sarcoplasmic vesicles is blocked by α-tocopherol (209). By disturbing the normal bilayer arrangement of membrane lipids, lipid peroxidation changed membrane permeability.

Decreased membrane fluidity (99) and increased rigidity of phospholipid bilayers (72) have been reported after lipid peroxidation. Rice-Evans and Hochstein (272) demonstrated an increase in lipid microviscosity with peroxidation of membrane lipids and suggested lipid-lipid interaction and lipid packing density increase while lipid-protein interactions decrease (296). These modifications of membranes structure have been correlated with alteration in membrane bound enzyme function (72). In isolated mitochondria, oxidative phosphorylation was uncoupled with lipoperoxidation (155,326). The degree of peroxidation which changed membrane fluidity was similar to that which alters mitochondrial function (72). Lipid peroxidation also damaged membrane proteins (326) and inactivates enzymes (43,172,330). Hydrogen ion abstraction by lipid peroxy radicals altered protein structure (326). Damaged proteins are able to cross-link with other proteins or phospholipids to form polymers (160,272). Malondialdehyde, a degradation product of peroxidized lipids (226,261), may facilitate amino group cross-linking within the membrane (108,160,174,272,326). Levin et al. reported cytochrome P450 breakdown during lipid peroxidation (207).

Random damage to cytochromes $a + a_3$, b, and $c + c_1$ has also been reported (326). Slight accumulation of lipoperoxidation products in cardiac microsomes uncoupled ATP hydrolysis from calcium transport and completely inhibited calcium transporting ability (170). The antioxidant α-tocopherol prevented accumulation of peroxidation products and protected microsomal membrane calcium transport. Inactivation of mitochondrial and microsomal enzymes (261,326,358) and membrane ATPases (272) also closely parallel lipid peroxidation. Alterations in enzyme function may be due to protein damage and subsequent polymerization or due to changes in the phospholipid microenvironment (123).

G. CELLULAR ANTIOXIDANTS

The human body has multiple lines of defense against oxidizing agents and free radicals encompassing enzymes systems and small molecular weight molecules with antioxidant capabilities. These antioxidant defense mechanisms include α-tocopherol and glutathione peroxidase.

1. α-Tocopherol

Alpha-tocopherol, the principle antioxidant constituent of vitamin E, is found in plasma and intracellular membranes of throughout the body including cardiac myocytes (122,163,218,226,247). The basic structure consists of a hydroxylated ring system and an isoprenoid side chain (240). The aromatic ring or chromanol group of α-tocopherol is oriented toward the membrane surface and a hydrophobic phytyl side chain is buried within the hydrocarbon region (38). This structure allows α-

tocopherol to be an effective scavenger of lipid free radicals (37,39,70), superoxide anion radicals ($O_2^{\cdot-}$) (100,102), hydroxyl radicals ($HO^{\cdot}$) (100,101,225), and singlet oxygen ($^1O_2$) (55,84,96,97,315) as well as a stabilizer of biological membranes (80,215).

The presence of fat-soluble antioxidants which inhibit or terminate free radical reactions of unsaturated lipids is essential to membrane integrity (154,226). Alpha-tocopherol, a principle defense against lipoperoxidation (102,329), is an effective chain-breaking antioxidant (38,327). It does not prevent the initiation of lipid peroxidation (148,360) but traps the propagating lipid radicals thereby terminating the autoxidizing chain reactions (Reaction [6]) (37,39,102,150,327,329). The resulting phenoxy radical of α-tocopherol (αT-O$^{\cdot}$) is resonance stable and does not continue the chain reaction (38). The α-tocopherol radical can be destroyed by reaction with a second peroxyl radical (Reaction [7]) (38) or be reduced by ascorbic acid (38,102,225,251). Fukuzawa et al. (99) reported that one molecule of α-tocopherol prevented peroxidation of approximately 100 polyunsaturated fatty acid molecules.

[6] $\quad LOO^{\cdot} + \alpha T\text{-}OH \rightarrow LOOH + \alpha T\text{-}O^{\cdot}$

[7] $\quad LOO^{\cdot} + T\text{-}O^{\cdot} \rightarrow$ unreactive products

The antioxidant capability of α-tocopherol has been demonstrated in vitro and in vivo (89,102,164,181). Lipid oxidation in egg yolk phosphatidylcholine liposomes was inhibited after a critical concentration of α-tocopherol was present (102). As α-tocopherol concentration increased above this critical level, peroxidation decreased

then stopped entirely although the tocopherol was still being oxidized. At this point α-tocopherol competed successfully with oxygen for lipid radicals. The efficacy of α-tocopherol is dependent on the membrane concentration (360).

Having membranes rich in polyunsaturated lipids and numerous catalysts of lipid peroxidation, mitochondria are highly susceptible to oxidation damage (326). α-Tocopherol, normally present on the inner mitochondrial membrane (226), has been associated with enzymatically active lipoprotein complexes of the electron transport system (85,247,289). Mitochondria from α-tocopherol deficient rabbit hearts had depressed function, increased lipoperoxidation and enhanced production of superoxide anion by electron transport enzyme systems (123). The increased formation of superoxide anion was beyond the neutralizing capacity of the superoxide dismutase (227) and resulted in loss of polyunsaturated lipids from mitochondrial membranes (226). Further lipid peroxidation resulted in the swelling, lysis and disintegration of membranes from isolated mitochondria (326). The presence of α-tocopherol in membranes of the cardiac mitochondria protected them from oxidative damage and membrane deterioration (123,326). Reduced cytochrome levels in liver mitochondria and decreased oxidative ability of the mitochondria have been reported in vitamin E deficient rats (289). With vitamin supplementation cytochrome levels and oxidative ability of the mitochondria were restored. Vitamin E may protect the membrane bound proteins from damage maintaining normal enzyme concentration within the membrane.

Increased susceptibility to lipid peroxidation is associated with α-tocopherol

deficiency (240,320). Janero and Burghardt (164) have shown phospholipid damage characterized by acute onset and rapid progression in cardiac membranes from rats with a 3-fold lower α-tocopherol content compared to control rat hearts. This relative α-tocopherol deficiency increased the susceptibility of the myocardial lipids to peroxidative injury and reduced the efficacy of other antioxidant interventions. Similar results have been reported for the mouse myocardium (280). Rats fed diets with large amounts of unsaturated fats had increased malonaldehyde in bone marrow, heart and spleen compared to rats fed normal diets (330). Peroxidation was proportional to polyunsaturated fatty acids content and inversely related to α-tocopherol (134,330).

Experiments of Lucy and Diplock (71,215,216) indicated that α-tocopherol modulated the structure and permeability of the phospholipid bilayer of biomembranes. Studies using monolayers of phospholipids and α-tocopherol demonstrated a molecular interaction between α-tocopherol and unsaturated phospholipids but gave no evidence of tocopherol interacting with saturated lipids (216). Ultraviolet absorption spectra and flourometric assays also suggested that α-tocopherol forms complexes with free fatty acids in membrane systems (80). Using molecular modeling, Lucy and Diplock (215) proposed a dynamic interaction between α-tocopherol and phospholipids containing arachidonyl residues or similar fatty acyl chains. Specifically, methyl groups of the phytyl side-chain of α-tocopherol move into pockets created by *cis* double bonds of fatty acyl chains. Remaining methylene groups in the backbone of the phytyl and fatty acyl chains interact through Van der Waals forces, stabilize the

membrane and preserve the integrity of cell membranes (80,215). Additionally, Urano and Matsuo (341) reported that the chromanol moiety of α-tocopherol also interacts with the double bonds of unsaturated lipids. This interaction between α-tocopherol and unsaturated phospholipids may render the lipids less susceptible to radical-induced injury (71,101,218,327).

In α-tocopherol deficient animals, sarcolemmal membranes were abnormally permeable (215,240). The addition of α-tocopherol to liposomes decreased permeability to glucose and chromate ion (216). Philipson and Ward (259) reported enhanced permeability of cardiac sarcolemmal vesicles to calcium as the content of unsaturated fatty acids within the vesicles increased. Tocopherol-lipid interactions reduced the permeability of biological membranes containing relatively high levels of polyunsaturated fatty acids (70,71). To assess the interaction of α-tocopherol, lipids and proteins within biological membranes, glucose transport was measured as α-tocopherol was added to the culture media of mouse fibroblasts (105). In this tissue culture system, the addition of α-tocopherol increased fibroblast glucose uptake. Since the transport of glucose into cells is a carrier-mediated process, Giasuddin and Diplock (105) suggested that the integrity and maximal function of the plasma membrane was dependent on a structural interaction of α-tocopherol and other membrane components.

Some authors suggest a metabolic role for vitamin E. Fedelesova et al. (85) reported decreased high energy phosphate stores in hearts from vitamin E deficient rats. Lactate dehydrogenase and malate dehydrogenase activities were decreased while

sarcolemmal sodium-potassium ATPase and sarcoplasmic reticular calcium ATPase activities increased. The increased sarcoplasmic reticular ATPase may be a compensatory mechanism for an increased intracellular calcium which has been reported with vitamin E deficiency. The ten week vitamin E deficient diet used by Fedelesova (85) depressed myocardial contractility even though there was no evidence of ultrastructural damage. These results may indicate that in the early stages of vitamin E deficiency myocardial energy production and utilization are altered.

2. Glutathione Peroxidase

Selenium-dependent glutathione peroxidase is another major defense against oxidizing agents and lipid peroxidation (150,212,328). It is found in plasma, the cytosol and the mitochondrial matrix (58). The enzyme is a tetramer with four identical subunits each containing one selenium atom (150,246,330). Selenium or more specifically a selenocystine moiety has been identified as the catalytic site for glutathione peroxidase (278,279,326).

Glutathione peroxidase protects cellular and subcellular membranes from peroxidative damage. Using glutathione as a hydrogen donor (50), glutathione peroxidase catalyzes the reduction of hydrogen peroxide to water (212) thereby preventing the initiation of peroxidation (278,330). This enzyme also detoxifies membranes of fatty acid hydroperoxides preventing these peroxides from altering membrane structure and function (126). Working with phospholipase A_2, glutathione peroxidase reduces polyunsaturated lipid hydroperoxides to hydroxy acids in the hydrophilic region of a membrane (212,279,290,327,330). Phospholipase A_2

hydrolyses peroxidized fatty acids of membrane phospholipids (343). After release from the phospholipid, peroxidized fatty acids are available for reduction by glutathione peroxidase (36,58,325,330). It is estimated that 50 to 250 fatty acid hydroperoxides may be destroyed by one selenocystine moiety (360). In addition to destroying peroxides, glutathione peroxidase reduces secondary lipid peroxidation occurring with the breakdown of lipid hydroperoxides (360).

Glutathione peroxidase activity increases with lipid peroxidation (330). If present in sufficient quantities glutathione peroxidase removes lipid peroxides as they are formed, before the peroxides alter membrane structure. Selenium deficiency results in reduced glutathione peroxidase activity (3,364), an inability to metabolize hydroperoxides through glutathione-dependent pathways (361), and decreased protection against free radicals (361,364). Cardiac muscle appears to be very sensitive to selenium depletion (46,150). Upon exposure to low dose oxygen radicals, decrease in function of isolated selenium-deficient rat hearts was significantly more pronounced than control hearts (364).

Free radicals generated by catecholamine autoxidation may be involved in the development of catecholamine-induced cardiomyopathy (131,243,258,304). Singal et al. (304) have shown that vitamin E pretreatment prevented the deleterious effects of high dose ISO. These investigators (302) have also demonstrated increased lipoperoxidation in rat myocardium with ISO that was prevented by α-tocopherol pretreatment. Plasma from rats injected with 5 mg/kg EPI was cytotoxic to isolated cardiac myocytes after intracellular glutathione levels decreased (243). Since

ultrastructural changes did not occur until glutathione levels decreased, Noronha-Dutra et al. (243) proposed that free radicals were the cytotoxic agent which initiated lipid peroxidation. The administration of selenium and vitamin E to pigs also afforded some protection to high dose ISO (344). The administration of selenium has also been shown to increase glutathione peroxidase activity and afford protection against lipid peroxidation (3,46,73,126).

This research indicates that adequate amounts of selenium-dependent glutathione peroxidase and α-tocopherol are essential for normal membrane physiology particularly if tissues are exposed to substances which directly or indirectly promote lipid peroxidation (59,150). Therefore, in the third study of this dissertation, α-tocopherol and selenium will be administered over a two week period to increase the antioxidant capacity of the heart and determine if this pretreatment protected the rabbit myocardium from NE-induced injury.

H. NO-REFLOW PHENOMENON

No-reflow phenomenon is the failure to achieve uniform reperfusion following temporary ischemia (183) and has been described in various organs of a number of experimental animals. In 1966, Krug et al. (190) reported that significant portions of the endocardium could not be reperfused after temporary occlusions of 60-120 minutes in the cat. But it was not until 1974 that Kloner et al. (184) provided the first direct evidence that the no-reflow phenomenon occurred in the heart. The pathological mechanism causing the no-reflow phenomenon has not been elucidated however

several mechanisms including endothelial and myocyte swelling, ischemic contracture and rigor mortis, have been suggested (103).

Brown and Egginton (31) demonstrated a loss of alkaline phosphatase staining in regions of ischemic injury in the heart. The loss of alkaline phosphatase, an enzyme normally present in capillary endothelium, indicated endothelial damage. Electron microscopic examination revealed no loss of capillaries 48 hours after NE infusion in the rabbit but capillary morphology was disrupted and endothelial cells were edematous. In areas of no-reflow present in the canine myocardium after 90 minutes of ischemia, Kloner demonstrated endothelial bleb formation, endothelial gaps, red blood cell aggregates and occasional thrombi within capillaries (184). Subsequently it was proposed that endothelial injury had prevented homogeneous reperfusion of the ischemic myocardium (183,184). However, the demonstration that colloidal thorium dioxide could pass through vessels with severely swollen endothelium showed vascular injury itself could not account for the no-reflow phenomenon.

Compressive stress on the coronary microcirculation during ischemic contracture may contribute to the development of no-reflow (211). Ischemic contracture in isolated rat hearts has been associated with reduced flow in the subendocardium (104,211). Gavin et al. (104) showed that rat hearts subjected to 60 minute global ischemia had no morphological evidence of endothelial swelling or constriction of any particular population of vessels and hypothesized that the endocardium of the left ventricle lost its ability to be reperfused because of

extravascular compression. Ischemic contracture closed subendocardial vessels and focal rigor mortis prevented vessels from opening with reperfusion (103). Vascular obstruction was not the essential cause of no-reflow.

During prolonged catecholamine infusion, α-receptor mediated coronary vasoconstriction decreases and redistributes myocardial blood flow (135,301). Currently it is unknown if normal blood flow is reestablished to the ischemic areas after catecholamine infusion is terminated. In this dissertation, coronary blood flow was measured in rabbits after 40 minutes of NE infusion and at one hour post infusion to determine if the no-reflow phenomenon occurs with catecholamine-induced injury.

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CHAPTER III

GENERAL METHODS

A. NOREPINEPHRINE-INDUCED CARDIOMYOPATHY

Male, New Zealand white rabbits weighing approximately 2 kg were anesthetized with sodium pentobarbital (30 mg/kg IV). 0.9% sterile saline or norepinephrine bitartrate (NE; 2, 4, or 6 μg/kg/min) in 0.9% sterile saline was infused as a constant rate of 0.238 ml/min (Harvard Apparatus infusion/withdrawal pump, model 906) for 90 minutes via a 25-gauge needle inserted into the marginal ear vein. In rabbits used for acute experiments, the femoral artery was cannulated for monitoring arterial pressure. A standard Lead II ECG was recorded. Hearts were removed at the end of the infusion period for functional studies or 1 hour post-infusion for blood flow measurements. Rabbits used in chronic experiments did not have the femoral artery cannulated and were allowed to recover from the anesthetic.

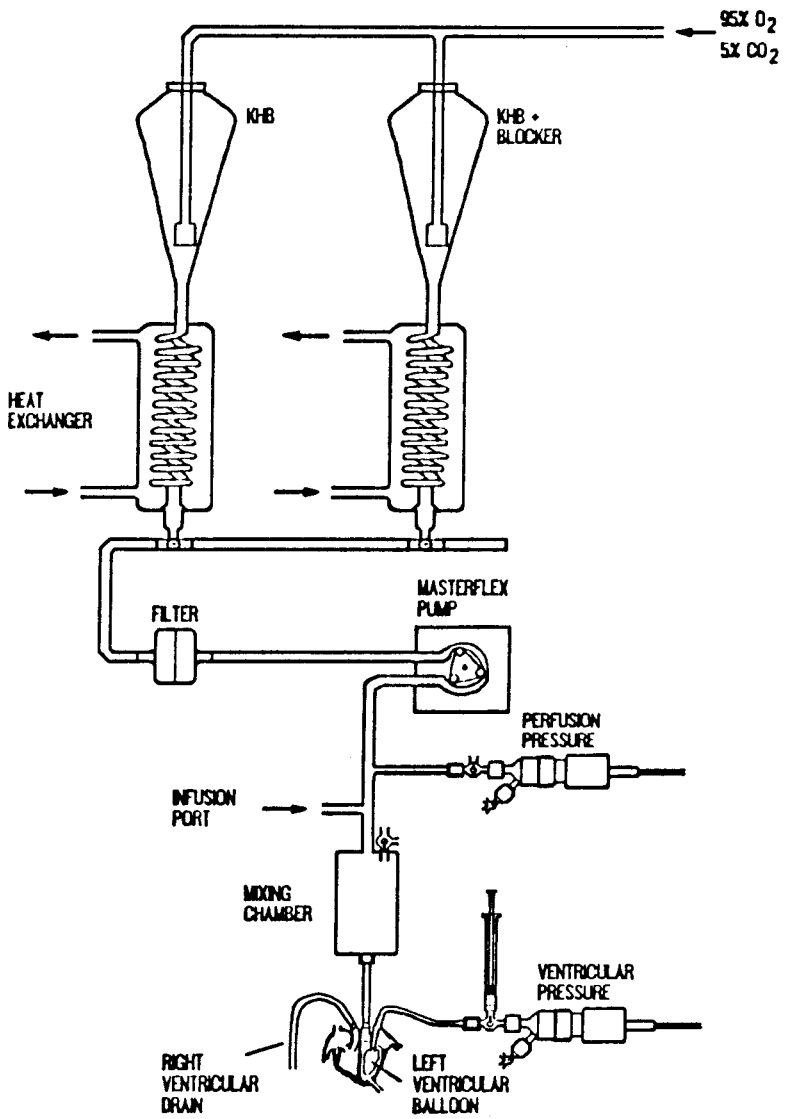
B. ISOLATED, NON-EJECTING HEART PREPARATION

1. Langendorff Perfusion Apparatus

A non-recirculating Langendorff apparatus with dual reservoirs and constant flow system was used in all isolated heart preparations (Figure 3-1). Hearts were perfused with Krebs-Henseleit buffer (KHB; 118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 24 mM $NaHCO_3$, and 1.2 mM KH_2PO_4) modified with the addition of 11 mM glucose, gassed with 95% O_2 and 5% CO_2 (pH=7.40 $\pm$ .03). Solutions were filtered through a 47μm inline filter and warmed by Graham condensers and constant temperature circulator (Model 1267-00; Cole-Parmer Instrument Co.) to maintain intraventricular temperature at 37 $\pm$ 0.5°C. A series of valves following the condensers permitted rapid switching between reservoirs. A Masterflex peristaltic pump (Cole-Parmer Instrument Co.) regulated coronary flow at 10 ml/min/g heart, wet weight (7). Although oxygen uptake was not measured, functional data indicated that this flow rate adequately perfused the isolated rabbit heart. Hearts increased ventricular function in response to increasing concentrations of NE and showed no signs of failure. Increasing coronary flow to 15 ml/min/g did not change perfusion pressure or ventricular function. However when flow was 15 ml/min/g significant edema was apparent within 60 minutes. A sideport positioned after the pump permitted the addition of adrenergic agonists to the perfusate. Perfusion pressure was measured by a Gould Statham pressure transducer located near the aortic cannula and was recorded continuously on a Gould 2400S chart recorder. A bubble trap was positioned before a stainless steel aortic cannula.

FIGURE 3-1

LANGENDORFF PERFUSION APPARATUS



Schematic diagram of the non-recirculating, constant flow Langendorff perfusion apparatus used in ventricular function studies.

The pulmonary artery was cannulated with a 10 cm length of plastic tubing. A thermistor probe (Model 43 TA; Yellow Springs Instrument Co., Inc.) was fed through this cannula, inserted into the right ventricular cavity and secured in place. Intraventricular temperature was monitored continuously.

A balloon tipped cannula was used to measure relative left ventricular pressure (LVP). The compliant, fluid-filled (50% saline/50% ethanol; v:v) latex balloon was inserted into the left ventricle and connected to a Gould Statham pressure transducer via a 9 cm PE-190 cannula. A Hamilton syringe mounted next to the pressure transducer and connected via a three way valve to the balloon cannula permitted serial inflation of the intraventricular balloon and measurable changes in preload. The frequency response characteristics were calculated from the transient oscillations occurring after a step change in pressure (5). The balloon tipped cannula had a natural frequency of 301 $\pm$ 22.5 Hz and a damping coefficient of 0.49 $\pm$ .02. Although slightly underdamped, this system had a constant output/input amplitude ratio for frequencies up to 20% of the natural frequency (3). With hearts paced at 220 beats/minute, the fundamental frequency of pressure generation was 3.7 Hz. Since the essential physiologic information was contained within frequencies up to 37 Hz (12.3% of the natural frequency), this system was adequate to measure ventricular pressure and LV dP/dt. The transducer, cannula and balloon were checked daily for leaks and air bubbles. The balloon was stored in a small vial of 50% saline/ 50% ethanol (v:v) and thoroughly rinsed with KHB before use. Left ventricular dP/dt was derived from the LVP signal using a Gould Differentiator Amplifier (Model 13-4615-

71). LVP and LV dP/dt were continuously recorded on a Gould 2400S chart recorder.

KHB, KHB plus 10^{-6} M phentolamine (Regitine HCl, Ciba) or KHB plus 10^{-6} M timolol (Timolol maleate; Merck) was stored in a reservoir of the Langendorff setup. Three-way valves below the Graham condensers allowed rapid switching between these perfusates. Norepinephrine, methoxamine, dobutamine and isoproterenol were infused via the sideport. Infusion rate was 2 ml/min. The amount of drug infused per minute was adjusted so the final concentration reaching the heart was that concentration stated in the methods. The bubble trap, containing approximately 10 ml solution, allowed for adequate mixing of the drug with the perfusate.

2. Heart Isolation and Instrumentation

After sodium pentobarbital anesthesia (30 mg/kg), 500 IU/kg sodium heparin was administered via the marginal ear vein. The abdomen was opened and the diaphragm cut off the rib cage. The thoracic cavity was opened with two axial incisions, from the diaphragm to the first or second rib. The anterior thoracic wall was lifted over the rabbit's head. The heart was excised quickly and placed in cold heparinized KHB. This procedure required 20 to 30 seconds from initial incision to immersion of the heart in cold KHB. All extraneous fat and connective tissue were removed from the aorta. The heart was weighed on a Mettler PL200 balance. Each heart was removed from the cold KHB, placed on a tared weighing boat, weighed and

quickly returned to the KHB. The weight of KHB remaining in the boat was subtracted from the heart weight.

The aorta was cannulated and immediately perfused with warmed KHB. The left atrium was opened and a balloon tipped cannula was inserted into the left ventricle through the mitral orifice and secured. Care was taken so the balloon cannula was not forced against the ventricular wall or through the ventricular septum. The fluid within the balloon contained a drop of Evans Blue so bulging of the balloon into the left atria upon inflation was identified easily and balloon position corrected before beginning an experimental protocol. Balloon volume was adjusted to left ventricular end-diastolic pressure (LVEDP) of 5 mmHg. To prevent KHB accumulation within the left ventricular chamber a small cannula was inserted through the apical wall. A bipolar electrode attached to the right atrial appendage was used to pace hearts at 220 beats/min.

After instrumentation, hearts were perfused for an additional 20 minutes before experiments were begun. During this time contractions become regular and intraventricular pressure fluctuations reach steady state. Only hearts meeting the following criteria were used in this study: 1) baseline peak systolic pressure greater than 80 mmHg (EDP = 5 mmHg) in hearts from rabbits infused with saline and $\geq$ 65 mmHg in hearts from rabbits treated with NE; 2) stable coronary perfusion pressure; and 3) heart following pace of 220 beats/minute.

C. MEASUREMENT OF DIASTOLIC FUNCTION

Two parameters of diastolic function, LV -dP/dt_{max} and tau, were evaluated in cardiac function experiments. Tau, the time constant for isovolumic relaxation, was measured from the LVP trace after the experiment. Isovolumic relaxation is delimited by the closure of the aortic valve and opening of the mitral valve. Negative LV dP/dt_{max} has been reported to be the best indicator of aortic valve closure (1). Therefore, tau has been defined as the time required for LV cavity pressure at -dP/dt to be reduced by a factor of 1/e (6). Normally LV -dP/dt_{max} occurs before peak systolic pressure has decreased by 40.0% (1,4,8). Using the balloon tipped cannula system in the isolated heart, LV -dP/dt_{max} did not occur until peak systolic pressure decreased by 63%. This point, being extremely low on the LVP trace, did not accurately represent the beginning of isovolumic relaxation. In these experiments a constant of 75% peak systolic pressure was designated as the beginning of isovolumic relaxation. Accordingly, tau was defined as the time required for ventricular pressure at 75% peak systolic pressure to be reduced by 1/e. Tau was measured at 5 mmHg LVEDP.

D. EXPERIMENTAL PROTOCOLS FOR ISOLATED HEART PREPARATION

1. Ventricular Function and β-Adrenoceptor Mediated Inotropy

These studies were designed to assess acute and chronic alterations in LV function with NE-induced injury. In acute studies, LV performance was assessed immediately after saline or NE infusion. Chronic experiments were performed 48

hours after the infusion. To eliminate neural and hormonal influences, the isolated perfused heart preparation was used in all functional studies.

With hearts paced at 220 beats/minute, baseline LV function was measured as LVEDP was increased from 0 to 25 mmHg (5 mmHg steps). Steady state LVP and LV dP/dt_{max} were recorded at each EDP. Hearts were perfused with incremental, cumulative concentrations of NE, 10^{-10} to 10^{-7} M. At each concentration, LVEDP was increased from 0 to 25 mmHg and steady state LVP and LV dP/dt_{max} were recorded. At the termination of each inflation series, the intraventricular balloon was deflated and LVEDP readjusted to 5 mmHg. Hearts were perfused with KHB until ventricular function returned to baseline.

β-Adrenoceptor mediated inotropy was assessed in the presence of α-adrenergic blockade (10^{-6} M phentolamine). α-Receptor blockade was tested with 10^{-7} M methoxamine then baseline ventricular function measurements were recorded. Hearts were perfused with increasing concentrations of dobutamine (10^{-9} - 10^{-6} M), a selective β_1-agonist. LVEDP was varied from 0 to 25 mmHg at baseline and each dobutamine concentration. Steady state developed LVP and LV dP/dt_{max} were recorded.

2. α-Adrenoceptor Mediated Changes in Ventricular Function

The inotropic response to α-adrenergic stimulation was measured in the isolated heart immediately after NE (4 μg/kg/min) infusion and 48 hours post saline or NE infusion. Isolated heart was paced at 220 beats/minute and LVEDP was maintained at 5 mmHg. Baseline ventricular function was recorded then each heart

was perfused with cumulative, decreasing concentrations of NE (10^{-6} - 10^{-7} M). The heart was washed for 20 minutes with KHB and LV function returned to baseline values. Timolol (10^{-6} M), a nonselective β-adrenergic antagonist, was added to the perfusate. After ten minutes, β-receptor blockade was tested with 10^{-7} M isoproterenol. Hearts were re-exposed to the incremental decreasing concentrations of NE (10^{-6} - 10^{-7} M). LVP and LV dP/dt_{max} were recorded at baseline, in the presence of timolol and after two minutes at each NE concentration. Changes in LV function attributed to NE stimulation of α-adrenergic receptor were determined.

3. α-Tocopherol and Selenium Administration

Thirteen male, New Zealand white rabbits (2.2 kg) were treated subcutaneously with α-tocopherol (25.0 mg; BO-SE, Schering Co., U.S.A.) and selenium (0.5 mg) every other day for two weeks. Twenty-four hours after the last treatment rabbits were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Saline (n=6) or 4 μg/kg/min NE (n=7) was infused for 90 minutes via the marginal ear vein. Infusion rate was constant at 0.238 ml/min. Ventricular function was studied two days post infusion using the isolated heart preparation.

Baseline LV function was measured as LVEDP was increased from 0 to 25 mmHg in 5 mmHg steps. Hearts were perfused with incremental, cumulative concentrations of NE (10^{-9} - 10^{-6} M). At each concentration of NE, LVEDP was increased from 0 to 25 mmHg and steady state developed LVP and LV dP/dt_{max} were recorded. LVEDP was readjusted to 5 mmHg following each serial increase in EDP.

E. MYOCARDIAL BLOOD FLOW

This study was designed to determine if rabbit heart exhibited the no-reflow phenomena after a 40 minute NE infusion. Blood flow measurements were made at 0 and 40 minutes of the NE (4 μg/kg/min) or saline infusion and at one hour post-infusion. In another group of rabbits myocardial blood flow was measured 48 hours after NE infusion.

1. Surgical Preparation

Male, New Zealand white rabbits were anesthetized with sodium pentobarbital (30 mg/kg IV). The trachea was cannulated with a glass Y-tube connected to a Harvard Apparatus respirator (Model 661). Each rabbit was ventilated with room air at an end-expiratory pressure of 1 cm of water. Lead II ECG was measured with needle electrodes. Body temperature was monitored and maintained at 38 $\pm$ 1°C with a heat exchanger and heating pad. The femoral arteries were cannulated with polyethylene catheters (PE-90). The right arterial cannula was connected to a Gould Statham pressure transducer for measurement of arterial blood pressure. The left arterial cannula was connected to a Harvard Apparatus infusion/withdrawal pump (Model 906) for the withdrawal of blood samples during microsphere injection. In acute experiments, a femoral vein was cannulated for the infusion of saline or NE. After a left thoracotomy, the left atrial appendage was cannulated with a short piece of PE-160 tubing. This cannula permitted atrial injection of microspheres. Arterial pressure and ECG were monitored throughout the experiment and recorded on a Gould chart recorder.

2. Blood Flow Determination

The following species of radioisotope labeled microspheres (3M Co, New England Nuclear) were used: Ce-141, Nb-95, Sr-85, and Ru-103. These microspheres (14.5 $\pm$ 0.6 μm) were suspended in 10% dextran with 0.05% polyoxyethelene-20 sorbitan mono-oleate (Tween 80) to deter the inherent microsphere aggregation. Vials containing the microspheres were sonicated for at least 20 minutes before use and were vigorously agitated in a vortex mixer immediately prior to injection. For each blood flow measurement approximately 1 x 10^6 microspheres were injected so a minimum of 400 spheres were trapped in each myocardial sample. Microspheres were injected through the atrial cannula over a 20-30 second period then the cannula was flushed with 0.5 ml warm saline. A reference blood sample was withdrawn from the femoral artery at a rate of 3-4 ml/min. Blood withdrawal began 30 seconds before the injection of microspheres and continued for 30-40 seconds post-injection. Withdrawal pump rate was calibrated at the end of each experiment. The reference blood sample was put into plastic counting tubes. The syringes which contained blood were rinsed and the rinse added to respective blood sample counting tubes. At the termination of the experiment, the heart was removed. The atria, right ventricular free wall and left ventricle were isolated and fixed for 24-48 hours in 10% formalin. The atria and right ventricular free wall were weighed and placed in counting tubes. The left ventricle was cut into 5-6 rings. Each ring was separated into endocardial and epicardial halves via a circumferential cut at a point midway between the epicardial and endocardial surfaces. Endocardial and epicardial samples were weighed

and placed in separate plastic counting tubes. The radioactivity of the heart and reference blood tissue samples were counted by a three inch NaI crystal within a TM Analytic gamma counter (Model 1185). Radioactivity counts were sent directly to a computer and stored on floppy disc for later analysis.

Blood flow was calculated using the theoretical organ technique. Using radioactivity counts, calculated withdrawal rate of reference blood sample and tissue weights, a Compaq 286 computer calculated blood flow in ml/min/ 100 g tissue. Standard equations with appropriate corrections and interference coefficients were used. The following equation was used to calculate blood flow: MBF = (Ct/TW) x (RBW/Cb), where MBF = myocardial blood flow in ml/min/100 g, Ct = tissue radioactivity in counts/min, TW = weight of tissue sample in grams, RBW = reference blood withdrawal rate in ml/min, and Cb = total radioactivity in reference blood sample.

F. HISTOLOGY

After termination of several LV function experiments, hearts were removed from the perfusion apparatus and weighed. The atria and right ventricular free wall were dissected from the left ventricle. A midlevel transverse section of LV and septum was taken from each heart and placed in a plastic tissue capsule. Tissue was fixed in 10% buffered formalin solution and further processed with an Autotechnicon Mono Model 2A (Technicon Corporation) tissue processor. Tissue processing included 6 hour tissue alcohol dehydration, 3 hour xylene clearing and 2 hour

impregnation with paraffin. The processing cycle occurred over 12 hours and was followed by embedding in paraffin. Tissue specimens were cut in 10 μ sections (820 Microtome, American Optical Spenser) and placed on microscope slides. Each slide was warmed slightly so the section would adhere to the glass slide during the staining procedure. After a series of xylene and alcohol washes, tissue sections were stained with hematoxylin and eosin (2). Staining was followed by another series of alcohol and xylene washes. The microscope slides were dried and the coverslips permanently mounted. LV sections were viewed to verify the presence or absence of myocardial injury. This injury was noted by edema, loss of nuclei, focal myofibrillar destruction, and the presence of cellular infiltration.

G. DATA ANALYSIS

All data was expressed as mean $\pm$ SEM. Analysis of variance (ANOVA) with repeated measures was used to test for differences over time in heart rate and blood pressure during saline or NE infusion. A two way ANOVA was used to test for differences between groups at each time point or drug concentration. The Student-Newman Keuls test was used to isolate differences. In the myocardial blood flow studies, an ANOVA with repeated measures was used to test for changes in heart rate, mean arterial pressure, rate-pressure product, blood flow and vascular resistance during and after NE or saline infusion. A t-test with a Bonferroni correction was used to isolate differences. A two way ANOVA was used to test for differences between groups. Values of $p < 0.05$ was considered statistically significant. Glantz Primer

of Biostatistics Program or SAS (Statistical Analysis System) was used for all statistical tests.

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CHAPTER IV

VENTRICULAR DYSFUNCTION IN NOREPINEPHRINE-INDUCED CARDIOMYOPATHY

A. INTRODUCTION

Myocardial lesions produced by excessive administration (19,23,26) or release of catecholamines from the sympathetic nervous system (7,14,21) have been well characterized. Typical lesions including myofibrillar degeneration, leukocyte infiltration and focal necrosis (10,13,16,20,25) can be induced by a variety of catecholamines (3,9,13) including norepinephrine (NE)(5,23,24,26). The severity and extent of myocardial injury is dependent on the dose and rate of catecholamine administration (13,22). Resultant injury has been termed catecholamine cardiomyopathy (8,21,22).

Cardiac dysfunction also follows prolonged exposure to high circulating levels of catecholamines. Left ventricular systolic function is depressed (2,16) and responsiveness to NE is attenuated (16) *in vivo* two days post NE infusion. Diastolic function, however has not been systematically evaluated. In addition, the time course of ventricular dysfunction has not been fully investigated. The isolated non-ejecting heart preparation was used to eliminate neural and endocrine factors.

Thus, the purpose of this study was threefold: (1) to evaluate ventricular systolic and diastolic function in hearts from rabbits infused with 2, 4, or 6 μg/kg/min NE for 90 minutes, (2) to determine if cardiac performance is impaired immediately after prolonged exposure to high catecholamines and (3) to determine if ventricular function has changed by two days post NE infusion.

B. METHODS

1. Norepinephrine-induced Cardiomyopathy

Forty-nine male, New Zealand white rabbits (1.5 - 2.3 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg). A modification of Downing and Lee's model of norepinephrine-induced cardiomyopathy (8) was used in this study. Saline or norepinephrine bitartrate (NE) in saline was infused for 90 minutes via the marginal ear vein. Infusion rate was constant at 0.238 ml/min. Ventricular function was studied in twenty-six hearts isolated immediately after the infusion period and in twenty-three hearts two days post infusion. For acute experiments, rabbits were infused with saline (n=6; SAL-A), 2 μg/kg/min NE (n=8; NE2-A), 4 μg/kg/min NE (n=6; NE4-A) or 6 μg/kg/min NE (n=6; NE6-A). A standard Lead II ECG and arterial pressure were monitored throughout the infusion period. For chronic experiments, rabbits were infused with saline (n=5; SAL-C), 2 μg/kg/min NE (n=6; NE2-C), 4 μg/kg/min NE (n=6; NE4-C) or 6 μg/kg/min NE (n=6; NE6-C). Rabbits used in chronic experiments were allowed to recover from the anesthetic.

2. Isolated Heart Preparation

Following administration of sodium pentobarbital (30 mg/kg, IV), heparin (500 IU/kg) was administered via the marginal ear vein. The thoracic cavity was opened and the heart was quickly excised and placed in cold heparinized Krebs-Henseleit buffer modified with 11 mM glucose (KHB; pH 7.4). The aorta was cleaned of fat and connective tissue and the heart was weighed. The heart was placed on a non-recirculating, constant flow Langendorff apparatus. Each heart was perfused with KHB at 10 ml/min/g wet weight. The solution was warmed by Graham condensers and constant temperature circulator to maintain intraventricular temperature at 37 $\pm$ 0.5°C. A right atrial bipolar electrode was used to pace hearts at 220 beats/min. The pulmonary artery was cannulated to hinder flow of effluent into the left ventricle. A small cannula was inserted through the apex of the left ventricle to prevent fluid accumulation within the chamber. To measure left ventricular pressure (LVP), a fluid-filled latex balloon was inserted into the left ventricle through the mitral valve orifice. The balloon was connected to a Statham pressure transducer with a 9 cm length of polyethylene tubing. A Hamilton syringe positioned between the balloon and pressure transducer allowed for changes in balloon volume. After securing the balloon in the LV, balloon volume was adjusted so LV end-diastolic pressure (LVEDP) was 5 mmHg.

3. Protocol

After a 30 minute equilibration period, LVEDP was varied from 0 to 25 mmHg (5 mmHg steps) then reset to 5 mmHg. Control LVP, LV $+dP/dt_{max}$ and LV $-dP/dt_{max}$ were recorded at each LVEDP. Hearts were exposed to cumulative,

increasing concentrations of NE (10^{-10} - 10^{-7} M). At each NE concentration, LVEDP was varied from 0 to 25 mmHg (5 mmHg increments) then reset to 5 mmHg. Steady state LVP, LV $+dP/dt_{max}$ and LV $-dP/dt_{max}$ were recorded.

4. Data analysis

All values are expressed as mean $\pm$ SEM. Analysis of variance (ANOVA) with repeated measures was used to test for differences in heart rate or blood pressure within each group over the 90 minute saline or NE infusion. A two way ANOVA was used to test for differences between groups. The Student-Newman Keuls test was used to isolate differences. Values of $p < 0.05$ were considered statistically significant.

C. RESULTS

Heart rates immediately prior to saline (281 $\pm$ 7.3 beats/min) or NE infusion at 2 μg/kg/min (291.7 $\pm$ 3.1 beats/min), 4 μg/kg/min (281.7 $\pm$ 8.6 beats/min) or 6 μg/kg/min (293 $\pm$ 3.0 beats/min) were not different. Over the 90 minute infusion period, heart rates decreased slightly (10-20 beats/min), though insignificantly, in all groups. Mean arterial pressure (Figure 4-1) increased 25 to 35 mmHg above baseline within two minutes of NE infusion (all doses) and remained elevated significantly during the entire infusion period. Arterial pressure did not change during saline infusion.

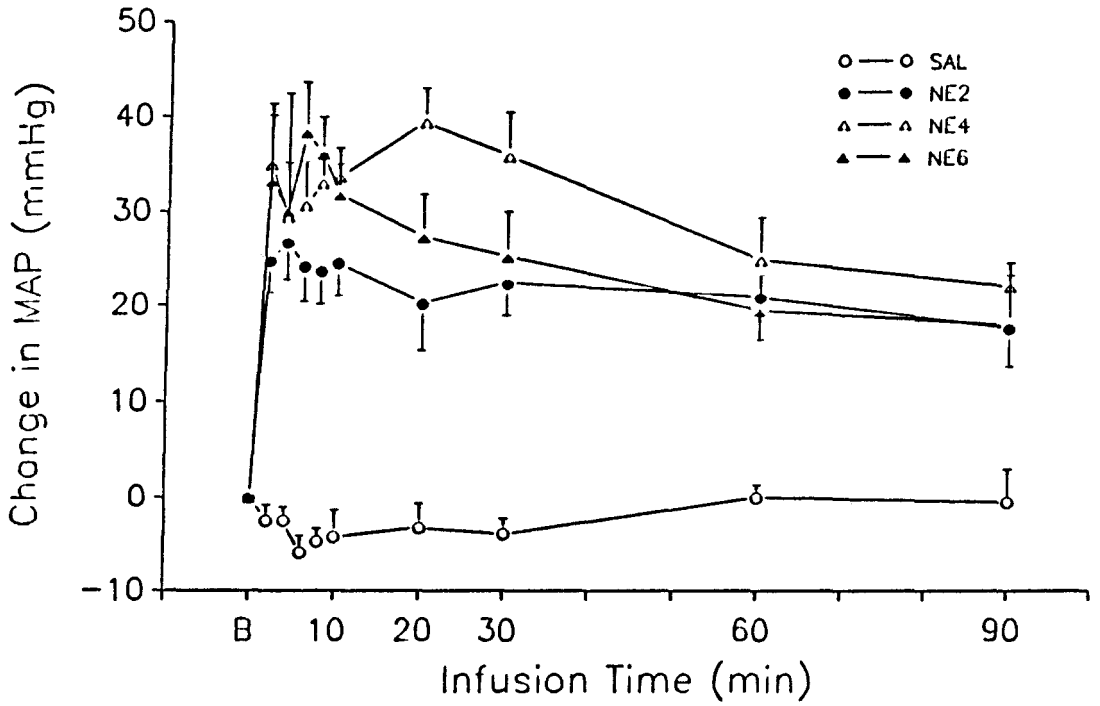
Baseline ventricular function is summarized in Tables 4-1 and 4-2. There was no difference between SAL-A and SAL-C in any measure of ventricular function so

these groups were combined (SAL). At the end of the infusion period, LVP developed by NE2-A, NE4-A, and NE6-A was less than SAL (Figure 4-2, panel A). The peak LVP developed in the NE treated groups was inversely related to the infused dose of NE. However, peak LVP was not significantly different between groups except at LVEDP of 0 and 5 mmHg. At these LVEDP's, NE6-A peak LVP was significantly lower than SAL, 76.0 $\pm$ 6.6 vs 99.4 $\pm$ 3.7 mmHg at EDP = 0 mmHg and 90.3 $\pm$ 4.7 vs 105.8 $\pm$ 3.8 at EDP = 5 mmHg. Two days post infusion, the peak LVP developed by NE6-C was depressed compared to SAL, NE2-C and NE4-C (Figure 4-2, panel B). NE2 and NE4 were not different from SAL. As demonstrated in figure 4-2, changes in LVP with increasing LVEDP (0-25 mmHg) were similar in NE treated hearts and SAL acutely and at 48 hours post infusion.

Immediately after infusion, LV $+dP/dt_{max}$ and LV $-dP/dt_{max}$ were depressed (LVEDP 0-25 mmHg) significantly in NE2-A, NE4-A, and NE6-A as compared to SAL (Table 4-1, Figure 4-4, panel A and Figure 4-5, panel A). At 48 hours, LV $+dP/dt_{max}$ of all NE injured hearts remained lower than SAL at each LVEDP but only NE6-C LV $+dP/dt_{max}$ was significantly less than SAL (Table 4-2 and Figure 4-4, panel B). Negative LV dP/dt_{max} also was depressed significantly at all LVEDP's in NE2-C, NE4-C, and NE6-C (Table 4-2). At LVEDP of 5 mmHg (Figure 4-5, panel B), LV $-dP/dt_{max}$ was -1235.7 $\pm$32.2 mmHg/sec for SAL, compared to -1066.9 $\pm$ 72.3, -1028.7 $\pm$ 333.7 and -926.3 $\pm$ 58.8 mmHg/sec for NE2-C, NE4-C and NE6-C, respectively.

FIGURE 4-1

CHANGE IN MEAN ARTERIAL PRESSURE DURING SALINE OR NOREPINEPHRINE INFUSION



Mean ± SEM for changes from baseline (B) in mean arterial pressure (MAP) during 90 minute infusion of saline (SAL) or norepinephrine at 2 (NE2), 4 (NE4), and 6 (NE6) μg/kg/min. Norepinephrine (all concentrations) significantly increased MAP above baseline ($p < 0.05$). MAP was elevated significantly during NE infusion compared to SAL.

TABLE 4-1

BASELINE VENTRICULAR FUNCTION IMMEDIATELY FOLLOWING HIGH DOSE NOREPINEPHRINE INFUSION

LVEDP (mmHg)	0	10	20
Peak LVP (mmHg)			
SAL	99.4 ± 3.7	114.6 ± 3.3	126.8 ± 3.3
NE2	87.6 ± 5.7	107.4 ± 4.3	115.3 ± 4.0
NE4	83.3 ± 5.6	97.8 ± 5.3	109.7 ± 5.7
NE6	76.0 ± 6.6*	97.0 ± 9.0	109.7 ± 7.8
LV +dP/dt (mmHg/sec)			
SAL	1762.0 ± 74.8	1809.9 ± 85.6	1885.3 ± 84.1
NE2	1400.3 ± 135.4*	1567.9 ± 119.5	1535.2 ± 119.8*
NE4	1293.3 ± 66.8*	1367.4 ± 58.9*	1400.6 ± 67.8*
NE6	1205.2 ± 122.7*	1390.9 ± 158.2*	1429.0 ± 144.0*
LV -dP/dt (mmHg/sec)			
SAL	-1272.5 ± 39.3	-1279.2 ± 38.7	-1273.9 ± 38.6
NE2	-962.0 ± 58.9*	-1028.3 ± 57.6*	-980.2 ± 55.0*
NE4	-925.1 ± 45.9*	-929.5 ± 39.1*	-925.0 ± 35.1*
NE6	-922.8 ± 87.1*	-986.4 ± 81.6*	-944.9 ± 63.7*

Hearts from rabbits infused with saline (SAL) or norepinephrine at 2 (NE2), 4 (NE4), and 6 (NE6) μg/kg/min for 90 min. LVP, left ventricular pressure; dP/dt, first derivative of LVP. Values are mean ± SEM. * Significantly different from SAL ($p < 0.05$).

TABLE 4-2

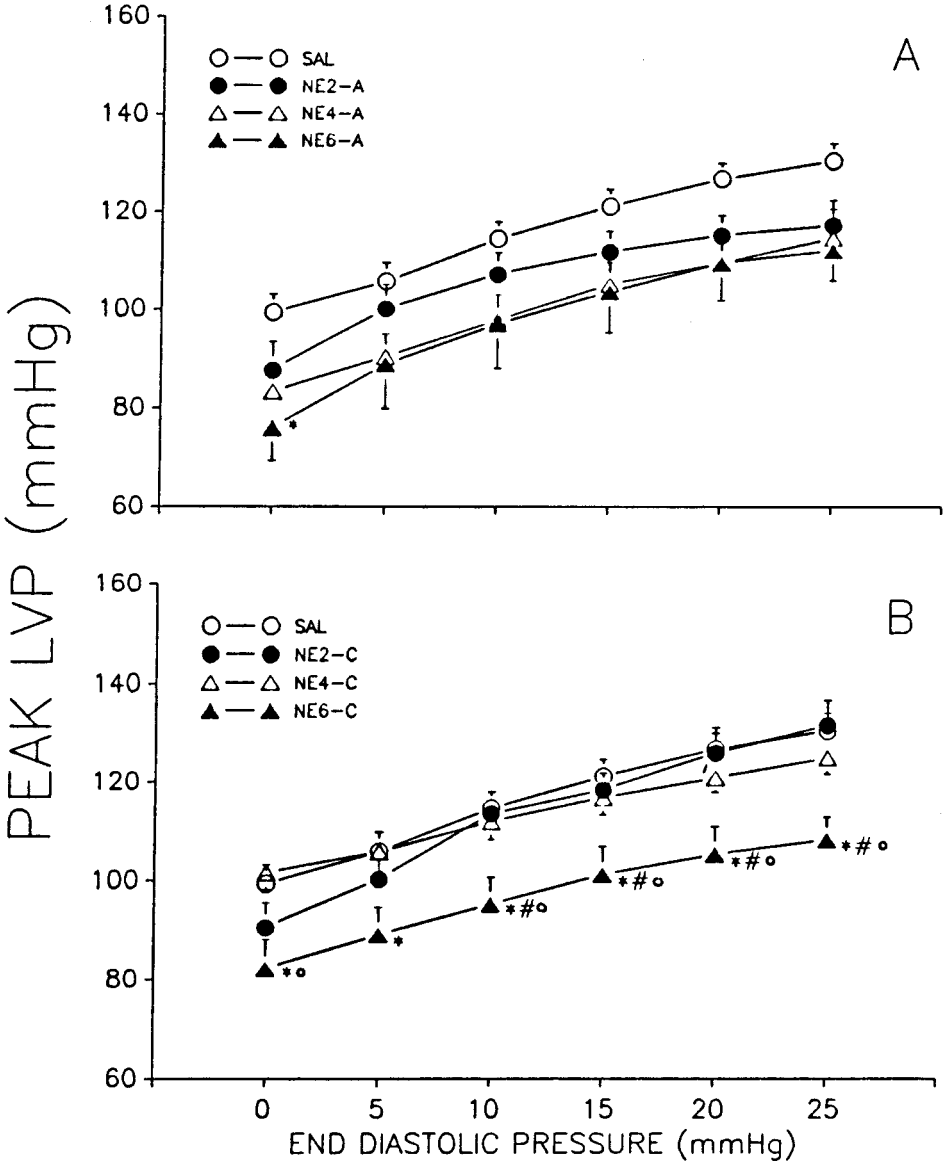
BASELINE VENTRICULAR FUNCTION 48 HOURS AFTER EXPOSURE TO HIGH DOSE NOREPINEPHRINE

LVEDP (mmHg)	0	10	20
Peak LVP (mmHg)			
SAL	99.4 ± 3.7	114.6 ± 3.3	126.8 ± 3.3
NE2	90.7 ± 4.9	113.5 ± 2.0	126.1 ± 5.1
NE4	101.5 ± 3.3	111.9 ± 3.6	120.9 ± 3.0
NE6	82.3 ± 6.0*†	95.3 ± 5.3 *†‡	105.3 ± 5.5*†‡
LV +dP/dt (mmHg/sec)			
SAL	1762.0 ± 74.8	1809.9 ± 85.6	1885.3 ± 84.1
NE2	1393.5 ± 116.7*	1589.3 ± 105.6	1731.7 ± 199.1
NE4	1624.2 ± 69.8	1650.2 ± 79.6	1679.4 ± 55.2
NE6	1304.6 ± 66.1*	1348.9 ± 43.1*	1361.6 ± 48.5*
LV -dP/dt (mmHg/sec)			
SAL	-1272.5 ± 39.3	-1279.2 ± 38.7	-1273.9 ± 38.6
NE2	-986.4 ± 75.1*	-1085.2 ± 68.3*	-1046.9 ± 90.3*
NE4	-1047.2 ± 35.5*	-1008.3 ± 36.4*	-960.1 ± 21.7*
NE6	-952.1 ± 48.4*	-910.7 ± 39.7*	-877.4 ± 10.6*

Values are mean ± SEM. Abbreviations as in table 4-1. * Significantly different from SAL ($p < 0.05$), † Significantly different from NE2, ‡ Significantly different from NE4.

FIGURE 4-2

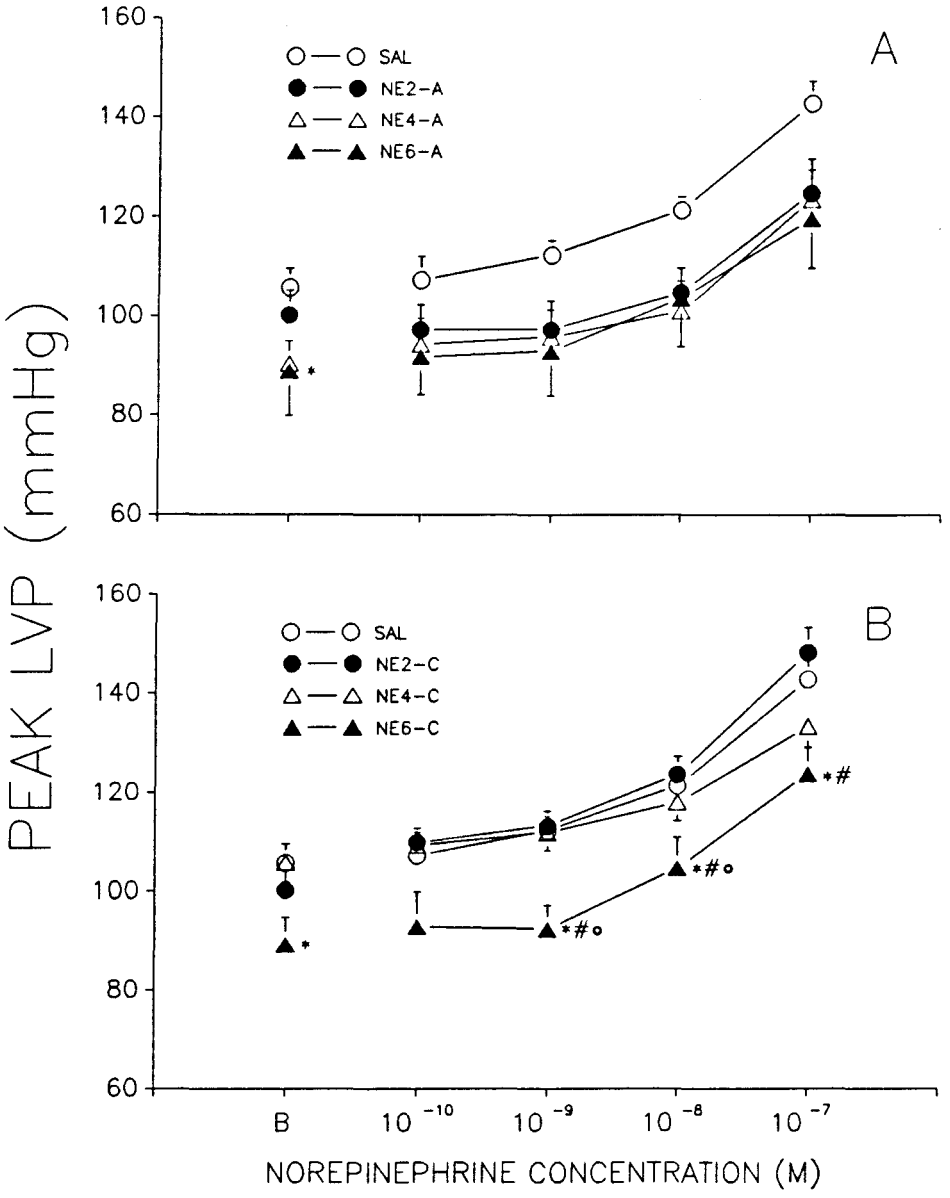
PRESSURE FUNCTION CURVES FOR NORMAL AND CARDIOMYOPATHIC HEARTS



Pressure function curves generated hearts from rabbits infused with saline (SAL) or norepinephrine at 2 (NE2), 4(NE4), and 6 (NE6) μg/kg/min for 90 min. Panel A: ventricular function assessed immediately following the infusion period. Panel B: ventricular function at 48 hours post infusion. Data presented as mean $\pm$ SEM. * Significantly different from SAL ($p < 0.05$), # Significantly different from NE2, ^{o} Significantly different from NE4.

FIGURE 4-3

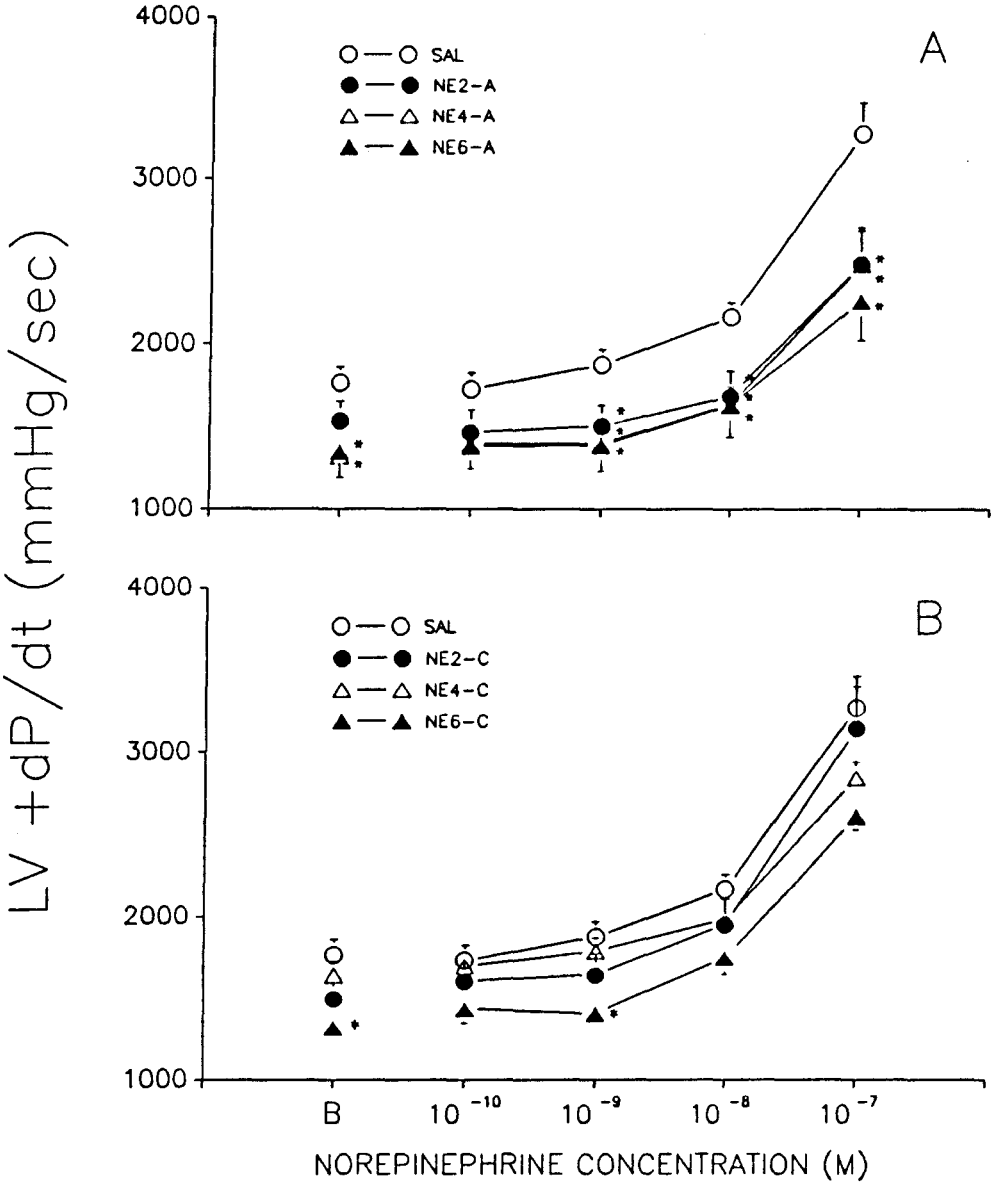
NOREPINEPHRINE LV PRESSURE DOSE-RESPONSE CURVES



Effect of norepinephrine on left ventricular pressure (LVP) development acutely (panel A) and at 48 hours (panel B) post infusion. End diastolic pressure is constant at 5 mmHg. Abbreviations as in Figure 4-2. * $p<0.05$ compared to SAL, # $p<0.05$ compared to NE2, ° $p<0.05$ compared to NE4.

FIGURE 4-4

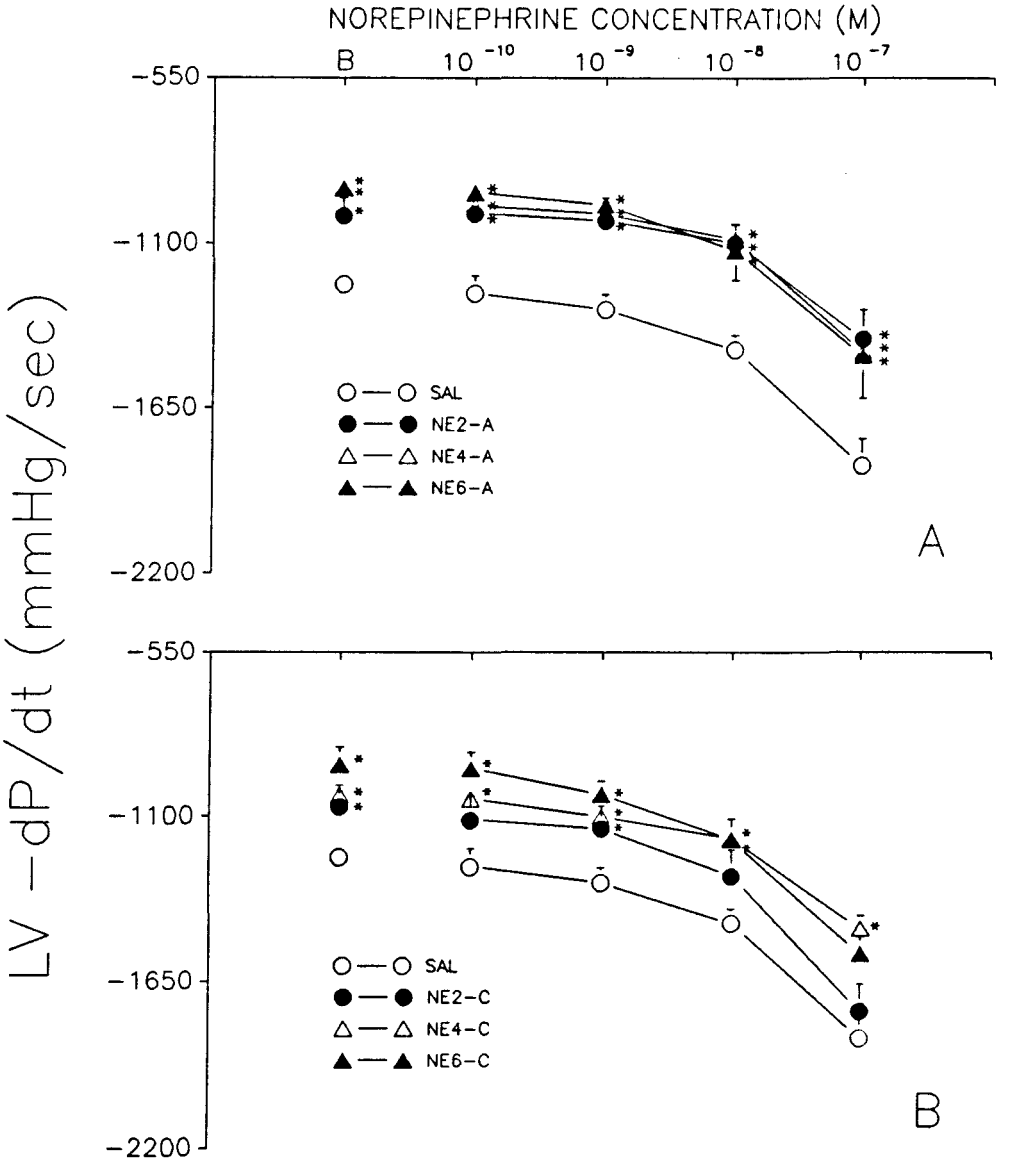
NOREPINEPHRINE LV +dP/dt$_{max}$ DOSE-RESPONSE CURVES



Norepinephrine dose-response curve generated immediately post infusion (panel A) or at two days (panel B). End diastolic pressure is 5 mmHg. LV +dP/dt, first derivative of LVP; other abbreviations as in Figure 4-2. * Significantly different from SAL ($p < 0.05$).

FIGURE 4-5

NOREPINEPHRINE LV -dP/dt_{max} DOSE-RESPONSE CURVES



Effect of increasing concentrations of norepinephrine on maximal rate of pressure decline (LV -dP/dt) in hearts isolated immediately after (panel A) or two days post (panel B) saline or norepinephrine infusion. Diastolic pressure = 5 mmHg. Abbreviations as in Figure 4-2. * $p<0.05$ compared to SAL.

LV systolic function, LVP and LV $+dP/dt_{max}$, of NE2 and NE4 showed improvement within 48 hours after NE infusion. Peak LVP increased slightly in all NE treated groups, however, the increase (LVEDP of 0 and 5 mmHg) was significant only in NE4-C. Positive LV dP/dt_{max} also improved significantly in NE4, but not in NE2 and NE6. Diastolic function, as indicated by LV $-dP/dt_{max}$, did not recover within two days post NE treatment.

Changes in cardiac function in response to increasing concentrations of NE are presented in Figures 4-3, 4-4 and 4-5. LVEDP shown in these figures is 5 mmHg and is representative of ventricular responses to NE at other diastolic pressures. In response to 10^{-7} M NE, LVP increased 37.0 $\pm$ 4.4, 24.6 $\pm$ 3.4, 33.2 $\pm$ 5.8 and 30.8 $\pm$ 7.9 mmHg above baseline in SAL, NE2-A, NE4-A, and NE6-A respectively. Increase in LVP with 10^{-7} M NE was similar in hearts studied two days post infusion. Although baseline function was depressed, myopathic hearts increased LV $+dP/dt_{max}$ and LV $-dP/dt_{max}$ the same amount as SAL at 10^{-7} M NE. In addition, there was no difference in percent increase in LVP, LV $+dP/dt_{max}$ and LV $-dP/dt_{max}$ of SAL, NE2, NE4, or NE6 in response to increasing concentration of NE in acute and chronic experiments.

E. DISCUSSION

Pressure function curves presented in our study for saline treated animals are consistent with those previously reported for the isolated, non-ejecting rabbit heart (15). We also show a downward shift of the function curve with NE injury acutely

with some functional improvement by 48 hours. Increases in LVP with changing LVEDP was similar in our SAL and NE groups. Using the open-chest rabbits, Werner and associates (27) reported in control animals very small increases in LVEDP with aortic constriction until LVP exceeds 150 mmHg. Hearts in rabbits treated with NE two days before assessment of LV function showed greater increases in LVEDP for a given change in LVP. The apparent difference between results published by Werner, et al. (27) and those reported here may be an inherent property of differences in the experimental models. Pressure function curves are afterload dependent. Afterload in the isolated, non-ejecting heart preparation is infinite whereas it is measurable in the whole animal. The conclusion however is the same. Ventricular function as measured by pressure function curves is depressed with NE-induced injury. In addition, cardiac output index, minute work index (2) and the ability to perform stroke work (4) are attenuated in catecholamine cardiomyopathy.

Cardiac function studies in vivo showed no difference in resting LV $+dP/dt_{max}$ two days post NE infusion between control and NE-treated rabbits (2,12). The result of the present series of experiments are consistent with this data. Positive LV dP/dt_{max} is depressed acutely with infusion of 2, 4, or 6 μg/kg/min NE. Two days post infusion NE2 and NE4 have returned toward control. However, systolic dysfunction (LV $+dP/dt_{max}$) does not improve within 48 hours after exposure to very high concentrations of NE (6 μg/kg/min). Prolonged NE infusion may have acutely depressed cross-bridge formation or actin-myosin ATPase activity resulting in decreased systolic function. Additionally, a greater number of cardiac myocytes may

have been permanently damaged by the 6 μg/kg/min NE infusion thereby hindering the recovery of systolic function by 48 hours post infusion.

In contrast to our results, Lee and Downing (12,16) have reported a decreased ability to improve myocardial contractility in rabbits two days after NE infusion (2 μg/kg/min for 90 min). In their studies (12,16), myopathic hearts in vivo did not increase LV $+dP/dt_{max}$ the same percent as control in response to increasing concentrations of NE. In the isolated heart preparation used in the current study, NE injured hearts increased LVP, LV $+dP/dt_{max}$, and LV $-dP/dt_{max}$ the same amount as SAL both acutely and at two days. It may be that extra-cardiac factors influenced LV response in situ.

During the pathogenesis of catecholamine-induced cardiomyopathy, elevated circulating catecholamines augment the flux of calcium into myocardial cells (1,11,22). Initially higher intracellular calcium levels are buffered by increased microsomal and mitochondrial transport (18). Later, microsomal calcium pump activity (18), sarcolemmal Na-Ca exchange and Ca pump activities (6,17) become depressed. These changes in calcium transport coupled with functional deterioration of mitochondria (11) result in elevated intracellular calcium (11,22). Higher levels of intracellular calcium and impaired removal of calcium during diastole may contribute to the decreased rate of ventricular relaxation. Cellular degeneration and intracellular and extracellular edema increase myocardial stiffness and may also contribute to diastolic dysfunction. The diastolic injury in norepinephrine-induced cardiomyopathy was not transient. Diastolic function was not improved 48 hours after

injury although systolic function showed improvement in NE2 and NE4. The time course and degree of recovery of diastolic function in cardiomyopathic rabbits was not assessed beyond two days post injury.

In summary, these experiments indicate that left ventricular diastolic function, in addition to systolic function is impaired acutely after exposure to high levels of catecholamines. Systolic function improved within 48 hours of injury whereas diastolic function remained depressed.

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CHAPTER V

ADRENERGIC RECEPTOR MEDIATED CHANGES IN VENTRICULAR FUNCTION IN NOREPINEPHRINE-INDUCED CARDIOMYOPATHY

A. INTRODUCTION

Prolonged exposure to catecholamines reduces responsiveness to adrenergic receptor stimulation in many organ systems and cell lines (3,6,13,19,24). This desensitization may result from the uncoupling of receptors from their effector enzyme systems or the removal of receptors from the plasma membrane (13-15). Both desensitizing mechanisms have been demonstrated for cardiac β-adrenergic receptors (11,12,16). In fact, uncoupling of rat cardiac β-receptors and a decrease in β-receptor density has been reported following prolonged norepinephrine (NE) infusion while cardiac α_1-receptors were not desensitized (5).

Exposure of cardiac tissue to high concentrations of catecholamines may induce myocardial damage. Characteristic histological lesions include contraction bands, myofibrillar degeneration and focal necrosis (8,9,21). A defect in the cardiac β-adrenergic receptor-adenylate cyclase system has also been identified. The guanine nucleotide regulatory (G) protein and/or adenylate cyclase enzyme were altered by high dose catecholamines so direct stimulation of the G protein by NaF or Gpp(NH)p

elicited a reduced response of adenylate cyclase (7,22).

If high levels of circulating catecholamines uncouple the β-receptor from its G protein (13,14) or alter the function of the G protein or adenylate cyclase enzyme (7,22), β-receptor mediated increases in ventricular function should be depressed. In this case, the α-adrenergic receptor system may be more important in increasing cardiac performance after catecholamine injury. The purpose of this study was (1) to evaluate α- and β-adrenergic receptor mediated changes in left ventricular function immediately after a 90 minute norepinephrine infusion and at 2 days post infusion and (2) to determine if the α-receptor system has a greater role in increasing cardiac function after NE induced injury.

B. METHODS

1. Norepinephrine-Induced Cardiomyopathy

NE cardiomyopathy was induced in sixty-six male, New Zealand white rabbits (1.5 - 2.3 kg). Following the administration of intravenous sodium pentobarbital (30 mg/kg), saline or NE bitartrate in saline was infused for 90 minutes via the marginal ear vein.

Ventricular responsiveness to β_1-adrenoceptor agonist dobutamine was studied in twenty-six hearts isolated immediately after the infusion period and in twenty-three hearts two days post infusion. For acute experiments, rabbits were infused with saline (n=6; SAL-A), 2 μg/kg/min NE (n=8; NE2-A), 4 μg/kg/min NE (n=5; NE4-A) or 6 μg/kg/min NE (n=6; NE6-A). A standard Lead II ECG and arterial pressure were

monitored throughout the infusion period. In chronic experiments, rabbits were infused with saline (n=5; SAL-C), 2 μg/kg/min NE (n=6; NE2-C), 4 μg/kg/min NE (n=6; NE4-C) or 6 μg/kg/min NE (n=6; NE6-C) then returned to their cages.

In a second series of experiments, myocardial responsiveness to α-adrenergic receptor stimulation was investigated immediately after 4μg/kg/min NE infusion (n=5) and at 48 hours post 4 μg/kg/min NE (n=6) or saline (n=7) infusion.

2. Isolated Heart Preparation

Following administration of sodium pentobarbital (30 mg/kg, IV) and heparin (500 IU/kg), the thoracic cavity was opened. The heart was quickly excised and immersed in cold heparinized Krebs-Henseleit buffer (KHB) modified with 11 mM glucose. The aorta was cleaned of fat and connective tissue. The heart was weighed and was placed on a non-recirculating, constant flow Langendorff apparatus. Flow rate was 10 ml/min/g wet weight. The KHB was warmed by Graham condensers and constant temperature circulator to maintain intraventricular temperature at 37 ± 0.5°C. A right atrial bipolar electrode was used to pace hearts at 220 beats/min. The pulmonary artery was cannulated to hinder flow of effluent into the left ventricle. A small cannula was inserted through the apex of the left ventricle to prevent fluid accumulation within the chamber. To measure left ventricular pressure (LVP), a fluid-filled latex balloon was inserted into the left ventricle through the mitral valve orifice. The balloon was connected to a Statham pressure transducer with a 9 cm length of polyethylene tubing. A Hamilton syringe positioned between the balloon and pressure transducer allowed for changes in balloon volume. After securing the

balloon in the LV, LV end-diastolic pressure was maintained at 5 mmHg. Hearts were allowed to equilibrate for 30 minutes

3. Tau

Tau, the time constant for isovolumic relaxation, was measured in experiments assessing responsiveness to α-receptor stimulation. The beginning of isovolumic relaxation was defined as 75% peak systolic pressure (17). Tau was the time required for ventricular pressure at 75% peak systolic pressure to be reduced by 1/e.

4. Protocol

In the first series of experiments, hearts were perfused with KHB plus the α-receptor antagonist phentolamine (Regitine, 10^{-6} M). α-Adrenoceptor blockade was tested with 10^{-7} M methoxamine. Receptor blockade was considered complete when there was no change in ventricular function or perfusion pressure when the heart was challenged with the appropriate agonist. Hearts were perfused with increasing, cumulative concentrations of dobutamine (10^{-9} - 10^{-6} M). Steady state LVP and LV dP/dt_{max} were recorded at baseline, i.e., in the presence of α-receptor blockade, and at each concentration of dobutamine.

To assess α-adrenoceptor responsiveness, other hearts were subjected to decreasing concentrations of NE (10^{-6} - 10^{-7} M). After a twenty minute wash, 10^{-6} M timolol was added to the perfusate. β-Receptor blockade was tested with isoproterenol (10^{-7} M). Hearts were re-exposed to NE (10^{-6} - 10^{-7} M). LVP and LV dP/dt_{max} were measured before exposure to NE and after two minutes at each NE concentration. Control measurements of tau were also recorded.

5. Data Analysis

All values are expressed as mean ± SEM. An analysis of variance was used to test for differences between groups. The Student-Newman Keuls test was used to isolate differences. Values of $p < 0.05$ were considered statistically significant.

C. RESULTS

Baseline peak LVP for hearts isolated after the 90 minute infusion was 93.4 ± 2.6 mmHg for SAL compared to 84.8 ± 3.4, 78.2 ± 5.7 and 80.8 ± 8.2 mmHg for NE2-A, NE4-A and NE6-A, respectively. Although peak LVP's were slightly lower in hearts from NE treated rabbits, there were no significant differences between the groups. At 48 hours post infusion, NE6-C peak LVP of 80.5 ± 4.4 mmHg was significantly lower than SAL (93.4 ± 2.6 mmHg), NE2-C (97.5 ± 5.1 mmHg) and NE4-C (95.8 ± 4.7 mmHg).

NE treated hearts increased LVP in response to increasing concentrations of dobutamine (Figure 5-1, panels A and B). Peak LVP generated by hearts acutely treated with NE was lower than SAL at each concentration, but the difference was significant only at 10^{-7} M dobutamine (panel A). Forty-eight hours post infusion, there were no differences in LVP for NE2-C, NE4-C and SAL at any concentration of dobutamine (panel B). NE6-C peak LVP was lower than SAL, NE2-C and NE4-C in the presence of dobutamine but NE6-C was significantly lower than SAL and NE2-C only at 10^{-7} M. Changes in LVP with exposure to dobutamine were less than SAL in NE2-A at 10^{-7} M and in NE4-C at 10^{-7} M and 10^{-6} M (Table 5-1). Changes in LVP

in other groups were not significantly different from SAL at any dobutamine concentration.

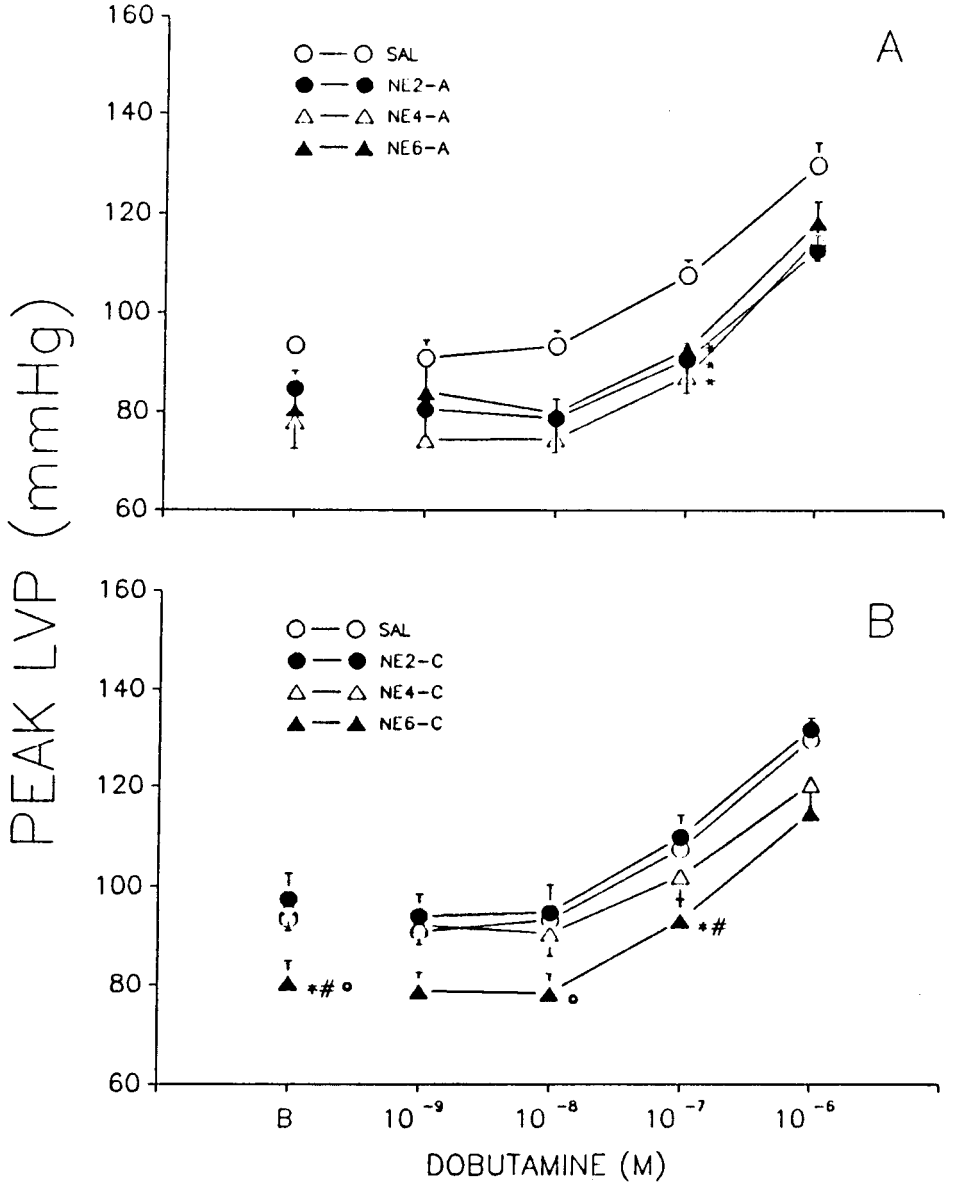
Baseline LV $+dP/dt_{max}$ of NE treated hearts were not different than SAL immediately after or at 48 hours post infusion (Figure 5-2, panels A and B). All hearts increased LV $+dP/dt_{max}$ in response to dobutamine yet LV $+dP/dt_{max}$ of NE2-A, NE4-A and NE6-A were significantly lower than SAL at 10^{-8} M, 10^{-7} M and 10^{-6} M dobutamine (Figure 5-2, panel A). Similarly, the change in LV $+dP/dt_{max}$ was significantly less than SAL for all NE-A groups at 10^{-7} and 10^{-6} M dobutamine (Table 5-1). In chronic experiments, NE4-C and NE6-C LV $+dP/dt_{max}$ were significantly lower than SAL at 10^{-6} M dobutamine (Figure 5-2, panel B) but the mmHg/sec increase in LV $+dP/dt_{max}$ was significantly less than SAL only in NE4-C (Table 5-1).

In acute experiments, LV $-dP/dt_{max}$ was depressed in NE2-A, NE4-A and NE6-A at baseline and 10^{-8} M through 10^{-6} M dobutamine compared to SAL (Figure 5-3, panel A). Two days post infusion (Figure 5-3, panel B), NE6-C LV $-dP/dt_{max}$ was reduced at baseline and at 10^{-6} M dobutamine while NE4-C LV $-dP/dt_{max}$ was lower than SAL at 10^{-6} M dobutamine. However,changes from baseline LV $-dP/dt_{max}$ when hearts were perfused with dobutamine were similar in SAL and NE treated hearts (Table 5-1).

Left ventricular pressure developed by NE2 at baseline and in the presence of dobutamine showed improvement within 48 hours of NE infusion. NE4-C LVP was significantly greater than NE4-A at baseline and at 10^{-8} M dobutamine (Figure 5-1). NE2-C LV $+dP/dt_{max}$ was greater than NE2-A at 10^{-7} and 10^{-6} M dobutamine. NE4-C

FIGURE 5-1

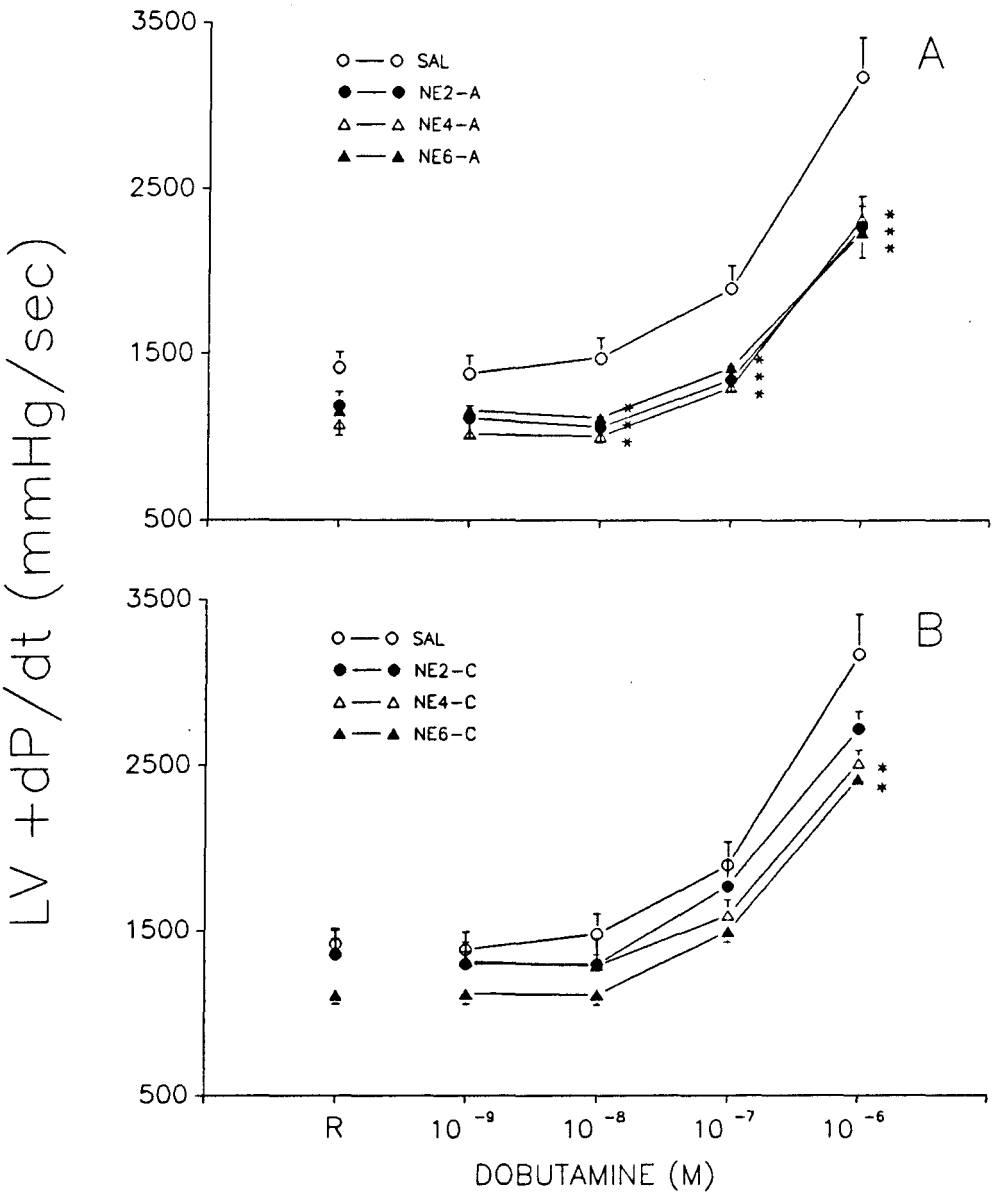
CHANGES IN LEFT VENTRICULAR PRESSURE WITH INCREASING CONCENTRATIONS OF DOBUTAMINE



Effect of increasing dobutamine concentrations on left ventricular pressure (LVP) development in hearts from rabbits infused with saline (SAL) or norepinephrine at 2 (NE2), 4 (NE4), and 6 (NE6) μg/kg/min. Panel A: LVP generated by hearts isolated immediately after infusion (-A). Panel B: LVP developed in hearts isolated 48 hours post infusion (-C). End diastolic pressure is 5 mmHg. Values are presented as mean $\pm$ SEM. B: baseline function. * $p<0.05$ compared to SAL, # $p<0.05$ compared to NE2-C, and ° $p<0.05$ compared to NE4-C.

FIGURE 5-2

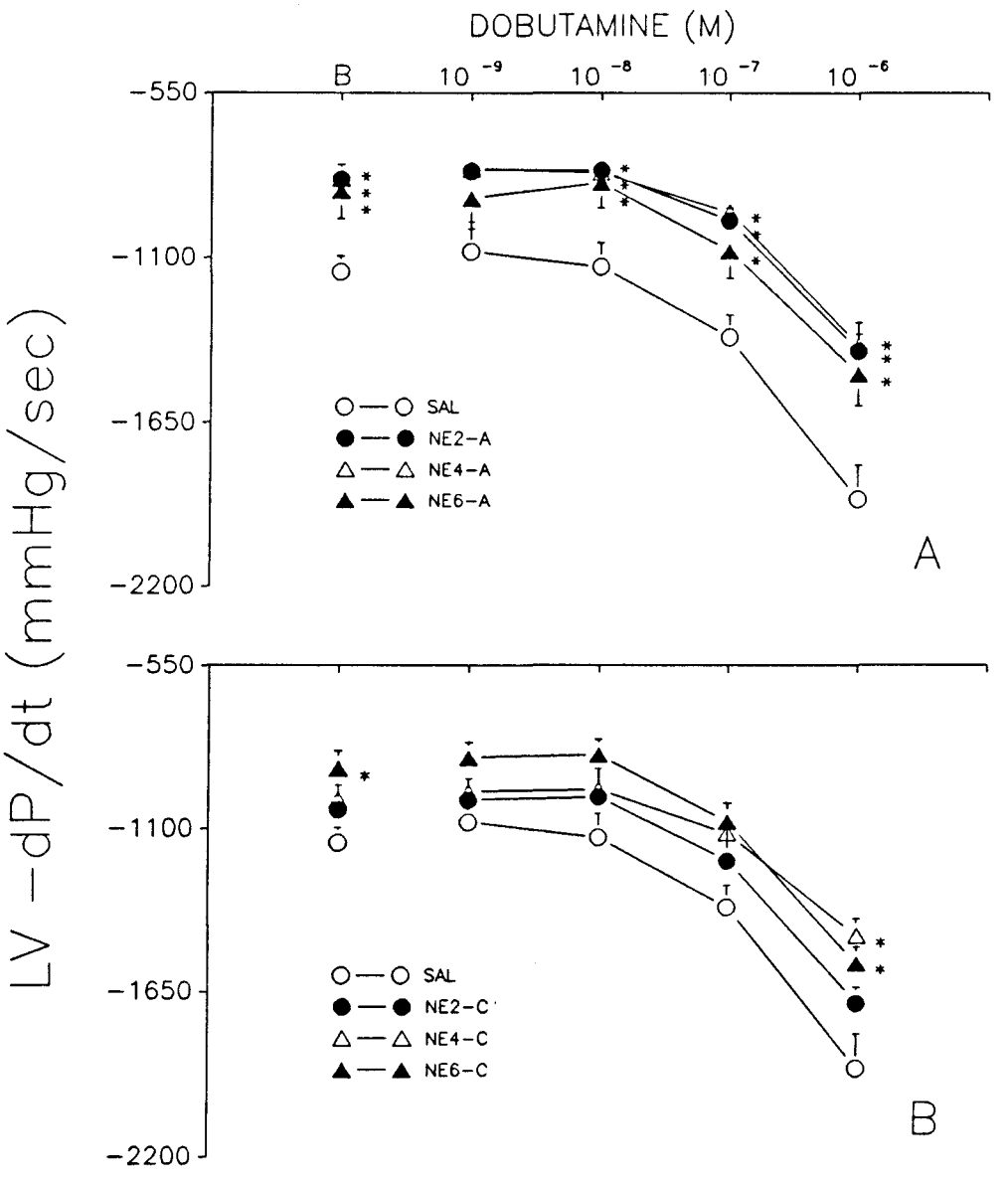
DOBUTAMINE LV +dP/dt DOSE-RESPONSE CURVES



Effect of dobutamine on LV +dP/dt generated by hearts isolated immediately post infusion (panel A) or at two days (panel B). End diastolic pressure is 5 mmHg. LV +dP/dt, first derivative of LVP; other abbreviations as in Figure 5-1. * Significantly different from SAL ($p < 0.05$).

FIGURE 5-3

DOBUTAMINE LV -dP/dt DOSE-RESPONSE CURVES



Effect of increasing concentrations of dobutamine on the first derivative of left ventricular pressure decline (LV -dP/dt) in hearts isolated immediately after (panel A) or two days post (panel B) saline or norepinephrine infusion. Diastolic pressure = 5 mmHg. Abbreviations as in Figure 5-1. * $p<0.05$ compared to SAL.

TABLE 5-1

CHANGE IN LEFT VENTRICULAR FUNCTION WITH DOBUTAMINE

LVP (mmHg)	10^{-7} M DOB	10^{-6} M DOB
SAL	13.4 ± 1.3	35.5 ± 2.7
NE2-A	5.8 ± 1.3*	27.9 ± 3.9
NE4-A	9.0 ± 0.6	37.0 ± 2.0
NE6-A	9.8 ± 3.3	35.6 ± 7.2
NE2-C	12.5 ± 1.5	32.1 ± 3.1
NE4-C	6.4 ± 1.1*	24.8 ± 1.5*
NE6-C	12.8 ± 1.4	34.5 ± 3.3
LV +dP/dt (mmHg/sec)	**10^{-7} M DOB**	**10^{-6} M DOB**
SAL	454.7 ± 66.3	1734.0 ± 165.6
NE2-A	136.6 ± 27.0*	1064.7 ± 99.7*
NE4-A	220.9 ± 32.0*	1242.9 ± 95.8*
NE6-A	220.7 ± 61.7*	1043.2 ± 160.5*
NE2-C	431.8 ± 23.2	1330.3 ± 51.9
NE4-C	219.0 ± 34.7*	1139.5 ± 24.0*
NE6-C	358.7 ± 45.4	1312.4 ± 63.4
LV -dP/dt (mmHg/sec)	**10^{-7} M DOB**	**10^{-6} M DOB**
SAL	-205.7 ± 26.8	-745.9 ± 69.8
NE2-A	-112.0 ± 36.2	-549.4 ± 72.5
NE4-A	-111.4 ± 15.3	-559.9 ± 51.2
NE6-A	-176.8 ± 51.3	-587.0 ± 108.9
NE2-C	-172.2 ± 42.4	-623.4 ± 50.2
NE4-C	-114.4 ± 14.2	-460.1 ± 24.2*
NE6-C	-180.1 ± 10.0	-658.8 ± 15.5

Listed values are means ± SEM for the increase in ventricular function above baseline values. DOB: dobutamine; other abbreviations as in Figure 5-1.
*$p < 0.05$ compared to SAL.

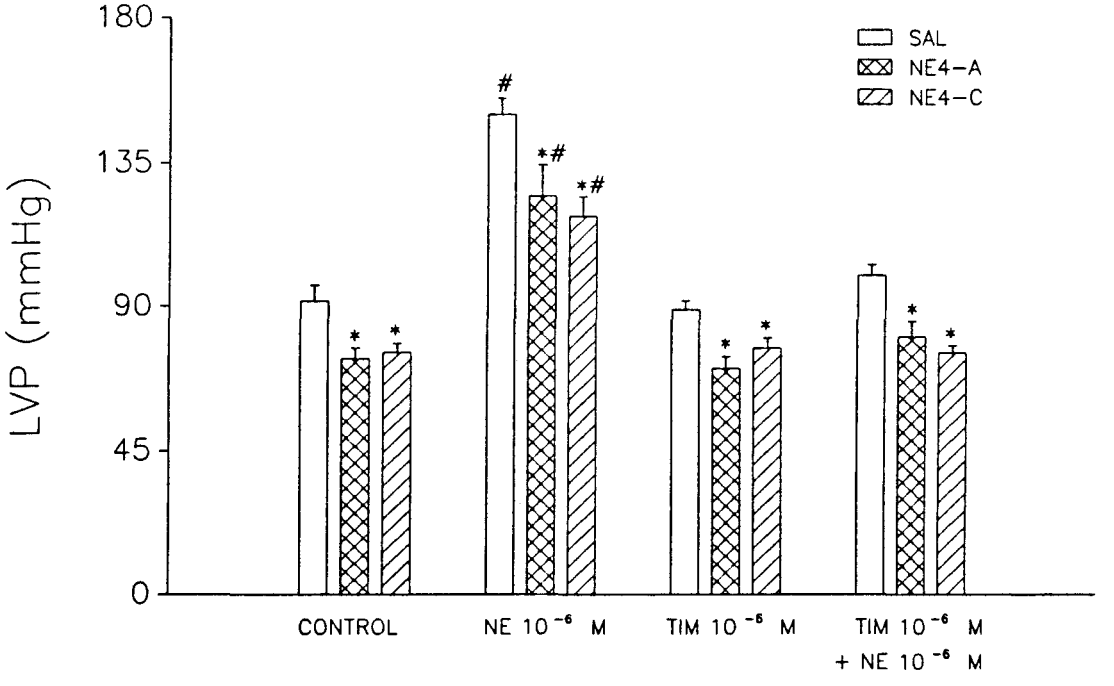
generated a higher LV $+dP/dt_{max}$ than NE4-A at baseline and concentrations of dobutamine to 10^{-7} M (Figure 5-2). Diastolic function of NE2 and NE4, as measured by LV $-dP/dt_{max}$, was improved at baseline and in the presence of dobutamine by the second day after the infusion (Figure 5-3). Systolic and diastolic function did not improve over the 48 hour period in NE6.

In the second series of experiments, LVP was depressed in NE4-A (73.7 $\pm$ 3.4 mmHg) and NE4-C (75.8 $\pm$ 2.7 mmHg) under control conditions when compared to SAL (91.7 $\pm$ 4.9 mmHg) (Figure 5-4). LVP increased significantly in all groups in response to 10^{-6} M NE, although, pressures developed by NE4-A and NE4-C were still lower than SAL. Perfusing hearts with KHB + timolol did not change LVP's. In the presence of timolol, 10^{-6} M NE increased LVP slightly, but insignificantly, in all groups.

There were no differences in control LV $+dP/dt_{max}$ between the groups (Figure 5-5) but control LV $-dP/dt_{max}$ of NE4-A and NE4-C was significantly lower than SAL (Figure 5-6). NE (10^{-6} M) increased LV $+dP/dt_{max}$ and LV $-dP/dt_{max}$ significantly in all groups. In the presence of timolol, NE4-A LV $+dP/dt_{max}$ was 1057.4 $\pm$ 38.2 mmHg/sec and was significantly lower than SAL (1342 $\pm$ 60.7 mmHg/sec) and NE4-C (1306.7 $\pm$ 58.4). LV $-dP/dt_{max}$ generated in the presence of timolol was not different from control values. With the β-adrenoceptors blocked, NE increased LV $+dP/dt_{max}$ slightly but insignificantly in all groups. Similarly, LV $-dP/dt_{max}$ generated by α-receptor stimulation was not significant except in NE4-A in presence of 10^{-6} M NE.

FIGURE 5-4

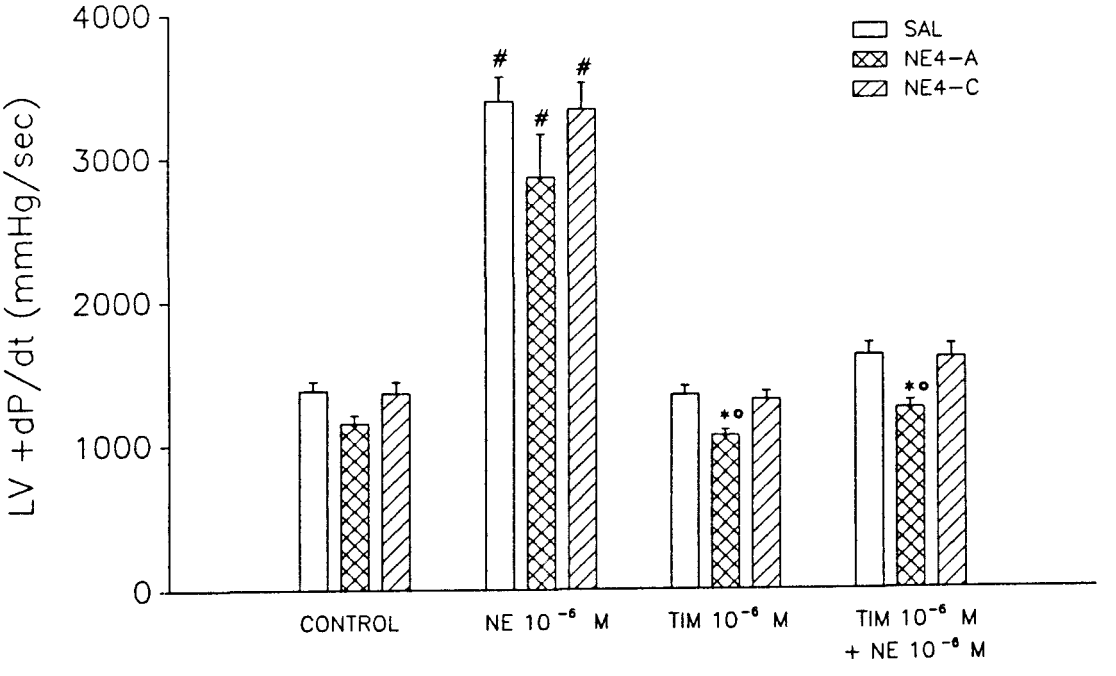
NOREPINEPHRINE INDUCED CHANGES IN VENTRICULAR PRESSURE BEFORE AND AFTER β-ADRENERGIC RECEPTOR BLOCKADE



Left ventricular pressure (LVP) developed in the presence of norepinephrine (NE) before and after β adrenergic receptor blockade by timolol (TIM). Hearts were isolated immediately after 4 μg/kg/min NE infusion (NE4-A) or 48 hours after saline (SAL) or 4 μg/kg/min infusion (NE4-C). * $p < 0.05$ as compared to SAL, # $p < 0.05$ as compared to corresponding control pressure.

FIGURE 5-5

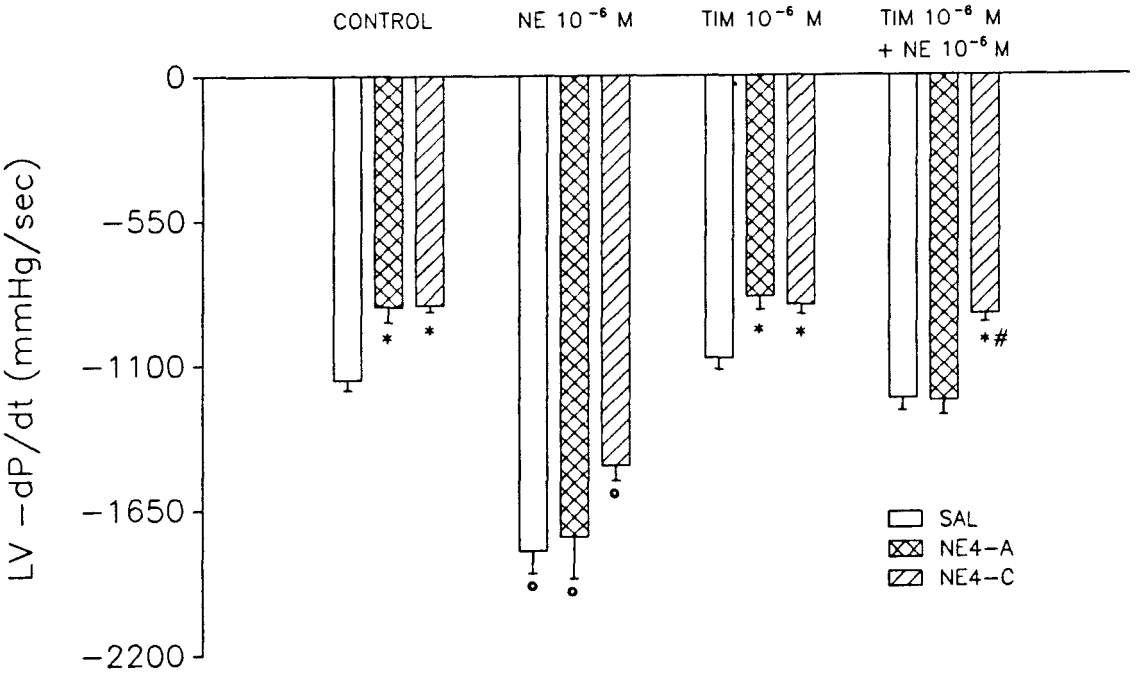
CHANGES IN LV +dP/dt INDUCED BY NOREPINEPHRINE BEFORE AND AFTER β-RECEPTOR BLOCKADE



Effect of norepinephrine on the first derivative of LVP (LV +dP/dt) before and after the addition of TIM. Refer to Figure 5-4 for additional abbreviations. * Significantly different than SAL ($p<0.05$), # Significantly different from control value, ° Significantly different from NE4-C.

FIGURE 5-6

CHANGES IN LV -dP/dt INDUCED BY NOREPINEPHRINE BEFORE AND AFTER β-RECEPTOR BLOCKADE



Effect of norepinephrine on the first derivative of LVP decline (LV -dP/dt) before and after β-receptor blockade by timolol. Abbreviations as in Figure 5-4. * $p < 0.05$ when compared to SAL, # $p < 0.05$ compared to NE4-A, ° $p < 0.05$ compared to control value.

TABLE 5-2

CHANGE IN VENTRICULAR FUNCTION DUE TO
α-RECEPTOR STIMULATION

	NE 10^{-7} M	NE 5 X 10^{-7} M	NE 10^{-6} M
LVP			
SAL	7.3 ± 2.2	10.6 ± 2.0	10.9 ± 2.2
NE4-A	8.4 ± 1.4	13.0 ± 0.8	10.0 ± 2.3
NE4-C	6.2 ± 1.6	6.3 ± 1.6	3.5 ± 1.6*
LV +dP/dt			
SAL	175.9 ± 46.1	280.6 ± 46.6	275.9 ± 42.6
NE4-A	161.5 ± 28.8	236.2 ± 7.9	182.4 ± 34.3
NE4-C	169.0 ± 53.9	293.9 ± 52.8	288.5 ± 48.2
LV -dP/dt			
SAL	-124.6 ± 38.6	-157.5 ± 22.4	-157.1 ± 24.8
NE4-A	-149.1 ± 27.1	-210.5 ± 23.8	-397.5 ± 29.8*
NE4-C	-34.7 ± 25.5	-61.1 ± 26.1*#	-75.4 ± 18.7*#

Hearts were perfused with norepinephrine (NE) in the presence of timolol. Data are expressed as mean ± SEM. Abbreviations as in Figure 5-4. * $p<0.05$ compared to SAL. # $p<0.05$ compared to NE4-A.

Changes in LVP and LV $+dP/dt_{max}$ due to α-adrenoceptor stimulation were similar among the groups however, the LVP generated by NE4-C was significantly less than SAL at 10^{-6} M NE (Table 5-2). Changes in diastolic function, as measured by LV $-dP/dt_{max}$, were lower in NE4-C at all NE concentrations compared to SAL and NE4-A. The increase in NE4-C LV $-dP/dt_{max}$ was significantly less than SAL and NE4-A at both 5×10^{-7} M and at 10^{-6} M NE. The change in NE4-A LV $-dP/dt_{max}$ was significantly greater than SAL and NE4-C at the highest NE concentration.

Tau, an additional measure of diastolic function, was significantly depressed in NE treated hearts under control conditions. Tau was 22.0 ± 0.9 msec for SAL hearts and 24.9 ± 0.7 msec and 24.9 ± 0.6 msec for NE4-A and NE4-C, respectively.

D. DISCUSSION

Exposure of tissue to an adrenergic agonist results in a rapid desensitization of adrenergic receptor mediated events. Hayes et al. (10) reported that isoproterenol (ISO) infusion attenuated subsequent myocardial inotropic responses to ISO, a β-adrenergic agonist, but not to phenylephrine, an α-receptor agonist. Chang et al. (5) have also shown a selective desensitization of rat β-adrenergic receptors following a NE infusion with no change in the responsiveness of α-adrenergic receptors. In contrast, Corder et al. (7) reported a decrease in β-receptors and α-receptors after ISO injection. This difference in the results reported by these investigators may be related to the dose of catecholamines administered.

Schulze et al. (18) reported that adenylate cyclase isolated from necrotic areas of the rat left ventricle previously damaged by an injection of high dose ISO, was unable to increase enzyme activity to the same degree as the cyclase from normal tissue with subsequent ISO stimulation. The decline in the ability to stimulate adenylate cyclase activity by ISO may have been due to receptor uncoupling and internalization (5,16). However, several investigators have suggested that high dose catecholamines or prolonged exposure to catecholamines may induce a defect in the G protein or adenylate cyclase enzyme (7,22).

In our study, it was hypothesized that desensitization of β-adrenergic receptor-adenylate cyclase system or alteration in effector proteins with catecholamine administration would result in a depressed ability of dobutamine, a β-receptor agonist, to increase ventricular function after NE infusion. Our data demonstrated that hearts isolated from rabbits immediately after or 48 hours post NE administration, increased LVP and LV $-dP/dt_{max}$ the same amount as SAL hearts in response to β-receptor stimulation. These data suggest that the β-receptor was not functionally uncoupled from adenylate cyclase.

The ability of β-receptor stimulation to increase LV $+dP/dt_{max}$ in hearts acutely injured by NE was impaired. The findings of this study suggest that NE treatment acutely altered the formation of cross-bridges between actin and myosin, cross-bridge motion, rephosphorylation of the myosin head by ATP or the dissociation of the actomyosin complex. Conceivably, the maximal number of cross-bridges formed was not changed since the increase in LVP of NE treated hearts was not different than

SAL. Brown and Hudlicka have reported that hearts of NE treated rabbits increased $+dP/dt_{max}$ to the same degree as saline controls when challenged with intravenous NE 48 hours after treatment (4). Similarly, in our study the increase in LV $+dP/dt_{max}$ 48 hours after the infusion was not different among the groups.

Increase in cardiac function mediated by α_1-adrenoceptors in the presence of β-receptor blockade has been demonstrated in various mammals (1,2,20,23). In this study we also reported a slight, but insignificant, increase in left ventricular systolic and diastolic function with α_1-receptor stimulation. At the onset of these experiments it was proposed that α-receptor mediated changes in ventricular function would be greater after NE administration. However, changes in ventricular systolic performance with α_1-receptor stimulation were not altered either acutely or chronically after NE treatment. These results are in agreement with data reported by Hayes et al. (10) which showed that responsiveness of the rat myocardium to α-adrenergic agonists was not changed following a prolonged ISO infusion.

In summary, the results presented in this chapter indicate that (1) the responsiveness of the NE cardiomyopathic heart to β-adrenergic receptor stimulation was not impaired during these experiments and (2) the α_1-adrenergic receptor system was not more important in increasing left ventricular function in the rabbit after NE-induced injury. Furthermore the results suggest that NE treatment acutely altered a step in cross-bridges formation or cycling.

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CHAPTER VI

EFFECT OF α-TOCOPHEROL AND SELENIUM PRETREATMENT ON NOREPINEPHRINE-INDUCED CARDIOTOXICITY

A. INTRODUCTION

Excessive endogenous or exogenous catecholamines produce multifocal lesions within the myocardium (65). Characteristic damage includes edema, contraction band formation, myofibrillar disintegration, cellular infiltration and necrosis (18,64,65,67). The etiology of this injury has not been fully elucidated but multiple mechanisms have been suggested (50). Several investigators have proposed that products of catecholamine autoxidation are involved in the cardiotoxicity of catecholamines (27,43,45,53).

The autoxidation of catecholamines generates free radicals and unstable, cytotoxic metabolites (5,24,53,58). Free radicals cause tissue damage by reacting with polyunsaturated lipids within membranes (10,35,48), nucleotides in DNA (35), or sulfhydryl bonds in proteins (35,40). The peroxidation of cellular membranes initiated by free radicals (35,52) alters membrane structure, integrity and function (32,34,37,60). Oxidative metabolites induce ultrastructural damage similar to that produced by high dose catecholamines (14,71,72,74). Enzyme activity (57,58),

mitochondrial function (14,59) and ventricular function (14,71-73) are also impaired by the metabolites of catecholamine oxidation.

α-Tocopherol, a constituent of vitamin E, is an antioxidant (7) which scavenges free radicals and terminates peroxidation of lipids within biological membranes (9,19,21,61). Singal et al. have shown that vitamin E protected against the deleterious effects of isoproterenol in the rat (53). These investigators have also demonstrated increased lipoperoxidation in the rat myocardium with ISO that is prevented by α-tocopherol pretreatment (52). Selenium is an essential trace element and integral component of glutathione peroxidase (28,62). Selenium-dependent glutathione peroxidase is an enzyme that reduces oxidized lipids. The administration of selenium has been shown to increase glutathione peroxidase activity and afford protection against lipid peroxidation (2,12,17,26).

Adequate amounts of selenium-dependent glutathione peroxidase and α-tocopherol are essential for normal cellular physiology particularly if tissues are exposed to substances which directly or indirectly promote lipid peroxidation. If catecholamine cardiotoxicity is mediated by lipid peroxidation, the administration of α-tocopherol and selenium should protect the myocardium from injury. The purpose of this study was to determine if a two week pretreatment with α-tocopherol and selenium would protect the rabbit myocardium from norepinephrine induced injury.

B. METHODS

1. Norepinephrine-Induced Cardiomyopathy

Thirteen male, New Zealand white rabbits (2.2 kg) were treated subcutaneously with α-tocopherol (25.0 mg; BO-SE, Schering Co., U.S.A.) and selenium (0.5 mg) every other day for two weeks. Twenty-four hours after the last treatment rabbits were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Saline (SAL-E; n=6) or 4 μg/kg/min norepinephrine bitartrate (NE4-E; n=7) was infused for 90 minutes via the marginal ear vein. Infusion rate was constant at 0.238 ml/min. Seventeen rabbits not receiving vitamin pretreatment were infused with saline (SAL; n=11) or NE (NE4; n=6). Ventricular function was studied two days post infusion.

When the rabbits were deeply anesthetized (sodium pentobarbital, 30 mg/kg, IV), heparin (500 IU/kg) was administered and the thoracic cavity was opened. The heart was quickly excised and placed in cold heparinized Krebs-Henseleit buffer modified with 11 mM glucose (KHB; pH 7.4). The aorta was cleaned of fat and connective tissue and the heart was weighed. The heart was placed on a non-recirculating, constant flow Langendorff apparatus and perfused with KHB at 10 ml/min/g wet weight. KHB was warmed by Graham condensers and constant temperature circulator to maintain intraventricular temperature at 37 $\pm$ 0.5°C. A right atrial bipolar electrode was used to pace hearts at 220 beats/min. The pulmonary artery was cannulated to hinder flow of effluent into the left ventricle. A small cannula was inserted through the apex of the left ventricle to prevent fluid accumulation within the chamber. To measure left ventricular pressure (LVP), a fluid-filled latex balloon was inserted into the left ventricle through the mitral valve

orifice. The balloon was connected to a Statham pressure transducer with polyethylene tubing. A Hamilton syringe positioned between the balloon and pressure transducer allowed for changes in balloon volume. After securing the balloon in the LV, balloon volume was adjusted so LV end-diastolic pressure (LVEDP) was 5 mmHg.

2. Protocol

After a 30 minute equilibration period, LVEDP was varied from 0 to 25 mmHg (5 mmHg steps) then reset to 5 mmHg. Baseline LVP and LV dP/dt_{max} were recorded at each LVEDP. Hearts were exposed to cumulative, increasing concentrations of NE (10^{-10} - 10^{-6} M). At each NE concentration, LVEDP was varied from 0 to 25 mmHg (5 mmHg increments) then reset to 5 mmHg. Steady state LVP and LV dP/dt_{max} were recorded.

3. Histology

At the end of several experiments, the atria and right ventricular free wall were dissected from the left ventricle. A midlevel transverse section of LV and septum was fixed in 10% buffered formalin and stained with hematoxylin and eosin. LV sections were viewed (blindly) to verify the presence or absence of myocardial injury. This injury was characterized by edema, loss of nuclei, focal myofiber destruction, and the presence of cellular infiltration. Sections were assessed by a pathologist who had no knowledge of experimental design.

4. Data analysis

All values are expressed as mean $\pm$ SEM. An analysis of variance (ANOVA)

was used to test for differences between groups. The Student-Newman Keuls test was used to isolate differences. Values of $p < 0.5$ were considered statistically significant.

C. RESULTS

1. Heart Weights

Body weights were similar in α-tocopherol and selenium treated and untreated rabbits. The weights of cardiomyopathic hearts (NE4 5.0 $\pm$ 0.1 g; NE4-E 5.5 $\pm$ 0.2 g) were not different than normal hearts (SAL 4.9 $\pm$ 0.2 g; SAL-E 5.1 $\pm$ 0.2 g).

2. Left Ventricular Function

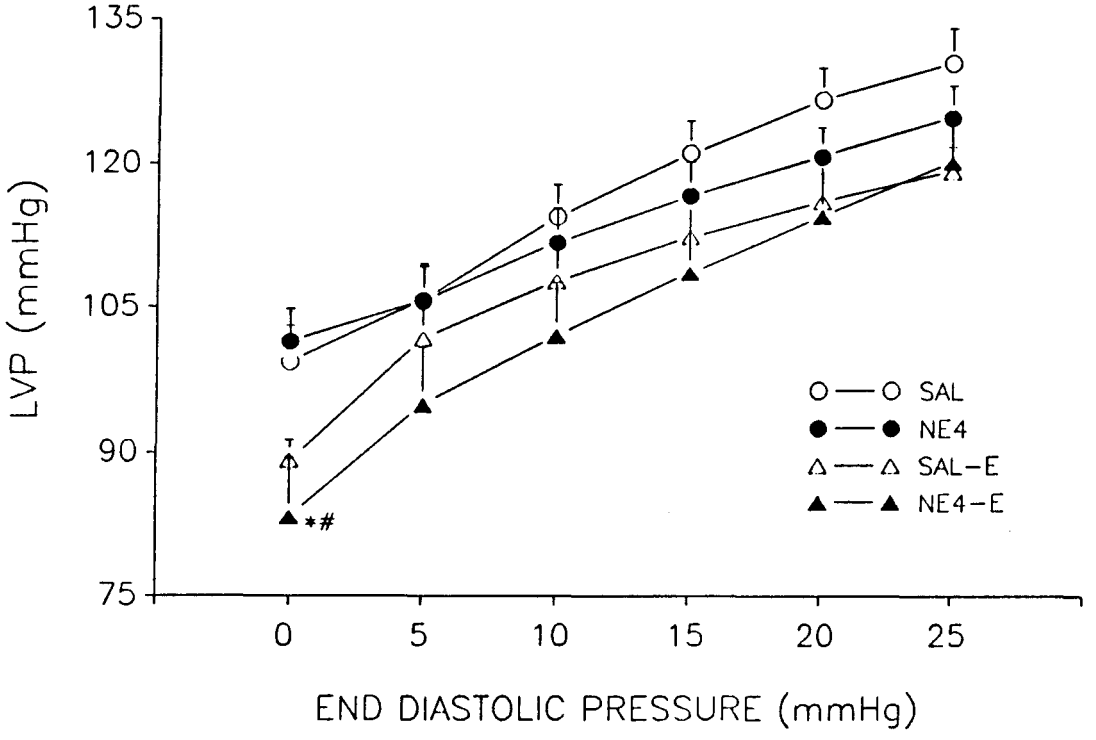
There was no difference in baseline LVP for LVEDP's of 5 to 25 mmHg among the groups (Figure 6-1). At EDP of 0 mmHg, LVP developed by NE4-E was significantly lower than SAL and NE4. Increases in LVP with changing LVEDP were similar in all groups.

Positive LV dP/dt_{max} was reduced significantly in hearts from rabbits treated with α-tocopherol and selenium (Figure 6-2) as compared to SAL at all EDP's. At 5 mmHg EDP, LV $+dP/dt_{max}$ was 1498.0 $\pm$ 67.7 and 1348.6 $\pm$ 97.5 mmHg/sec for SAL-E and NE4-E respectively, compared to 1762.4 $\pm$ 95.9 mmHg/sec for SAL and 1635.6 $\pm$ 82.6 mmHg/sec for NE4. Negative LV dP/dt_{max} also was depressed significantly in NE4, SAL-E and NE4-E compared to SAL (Figure 6-3).

Left ventricular systolic function improved in all groups in response to increasing concentrations of NE (Figure 6-4). NE4-E LV $+dP/dt_{max}$ was lower than

FIGURE 6-1

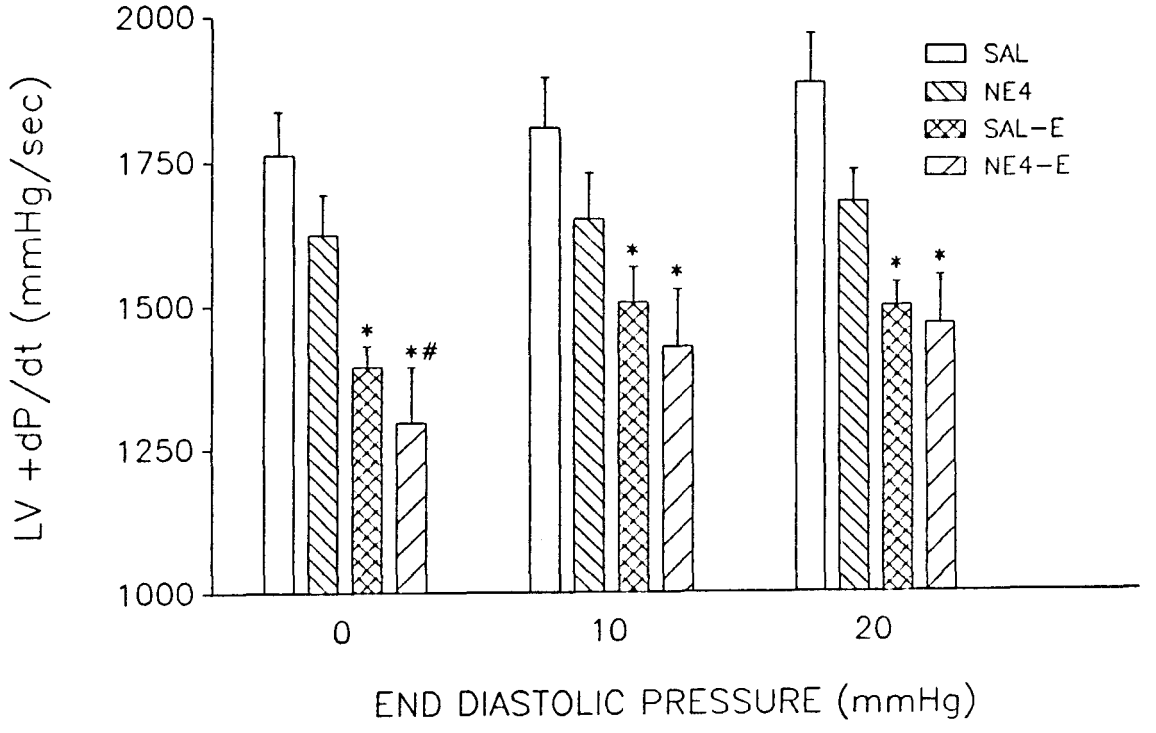
LEFT VENTRICULAR FUNCTION CURVES



Mean ± SEM for left ventricular systolic pressure (LVP) as a function of end diastolic pressure. SAL: hearts isolated from rabbits infused with saline. NE4: hearts from rabbits infused with 4 μg/kg/min norepinephrine. SAL-E: hearts from rabbits infused with saline after pretreatment with α-tocopherol and selenium. NE4-E: rabbits infused with 4 μg/kg/min norepinephrine after pretreatment with α-tocopherol and selenium. * indicates a significant difference compared to SAL. # indicates a significant difference compared to NE4.

FIGURE 6-2

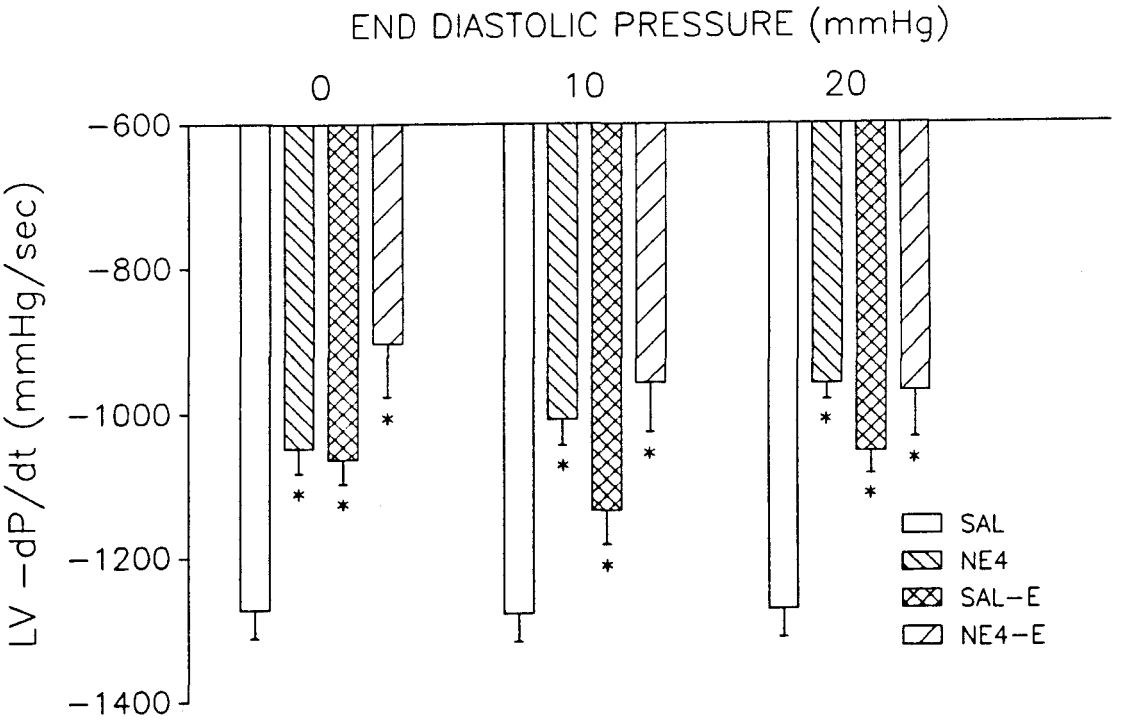
BASELINE LEFT VENTRICULAR +dP/dt_{max} AS A FUNCTION OF END-DIASTOLIC PRESSURE



Mean ± SEM for the first derivative of increasing left ventricular pressure, LV +dP/dt_{max}, as a function of end-diastolic pressure. Abbreviations as in Figure 6-1. * indicates a significant difference from SAL. # indicates a significant difference from NE4.

FIGURE 6-3

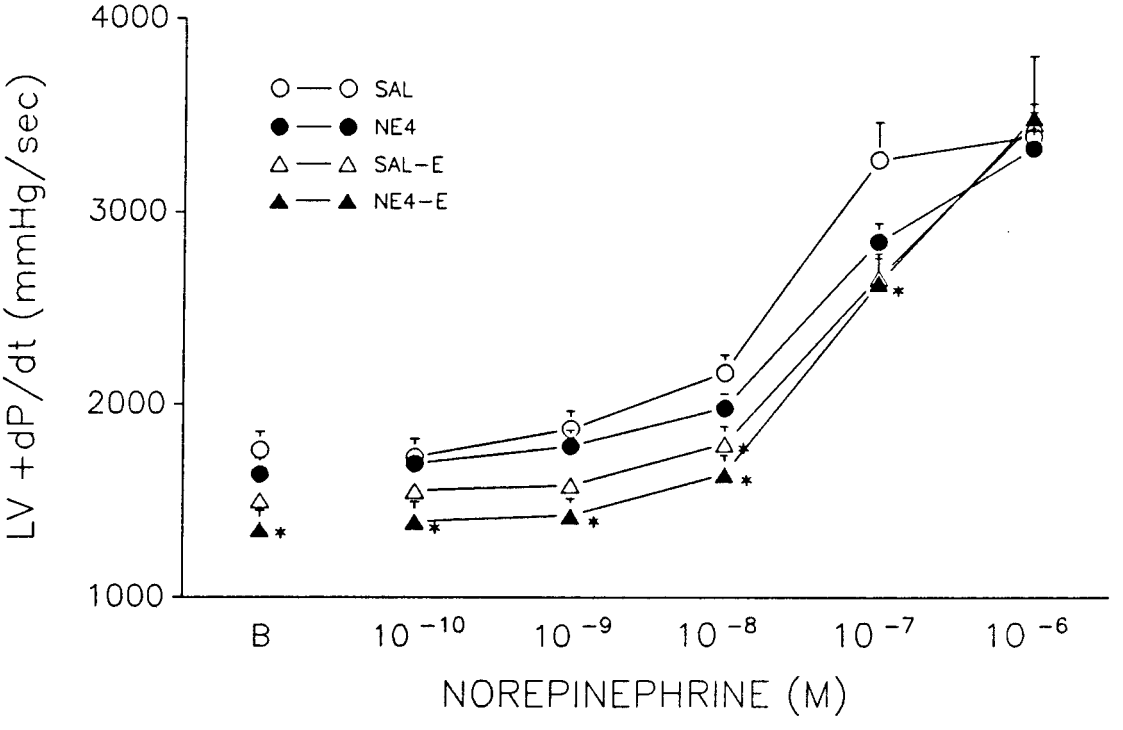
BASELINE LV -dP/dt$_{max}$ AS A FUNCTION OF END-DIASTOLIC PRESSURE



Mean ± SEM for the first derivative of decreasing left ventricular pressure, LV -dP/dt$_{max}$, as a function of end-diastolic pressure. Abbreviations as in Figure 6-1. * $p<0.05$ compared to SAL.

FIGURE 6-4

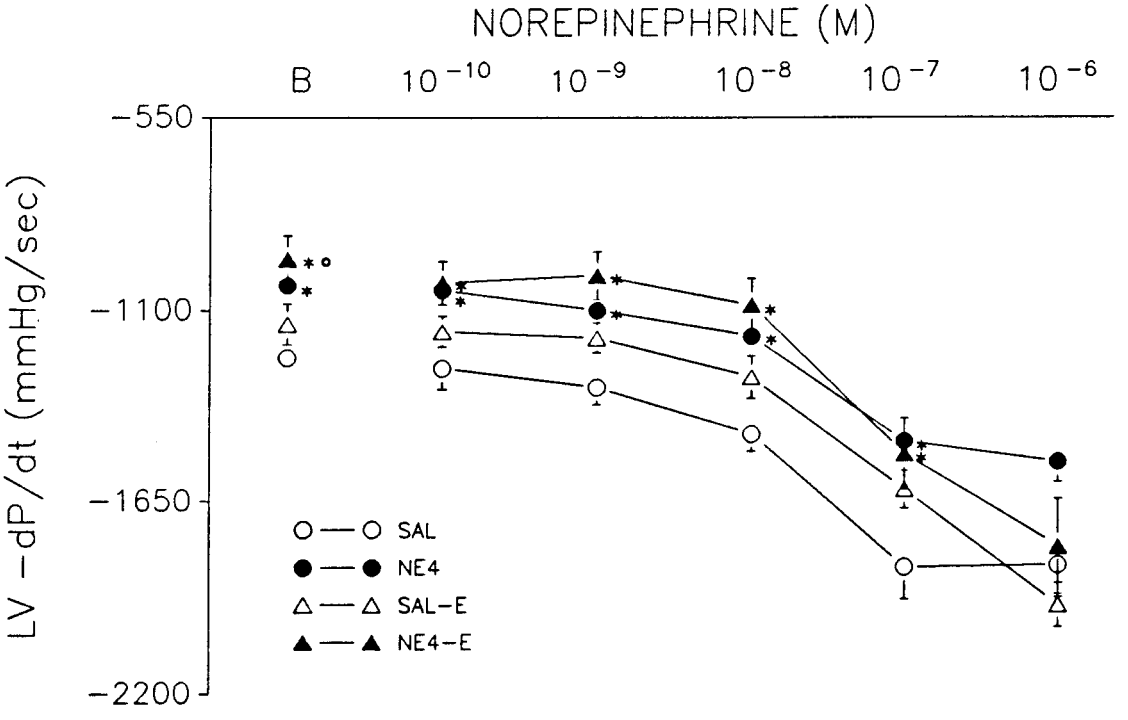
EFFECTS OF NOREPINEPHRINE ON
LEFT VENTRICULAR SYSTOLIC FUNCTION



Mean ± SEM values for the first derivative of increasing left ventricular pressure (LV $+dP/dt_{max}$) at baseline (B) and in response to increasing concentrations of norepinephrine. Measurements recorded at 5 mmHg end diastolic pressure. Other abbreviations as in Figure 6-1. * indicates a significant difference from SAL.

FIGURE 6-5

CHANGES IN DIASTOLIC FUNCTION IN
RESPONSE TO NOREPINPHRINE



Mean ± SEM values for the first derivative of decreasing left ventricular pressure (LV $-dP/dt_{max}$) in response to increasing norepinephrine concentrations. Measurements recorded at 5 mmHg end diastolic pressure. Refer to Figure 6-1 for other abbreviations. * indicates a significant difference from SAL. ° indicates significant difference from SAL-E.

SAL at baseline and at NE concentrations to 10^{-7} M. At 10^{-6} M NE, LV $+dP/dt_{max}$ was virtually the same in all groups. Increased diastolic function was also observed during perfusion with NE (Figure 6-5). At baseline and NE concentrations to 10^{-7} M, LV $-dP/dt_{max}$ was significantly lower in NE4 and NE4-E compared to SAL. There was no difference in LV $-dP/dt_{max}$ among the groups at 10^{-6} M NE.

3. Histology

Histological sections showed no abnormalities in the left ventricles of SAL and SAL-E. Edema, contraction bands, loss of myofibrillar material and nuclei and cellular infiltrates were observed in NE treated hearts. This histological injury was not quantified.

D. DISCUSSION

The results of the present study show that LV diastolic function is depressed two days post NE infusion. Treating rabbits for two weeks with α-tocopherol and selenium did not protect the myocardium from NE-induced injury. Surprisingly, treatment significantly decreased baseline LV function. However, all groups increased ventricular function similarly in response to increasing concentrations of NE. At the highest concentration of NE, there was no difference in LV diastolic and systolic function among the groups.

Circulating NE is normally inactivated by tissue uptake with subsequent storage and metabolism, conjugation, or urinary excretion of the free catecholamine. During prolonged infusion of high dose NE, these mechanism may be saturated leaving more

free catecholamine to undergo autoxidation. Spontaneously oxidized isoproterenol (14,72), adrenochrome (54,57-59,74) and free radicals (29,47) damage the myocardium and decrease left ventricular function. Free radicals attack unsaturated bonds of membrane phospholipids, abstract an hydrogen ion and initiation lipid peroxidation (11,35,46,62). Membrane permeability (6,30,37) and fluidity (16,20) are altered by lipoperoxidation. Additionally, mitochondrial and microsomal enzymes (46,60,70) and membrane ATPases (49) are inactivated. Alterations in enzyme function may be due to protein damage and subsequent polymerization or due to changes in the phospholipid microenvironment (23,25). Noronha-Dutra et al. (43) have shown that incubation of isolated heart cells with plasma from rats given high dose epinephrine induced the formation of membrane microblebs and microvilli. Sarcolemmal permeability was also increased. These changes in membrane characteristics are evidence of lipid peroxidation.

α-Tocopherol, an effective chain-breaking antioxidant (8,61), terminates lipid peroxidation in sarcolemmal and intracellular membranes (22,63) and preserves membrane structure and function (34). Glutathione peroxidase reduces lipid hydroperoxides formed by peroxidation to hydroxy acids (38,51,61,62) preventing these peroxides from altering membrane structure and function (26). Tappel has reported that selenium-dependent glutathione peroxidase activity increased with lipid peroxidation (62). Additionally, selenium administration was reported to increase glutathione peroxidase activity (2,12,17,26). Pretreatment with α-tocopherol and selenium should protect myocardial membranes from peroxidative damage. Singal et

al. (53) reported that pretreating rats with vitamin E (10 mg/kg/day) for two weeks protected the myocardium from isoproterenol induced damage and reduced high energy phosphate levels. A water-soluble analogue of vitamin E, Trolox C, has also been shown to be effective in preventing deleterious effects of isoproterenol in neonatal rat heart cultures (45). In contrast, the administration of selenium and vitamin E to pigs did not protect against myocardial damage with isoproterenol but did prolong survival (66). In the present study we have shown that pretreatment with α-tocopherol and selenium did not protect the rabbit heart exposed to high dose norepinephrine. In fact, treatment with these agents tended to decrease baseline ventricular function (Figures 1, 2, and 3).

The cause of depressed baseline ventricular function in pretreated animals (SAL-E) compared to SAL is unclear. The incidence of α-tocopherol toxicity is very low (4,39,44,68). Rabbits, cats, dogs and monkeys can tolerate 200 mg/kg body weight without toxic effects (4). When dosage exceeded 1 g/kg body weight per day, coagulation problems developed possibly due to vitamin E inhibition of platelet aggregation or increased requirement for vitamin K (1,4,41,55,56). Vitamin K supplementation has been reported to alleviate the coagulation problems in the rat (68). Megadose vitamin E has been associated with skeletal muscle degeneration (15) and a necrotizing, nonprogressive skeletal myopathy with unusual paracrystalline inclusion bodies in humans (3). Cardiac toxicity has not been reported. In our study, 25 mg α-tocopherol acetate administered every other day for two weeks was significantly below toxic levels. Left ventricular sections from α-tocopherol and

selenium pretreated, saline infused rabbits (SAL-E) had no pathology.

In contrast to α-tocopherol, selenium has been shown to be toxic in experimental animals. Characteristic signs of acute selenosis include "garlic breath", lethargy, excessive salivation, vomiting, dyspnea, muscle tremors and respiratory failure (13,69). Endocarditis and myocarditis have also been observed. In rabbits, Levine and Flaherty (36) reported an LD_{75} of 1.8 mg selenium/kg body weight for subcutaneous administration. Chronic selenosis is characterized by dermatitic lesions, anorexia with weight loss, and increased serum transaminases and alkaline phosphatase (13,33). Rabbits used in this study did not exhibit signs of acute or chronic selenium toxicity. Body and heart weights were not different between treated and untreated animals. Histological sections of the left ventricle from rabbits treated with α-tocopherol and selenium (SAL-E) had no signs of endocarditis or myocarditis. Serum levels of transaminase and alkaline phosphatase were not measured.

Vitamin E administered to rabbits in conjunction with selenium increased the LD_{50} from 2.53 mg/kg body weight to 2.73 mg/kg (69). This reduction in selenium toxicity (31,42,69) may be the result of vitamin E decreasing tissue selenium concentrations (42). In the present study, the administration of α-tocopherol may have decreased any overt toxic signs of selenium so they were not observed by the investigators. Alternatively, myocardial selenium or α-tocopherol concentrations elevated above some optimum may intrinsically depress myocardial function.

The data presented in this study shows that the administration of α-tocopherol and selenium every other day for two weeks does not protect the rabbit myocardium

from NE induced injury. In fact, pretreatment depressed baseline function. However, it should be noted that this pretreatment did not significantly exacerbate the functional injury created with high dose NE.

Further investigation is needed to separate the effects of α-tocopherol and selenium on cardiac function and to determine if the protective effect of these agents against catecholamine injury is species specific. The role of oxidized metabolites and lipid peroxidation in the etiology of catecholamine cardiomyopathy remains to be elucidated.

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CHAPTER VII

MYOCARDIAL BLOOD FLOW DURING AND AFTER A HIGH DOSE NOREPINEPHRINE INFUSION

A. INTRODUCTION

Catecholamines can modulate myocardial blood flow directly by the activation of adrenergic receptors within the coronary vasculature (3,6,9,10,20) or indirectly by stimulating myocardial function and metabolism (3,15,16,24). Stimulation of the β-adrenergic receptor produces dilation of coronary vessels (14,23,24). Similarly, intracoronary infusion of isoproterenol (ISO) has been shown to increase blood flow to the canine myocardium in a dose dependent manner (9). Blockade of the β_1 and β_2-adrenergic receptors eliminated this response.

Under resting conditions, the α-adrenergic receptors mediate a tonic vasoconstrictor tone in the coronary vasculature. Further vasoconstriction of coronary vessels with the administration of catecholamines, particularly in the presence of β-receptor blockade, has been demonstrated in several laboratories (1,16,18,22,25,26). In experiments where the dosage of a catecholamine was excessive, total myocardial blood flow was reduced below control with significantly less flow reaching the endocardium (4,8,9,21,27). Simons and Downing (21) reported decreased myocardial

blood flow during an infusion of norepinephrine (NE) at a dose known to induce myocardial injury. It was suggested that coronary vasoconstriction and localized ischemia mediated by α-adrenergic receptor stimulation contributed to catecholamine-induced cardiac injury.

If the cardiac injury induced by high dose catecholamines is the result of ischemia, perfusion abnormalities may develop. Kloner et al. (11,12) have shown abnormal myocardial perfusion following a temporary coronary artery occlusion. This defect, the no-reflow phenomenon, is characterized by poor perfusion or possibly regions of no perfusion in the myocardium after an ischemic insult (5,11).

The purpose of this study was to determine if the myocardium was perfused normally after a 40 minute NE infusion (4 μg/kg/min). Simons and Downing have reported that coronary blood flow was decreased at 40 minutes with the administration of NE (3 μg/kg/min) (21). Flow changes for a longer time course have not been investigated. In the present study, a NE concentration higher than that used by Simons and Downing was chosen in attempt to exacerbate the ischemic response. Coronary blood flow was measured at control, 40 minutes of NE infusion and at one hour and two days post infusion to further characterize the response.

B. METHODS

1. Norepinephrine-Induced Cardiomyopathy

Nineteen New Zealand white rabbits were anesthetized with sodium pentobarbital (30 mg/kg IV) and infused with saline or norepinephrine (NE;

4μg/kg/min) for 40 minutes. Coronary blood flow was measured at 0 and 40 minutes infusion and at one hour post-infusion in seven NE treated rabbits (NE4-A) and seven saline controls (SAL-A). Five NE treated rabbits (NE4-C) were returned to their cages post-infusion and myocardial blood flow was measured 48 hours after infusion.

2. Myocardial Blood Flow

After sodium pentobarbital anesthesia (30 mg/kg IV), the trachea was cannulated and the rabbit was ventilated with room air. Body temperature was monitored and maintained at 38 $\pm$ 1°C with a heat exchanger and heating pad. Femoral arteries were cannulated with polyethylene catheters. One arterial cannula was connected to a Statham pressure transducer for continuous monitoring of arterial blood pressure. The second arterial cannula allowed for withdrawal of reference blood samples during microsphere injection. In acute experiments, a femoral vein was cannulated for infusion of NE or saline. After a left thoracotomy, the left atrial appendage was cannulated. Standard lead II ECG was recorded.

Radiolabeled 15 μm spheres (Ce-141, Nb-95, Ru-103, and Sr-46 3M Co, New England Nuclear) were suspended in 10% dextran with 0.05% Tween 80. Microsphere vials were sonicated for 20 minutes before use and were vigorously agitated immediately prior to injection. For each flow measurement, approximately 1 x 10^6 microspheres were injected into the left atrium over 20-30 seconds. Reference blood sample was withdrawn from the femoral artery at a rate of 3-4 ml/min. Blood withdrawal began 30 seconds before injection of microspheres and continued for 30-40 seconds post-injection. The reference blood sample was put into labeled counting

tubes. The withdrawal syringe was rinsed and the rinse was added to respective blood sample counting tubes. At the termination of the experiment, the heart was removed. The right ventricular (RV) free wall and left ventricle (LV) were isolated and fixed for 24-48 hours in a 10% formalin solution. The RV was weighed and placed in counting tubes. The LV was cut into 5-6 rings. Each ring was separated into endocardial and epicardial halves via a circumferential cut at a point midway between the epicardial and endocardial surfaces. Endocardial and epicardial samples were weighed and placed in separate plastic counting tubes. Radioactivity of the myocardium and reference blood tissue samples was counted by a NaI crystal (3 inches) in a TM Analytic gamma counter (Model 1185). Blood flows were calculated by computer using standard equations with appropriate corrections and interference coefficients, previously used in this laboratory (2,7,19). The following equation was used to calculate blood flow: MBF = (Ct/TW) x (RBW/Cb), where MBF = myocardial blood flow in ml/min/g, Ct = tissue radioactivity in counts/min, TW = weight of tissue sample in grams, RBW = reference blood withdrawal rate in ml/min, and Cb = total radioactivity in reference blood sample.

3. Data Analysis

Flows were expressed in ml/min/100 g tissue. Rate-pressure product was calculated from heart rate and mean arterial pressure measurements taken immediately prior to the injection of the radiolabeled microspheres. Coronary vascular resistance was calculated from arterial blood pressure and myocardial blood flow. An analysis of variance (ANOVA) with repeated measures was used to test for changes in heart

rate, arterial pressure, the double product, coronary blood flow and coronary vascular resistance during norepinephrine or saline infusion. A t-test with a Bonferroni correction was used to isolate differences. A two-way ANOVA was used to test for differences between groups. Values of $p < 0.05$ were considered statistically significant.

C. RESULTS

There were no differences in control measurements between SAL-A and NE4-A for LV blood flow, coronary vascular resistance, mean arterial pressure, heart rate and rate-pressure product (Tables 7-1 and 7-2). Control RV flows of 161.1 $\pm$ 13.2 ml/min/100 g for SAL-A and 146.8 $\pm$ 33.2 ml/min/100 g for NE4-A were not different.

During saline infusion, there were no significant changes in blood flow to the LV (Table 7-1) or RV. Additionally, coronary vascular resistance, mean arterial pressure and heart rate were not different (Table 7-2). Epicardial, endocardial and total LV blood flows were significantly increased at 40 minutes of NE infusion compared to control and SAL-A. Right ventricular flow was also increased to 240.9 $\pm$ 35.5 ml/min/100 g ($p<0.05$). Coronary vascular resistance was decreased slightly, but insignificantly at 40 minutes infusion. Mean arterial pressure in NE4-A was elevated at 40 minutes infusion compared to control and SAL-A while heart rate was depressed. Rate pressure product increased slightly, but insignificantly, in NE4-A by 40 minutes. By one hour post infusion, myocardial blood flows, arterial

TABLE 7-1

REGIONAL BLOOD FLOW VALUES (ML/MIN/100 G)
AND CORONARY VASCULAR RESISTANCE (mmHg*ML/MIN/100 G)

	LV	LV-ENDO	LV-EPI	CVR
CONTROL				
SAL-A	223.6 ± 16.8	216.1 ± 25.6	231.0 ± 19.1	0.36 ± .O3
NE4-A	242.3 ± 47.0	256.0 ± 49.1	228.5 ± 45.0	0.39 ± .09
40 MINUTES				
SAL-A	243.0 ± 35.7	270.5 ± 45.3	215.5 ± 31.1	0.38 ± .06
NE4-A	395.1*#° ± 37.0	450.0*#° ± 37.3	340.6*#° ± 39.9	0.27 ± .02
1 HOUR POST INFUSION				
SAL-A	263.5 ± 25.0	290.8 ± 26.8	241.9 ± 24.O	0.30 ± .03
NE4-A	228.5 ± 13.9	248.4 ± 14.6	208.4 ± 14.6	0.32 ± .03
2 DAYS POST INFUSION				
NE4-C	240.2 ± 29.5	232.0 ± 29.0	248.4 ± 33.2	0.35 ± .04

Myocardial blood flow (ml/min/100g) for the left ventricle (LV) during a 40 minute infusion of saline (SAL-A) or 4 μg/kg/min norepinephrine (NE4-A). Blood flow was measured in a third group (NE4-C) two days after norepinephrine infusion. Left ventricular blood flow was separated into endocardial (LV-ENDO) and epicardial (LV-EPI) flows. CVR, coronary vascular resistance in mmHg*ml/min/100 g. Data is presented as means ± SEM. * $p<0.05$ compared to SAL-A, # $p<0.05$ compared to control, and ° $p<0.05$ compared to 1 hour post.

TABLE 7-2

ARTERIAL PRESSURE, HEART RATE AND RATE-PRESSURE PRODUCT DURING SALINE OR NOREPINEPHRINE INFUSION AND AT TWO DAYS POST INFUSION

	MAP	HR	RPP
CONTROL			
SAL-A	77.7 ± 3.9	285.2 ±14.6	22059.6 ±1265.4
NE4-A	80.8 ± 2.4	293.3 ±11.9	23635.8 ± 853.1
40 MINUTES			
SAL-A	79.1 ± 5.5	278.9 ±13.4	22063.2 ±1795.5
NE4-A	104.5*#° ± 2.9	245.8*#° ±16.9	25626.7 ±1682.9
1 HOUR POST INFUSION			
SAL-A	75.9 ± 4.5	284.5 ±17.9	21761.1 ±2035.0
NE4-A	72.0 ± 5.1	279.6 ±11.7	20180.7 ±1754.7
2 DAYS POST INFUSION			
NE4-C	78.8 ± 1.2	305.0 ± 6.4	24055.0 ± 796.5

Values for mean arterial pressure (MAP, mmHg), heart rate (HR, beats/min) and rate pressure product (RPP, mmHg*beats/min) are expressed as means ± SEM. Abbreviations as in Table 7-1. * $p < 0.05$ compared to SAL-A, # $p < 0.05$ compared to control, and ° $p < 0.05$ compared to 1 hour post infusion.

pressures, heart rates and the rate-pressure products were not different than control values. Similarly, LV myocardial blood flow and coronary vascular resistance at two days post NE infusion were not different than NE4-A at one hour post infusion.

D. DISCUSSION

Several investigators have observed a biphasic response in coronary vascular resistance and blood flow after the administration of NE in the conscious dog (13,22). Initially, there was a brief coronary vasodilation with increased myocardial flow. This was followed by a sustained increase in coronary resistance and decrease in coronary blood flow (9,22).

A decrease in myocardial blood flow, particularly to the subendocardium, with a prolonged infusion of catecholamines or the administration of high dose catecholamines has been reported (4,21,27). Simons and Downing (21) showed an initial increase in coronary blood flow in the rabbit during a NE infusion (3 μg/kg/min) followed by a significant reduction in flow at 40 minutes. Collins and Billings (4) reported that ISO injection decreased blood flow to the LV subendocardium of the rat. Similar results were reported in the dog after ISO administration (8). In the isolated, supported canine heart, an intracoronary infusion of NE reduced blood flow to the endocardium (27). The decreased flows were attributed to α-receptor mediated coronary vasoconstriction (21) or the underperfusion of the endocardium due to mechanical forces (4). It has been further hypothesized

that the depressed myocardial flow was a mechanism of catecholamine-induced cardiac injury.

In contrast, Moir and DeBra (17) have shown a 64% increase in total coronary blood flow with a slightly greater increase in endocardial flow with NE administration in the dog. The result presented in this chapter demonstrate a significant increase in myocardial blood flow with a 40 minute NE infusion. The discrepancy in the findings may be due to the catecholamine used, dose administered in each study or an undetermined factor. Collins and Billing (4) and Fortuin et al. (8) administered ISO, a potent β-agonist, while Moir and DeBra administered NE, a mixed α/β agonist. The effects of ISO on coronary blood flow were prevented by β-receptor blockade (4). When Fortuin et al. (8) administered NE in the presence of propranolol, endocardial flow was significantly augmented.

In the present study, endocardial, epicardial and total LV flows were increased and coronary vascular resistance was decreased at 40 minutes NE infusion in the rabbit (4 μg/kg/min). These results provided no evidence to support the hypothesis that a 40 minute NE infusion induced coronary vasoconstriction. A relatively unchanged rate-pressure product, an index of myocardial oxygen demand, coupled with a 63% increase in LV blood flow, also suggested the myocardium was not hypoxic during NE infusion. Further study is needed to determined if NE infusion of a longer duration or higher dosage results in ischemic injury and a defective reperfusion of the myocardium.

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CHAPTER VIII

SUMMARY

1. A 90 minute infusion of high dose norepinephrine in the rabbit acutely impairs left ventricular diastolic and systolic function.
2. Systolic function improved within 48 hours after norepinephrine infusion whereas diastolic function remained depressed.
3. Myocardial responsiveness to α- or β-adrenergic receptor activation was not altered acutely or chronically after norepinephrine administration.
4. The administration of α-tocopherol and selenium every other day for two weeks did not protect the rabbit myocardium from norepinephrine-induced injury.
5. Coronary blood flow was significantly increased in the left and right ventricular myocardium at the end of a 40 minute, high dose norepinephrine infusion. Myocardial blood flow returned to control levels by one hour post infusion.

CHAPTER IX

CONCLUSIONS

The myocardial lesions produced when the heart was exposed to excessive catecholamines have been intensely studied and characterized by numerous investigators. Alterations in cardiac function following catecholamine-induced injury, however, have not been fully investigated.

Ventricular function studies utilizing rat and rabbit models of catecholamine-induced cardiac injury indicated that systolic function was depressed forty-eight hours after catecholamine administration. In the first study of this dissertation, it was hypothesized that cardiac function was impaired acutely after catecholamine injury and some functional recovery occurred by 48 hours. To test this hypothesis, left ventricular function was measured immediately after a 90 minute norepinephrine (NE) infusion and at two days post infusion. To further characterize functional alterations, myocardial function was assessed at three levels of norepinephrine-induced injury.

This study is the first to demonstrate impairment in left ventricular systolic function of rabbit hearts acutely after norepinephrine injury with significant improvement by 48 hours, except in hearts with the most severe injury. Additionally,

this study is the first to report diastolic function in norepinephrine cardiomyopathy. Diastolic function, as measured by LV -dP/dt and tau, was impaired but unlike systolic function, the dysfunction observed immediately after the 90 minute NE infusion were still present at two days.

Depression in systolic function observed in norepinephrine-induced cardiomyopathy may be due to a loss of functional myocytes. Histological examination of the myocardium reveals the hypercontraction of sarcomeres and the loss of myofilament structure in some myocytes. The loss of normal structure of contractile elements reduces contractile function in those cells. Damage of a critical number of cardiac myocytes could depress systolic function of the entire myocardium. To date, there is no direct evidence that the myofilaments in cells with normal histology are injured. If the contractile elements are defective and function abnormally, even normal appearing cells would contribute to the impairment in systolic and possibly diastolic function observed in catecholamine cardiomyopathy.

Multiple factors may contribute to diastolic dysfunction. In the initiation of contraction, calcium moves into a myocyte through ion channels and is released by the sarcoplasmic reticulum so intracellular calcium concentration increases. If the cell is functioning normally, the calcium is later extruded via the sodium-calcium exchange and calcium pump or transported into the sarcoplasmic reticulum. The decrease in cytosolic calcium levels allows the muscle to relax. In catecholamine injury, removal of calcium from the cytosol is impaired. The sodium-calcium exchange and calcium pump activities are depressed so calcium is not extruded from the cell. Additionally,

the uptake of calcium by the sarcoplasmic reticulum is impaired. These defects allow intracellular calcium levels to rise. High cytosolic calcium concentrations coupled with impaired removal of calcium may contribute to the decreased rate of ventricular relaxation. Cellular degeneration and myocardial edema, characteristic signs of catecholamine injury, increase myocardial stiffness and may augment the diastolic dysfunction seen in norepinephrine-induced cardiomyopathy.

Exposure of the myocardium to catecholamines decreases the responsiveness of the adrenergic receptor system to subsequent stimulation (4,6). This process is known as desensitization. In the second study (Chapter V), it was hypothesized that the β-adrenergic receptor would be desensitized during the NE infusion. Stimulation of the isolated heart by a β-receptor agonist would then elicit smaller changes in ventricular function. It was also proposed that the changes in ventricular function with α-adrenergic receptor activation would be more important after NE induced injury.

Although adenylate cyclase activity was not measured directly, the results presented in Chapter V indicate the β-receptor was not uncoupled from adenylate cyclase. Beta-receptor stimulation by dobutamine increased function in NE pretreated hearts the same amount as hearts from saline treated rabbits with one interesting exception. In acute experiments, the ability of β-receptor stimulation to increase LV +dP/dt in hearts injured by NE was impaired. It was hypothesized that the NE infusion acutely altered a step in cross-bridge formation or cycling. The ability of NE injured hearts to increase LV +dP/dt with β-receptor activation was slightly improved

at 48 hours. The responsiveness of the α-adrenergic receptor to agonist stimulation was not changed following NE administration.

The pathogenesis of catecholamine cardiomyopathy involves multiple mechanisms. Several investigators have reported that toxic metabolites of catecholamine autoxidation induce myocardial injury (3,9,11). These metabolites, particularly the free radicals may initiate lipid peroxidation which alters membrane permeability and function.

The third study (Chapter VI) was designed to test whether pretreatment with α-tocopherol and selenium protected hearts from norepinephrine-induced injury. α-Tocopherol is an antioxidant located in cellular membranes. Selenium is an essential component of glutathione peroxidase, an enzyme which removes lipid peroxides from membranes before the peroxides damage the membrane. It was hypothesized that if metabolites of NE autoxidation are acting as membrane oxidants, increasing the anti-oxidant capacity of the heart would protect the myocardium from NE injury. The results of this study suggest however, that NE injury in the rabbit heart is not produced by oxidative damage. Pretreatment with α-tocopherol and selenium did not reduce the diastolic or systolic dysfunction which occurred with the administration of high dose NE.

One alternative explanation is that the oxidative capacity of the heart was exceeded by the dose of NE administered eventhough rabbits were pretreated with α-tocopherol and selenium. The oxidants produced by NE oxidation would then damage the myocardium unhindered. Another explanation is that α-tocopherol administered

with selenium is not the most effective combination of anti-oxidants. The protective effects of α-tocopherol and selenium may be species specific. Further investigation is needed to determine if α-tocopherol administered alone or in combination with another antioxidant protects the rabbit myocardium from NE-induced injury.

Alpha-receptor mediated coronary vasoconstriction with the administration of catecholamines is well documented (1,2,7). It has been proposed that high levels of circulating catecholamines produce coronary vasoconstriction with localized ischemia with the ischemic insult injuring the myocardium (8). If the cardiomyopathy induced by high dose catecholamines is the result of ischemia, perfusion abnormalities similar to those observed in models of temporary ischemia, may develop.

In the final study of this dissertation (Chapter VII), catecholamine induced coronary vasoconstriction or ischemia was not observed at 40 minutes of NE infusion. In fact, endocardial, epicardial and total left ventricular blood flows were elevated significantly while coronary vascular resistance was reduced slightly. Perfusion abnormalities were not apparent. This data does not support the hypothesis that NE injury is secondary to coronary vasoconstriction and/or ischemia. The increase in blood flow coupled with only a small change in rate-pressure product also suggest that the myocardium was not hypoxic during the NE infusion.

The administration of catecholamines initially increases myocardial blood flow and is quickly followed by an α-receptor mediated coronary vasoconstriction and a decrease in coronary blood flow (5,10). The effects of administering catecholamines for longer periods on myocardial blood flow has not been thoroughly investigated.

The data presented in Chapter VII suggests that myocardial blood flow increases again with prolonged exposure to a catecholamine. With time, metabolic factors may override α-vasoconstriction and induce coronary vasodilation.

In summary, it is clear that a few questions regarding ventricular dysfunction which occurs following the administration of high dose NE have been answered. Left ventricular function of rabbit hearts is depressed acutely after norepinephrine injury with significant improvement by 48 hours, except in hearts with the most severe injury. Diastolic function was impaired but unlike systolic function, the dysfunction observed immediately after the 90 minute NE infusion was still present at two days. Further investigation is needed into the time course of recovery of diastolic function, if indeed it does completely recover. Similarly, it would be interesting to determine if hearts damaged by the highest NE dose (6 μg/kg/min) gradually recovered ventricular systolic and diastolic function. Mechanisms of catecholamine-induced myocardial injury also need further study. The exact role of metabolites of catecholamine autoxidation in the pathogenesis of catecholamine cardiomyopathy remains to be elucidated. Likewise, the time course of changes in myocardial blood flow during a prolong catecholamine infusion needs to be determined.

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APPROVAL SHEET

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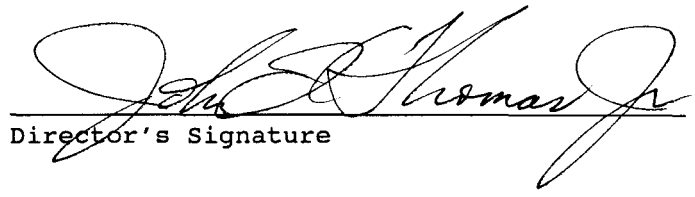
The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Physiology.

8/1/91
Date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