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The University of Arizona PH.D. 1984

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REGULATION OF MYOCARDIAL HYPERTROPHY BY EPINEPHRINE

by

Douglas F. Larson

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Douglas F. Larson

entitled Regulation of Myocardial Hypertrophy by Epinephrine

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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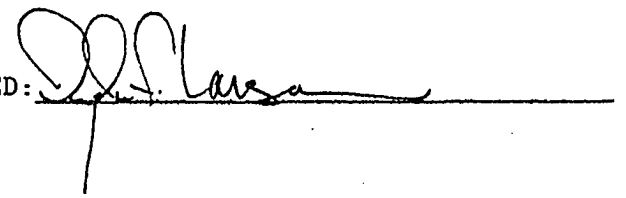
SIGNED: 

DEDICATION

To my wife and children, for their unwavering support through the years leading to the realization of this dissertation.

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ABSTRACT

Hormonal regulation of growth and of macromolecular synthesis in a variety of tissues is now well established. This dissertation addresses the role of circulating hormones, particularly epinephrine, in the physiological regulation of myocardial mass. Following hemodynamic overload of the right ventricle, the circulating epinephrine concentration increased significantly, and blood epinephrine exhibited a significant positive correlation with myocardial mass. Further, a nonspecific β-antagonist, propranolol, blocked the usual myocardial hypertrophy that occurs in response to hemodynamic overload. These studies strongly implicate β-adrenoceptors in the regulation of myocardial mass.

Theoretically, a circulating myocardial trophic hormone should result in biventricular hypertrophy. We found that a selective hemodynamic overload of the right ventricle produced significant hypertrophy of both the right and the left ventricles. A biochemical marker of β-receptor activity, ornithine decarboxylase, a key regulatory enzyme in growth, showed elevated activity in both the right and left ventricles following hemodynamic overload of the left ventricle.

To further evaluate possible circulating myocardial trophic hormones, we studied hypertrophy in a donor heart transplanted into the abdomen of a recipient animal. Myocardial hypertrophy of the donor heart occurred independently of innervation and of any hemodynamic parameters. Alteration in myocardial mass paralleled the extent of β-receptor activity as assessed by the administration of exogenous β-agonists or by the modulation of β-receptor number by denervation. β-Receptor activity was assessed by the ability of isoproterenol to elevate ornithine decarboxylase activity in either the donor or the recipient heart. Finally, alterations in the levels of circulating endogenous hormones in response to pulmonary artery banding of the recipient rat heart resulted in concomitant hypertrophy of both recipient and donor hearts.

These studies suggest that myocardial mass is regulated by the concentration of circulating epinephrine through its effect on myocardial β-adrenoceptors. This effect may be modified by the level of other hormones such as thyroid hormone, but does not appear to be altered to any extent by myocardial innervation or by the alteration of hemodynamic parameters except as they affect the circulating level of catecholamines.

CHAPTER 1

OVERVIEW OF PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF CARDIAC HYPERTROPHY

Introduction

There have been many theories and much speculation about the identity of the growth regulatory factors initiating and promoting the biochemical chain of events leading to cardiac hypertrophy. Most discussions of cardiac growth suggest that the initial stimulus is hemodynamic overload which directly mediates increased myocardial protein synthesis and concomitant adaptive ventricular hypertrophy (Zak, 1973; Wikman-Coffelt et al., 1976). The cascade of events leading to biochemical and architectural changes in the myocardium is proportionate to the workload (Linzbach, 1960; Grossman et al., 1975; Braunwald, 1980). In these studies, we tested the hypothesis that cardiovascular hemodynamic overload activates a specific hormonal cascade that modulates adaptive cardiac hypertrophy.

Clinical Implications of Cardiac Hypertrophy

Mild adaptive hypertrophy initially may be beneficial by coupling an increased capacity of the heart to an increased workload. However, pathological hypertrophy may develop as a consequence of extensive adaptive hypertrophy which results in deterioration of myocardial performance and heart failure (Spann et al., 1967). In fact,

chronic hypertension leads to marked cardiac hypertrophy and, prior to the advent of effective antihypertensive therapy, heart failure secondary to hypertrophy was the most common cause of death from hypertension (Pickering, 1968). Today, hypertension-induced left ventricular hypertrophy in adults remains the most common precursor of congestive heart failure (Kannel et al., 1969, 1972; McKee et al., 1971; Kannel and Sorle, 1981). To develop a medical therapy(s) to prevent or modify the hypertrophy process, the events triggering the biochemical cascade coupled to cardiac hypertrophy need to be understood. This rationale underlies the current research to elucidate hormonal mechanism(s) mediating cardiac hypertrophy.

Dissociation between Hypertension and Cardiac Hypertrophy

Human autopsy studies have failed to establish a close correlation between the degree of cardiac hypertrophy and the extent and duration of hypertension (Grant, 1953; Kannel, 1974; Tarazi et al., 1977). Initial observations in spontaneously hypertensive rats (Sen et al., 1974) which have been confirmed in man by echocardiographic techniques have demonstrated that the incidence of left ventricular hypertrophy in both the hypertensive rats and hypertensive patients correlated poorly with arterial blood pressure (Liard and Fixer, 1981). Further, antihypertensive therapy with minoxidil or hydralazine, which effectively controlled blood pressure in spontaneously hypertensive rats, did not reduce ventricular hypertrophy. α-Methyldopa reduced both arterial blood pressure and myocardial mass; in contrast, propranolol did not reduce blood pressure but significantly reduced the

ventricular weight (Sen et al., 1977). Furthermore, there are other examples of dissociation between cardiac overload states and hypertrophy in experimental models. In the spontaneously hypertensive rat, the development of cardiac hypertrophy occurs prior to the onset of hypertension (Sen et al., 1974). In addition, deoxycorticosterone treatment may induce a hypertensive state without concomitant cardiac hypertrophy (Fregly and Arean, 1959). Based on these well-documented dissociations between pressure overload and cardiac structural response in different types of hypertension, we suggested that the circulating levels of trophic hormones actually initiate the process of cardiac hypertrophy.

Hormonal Regulation of Cardiac Hypertrophy

Hormones such as catecholamines bind specifically to receptors in the cell membrane which result directly or indirectly in the synthesis and/or release of intracellular mediators, such as calcium ions or cyclic nucleotides, which are known to affect cellular growth. Catecholamines such as epinephrine, norepinephrine and isoproterenol, have been demonstrated to be coupled to calcium channeling and to the cyclic AMP-mediated biochemical cascade resulting in cellular growth (Frankfurt, 1968; Gans and Cater, 1970; Radley and Hodgson, 1971, 1973; Byus et al., 1976; Zimmer and Gerlach, 1982).

Comparing the sequential biochemical cascade brought about by pressure overload and by catecholamine (isoproterenol) administration, an identical sequence is observed (Table 1). It is well understood that general elevations in protein and RNA synthesis are conspicuous

Table 1: Metabolic Changes following Pressure Overload or Catecholamine Stimulation

	Isoproterenol Stimulation	Pressure Overload Stimulation
adenylate cyclase	Kumano et al., 1983	Schreiber, 1971
cyclic AMP-dependent protein kinase	Byus et al., 1976	
ornithine decarboxylase	Haddox et al., 1981; Bartolomé et al., 1982	Feldman & Russell, 1972; Matsushita et al., 1972
spermine	Selmeci et al., 1982; Caldarera et al., 1974	Caldarera et al., 1971; Russell et al., 1971
adenine	Fleckenstein, 1971	Zimmer et al., 1972
RNA synthesis	Wood, 1971	Nair et al., 1968; Nair et al., 1976
RNA polymerase	Nair et al., 1976	Schreiber et al., 1971
adenine synthesis	Zimmer & Gerlach, 1974	Zimmer et al., 1972
protein synthesis	Zimmer & Ibel, 1979	Schreiber et al., 1966; Schreiber et al., 1971
myosin synthesis	Morkin et al., 1972	Schreiber, 1971
hexose monophosphate shunt	Zimmer et al., 1979	Zimmer et al., 1980

features of experimentally induced cardiac hypertrophy (Fanburg and Posner, 1968; Nair et al., 1968; Morkin et al., 1972; Rabinowitz and Zak, 1972; Cutilletta et al., 1978). However, there are other specific metabolic events which occur following pressure overload or catecholamine stimulation that further indicate a common process. One of the earliest biochemical changes detectable in the cardiac trophic response is the rapid excursion of the rate-limiting enzyme for polyamine biosynthesis, ornithine decarboxylase (ODC), whose activity peaks 4 h after stimulation (Copeland et al., 1982). This enzyme decarboxylates ornithine to produce putrescine which is the diamine precursor for the synthesis of the polyamines, spermidine and spermine. As a result, spermine content also is elevated early after stimulation (Caldarera et al., 1971; Russell et al., 1971; Feldman and Russell, 1972). The adenine nucleotide content decreases early in the metabolic process, and at the same time, RNA synthesis and nuclear RNA polymerase activity are enhanced (Nair et al., 1968; Posner and Fanburg, 1968; Fizel and Fizelova, 1971; Zak and Rabinowitz, 1979). At 24 h, both adenine nucleotide biosynthesis and protein synthesis are markedly enhanced; at 48 h, the hexose monophosphate shunt activity is accelerated to maintain adenine nucleotide biosynthesis (Morkin et al., 1972; Zimmer et al., 1972, 1980). At a biochemical level, there appears to be no difference between experimentally induced hypertrophy with pressure overload or catecholamine treatment which underscores the importance of a catecholamine mechanism in the regulation of cardiac hypertrophy.

β-Receptor Coupled to Ornithine Decarboxylase Activity

Ornithine decarboxylase (ODC) is the rate-limiting enzyme in polyamine biosynthesis (Cohen, 1971; Russell, 1973; Russell and Durie, 1978). ODC activity is universally elevated following target organ stimulation by growth-promoting agents or events (Pegg and Williams-Ashman, 1968; Russell and Snyder, 1969; Russell et al., 1970; Russell and Taylor, 1971; Russell et al., 1976; Russell and Durie, 1978; Russell, 1981). The enzyme has an extremely rapid half-life (10-20 min) and, following stimulation, exhibits peak activity within 4 h. Because of these two characteristics, ODC has been useful as a biological marker of the multiple cases of cyclic AMP-stimulated growth. In myocardial tissue, β-receptor stimulation results in a dose-dependent increase in ODC activity which serves as a physiological signal of the extent of growth stimulation and parallels the later elevation in myocardial mass (Haddox et al., 1981; Copeland et al., 1982).

During compensatory cardiac hypertrophy, the number of β-receptors has been described to decrease, increase or not to change; in addition, an increase or no change in affinity has been reported (Limas and Limas, 1978; Limas, 1979; Giachetti et al., 1979; Tse et al., 1979; Bhalla et al., 1980; Woodcock and Johnston, 1980a, 1980b; Blumenthal et al., 1982). Also, measurement of the β-receptor number presents methodological problems since recent reports demonstrated a decreased number of β-receptors in failing hearts according to the methods of Williams and Lefkowitz (1978) and an increased number of β-receptors using methods proposed by Baker and Potter (1980). Kumano

et al., (1983) reported uncoupling of β-receptors to adenylate cyclase during hypertrophied states which could be reinstated following reversal of hypertrophy. Thus, measurement of the number of β-receptors in the hypertrophied heart may not describe the potential for β-receptor stimulation. Therefore, we characterized β-receptor activity in the following studies through the ability of a β-agonist to stimulate myocardial ODC activity.

Purpose

The purpose of these studies was to further demonstrate that myocardial hypertrophy is hormonally regulated. We are concerned with demonstrating the presence and action of the circulating hormone(s) link that initiates the biochemical chain of events leading to heart growth. We will test the hypothesis that the response to cardiac overload stress, provided by increased volume or pressure overload, activates sympathetically-mediated release of epinephrine from the adrenal medulla which, presumably through β_2-adrenergic activity, modulates myocardial mass. Studies also are designed to test the hypothesis that epinephrine may not be the sole modulator of cardiac mass as other hormones may affect its action or may directly affect cardiac mass.

Statistics

Student's t-test was used to determine the significance of difference between two means. Significance levels of difference between two means in time course or dose response experiments were determined by the Newman Keuls procedure. Correlations between two

sets of data were performed by linear regression analysis. All these statistical procedures were computed with the integrated system of Statistical Package for Social Sciences (SPSS) programs.

CHAPTER 2

OVERVIEW OF HORMONAL MODULATION OF CARDIAC MASS

Development of cardiac hypertrophy is a rapid, well-documented consequence of increased volume or pressure load of the ventricles. It is clear that elevated ventricular load is not directly responsible for the development of hypertrophy and that endocrine mechanisms may serve as intermediates between the mechanical stimulus and the cellular biochemical processes eventually leading to hypertrophy. These hormones together may act as: 1) modulating factors through direct trophic roles regulating myocardial mass, or 2) permissive factors which are necessary but not sufficient as sole agents to cause hypertrophy. To characterize the myocardial trophic effects of hormones, investigators have used models of ventricular overload and ablation of specific endocrine sources.

Role of the Adrenal Gland in the Regulation of Cardiac Hypertrophy

In experiments performed over 30 years ago, Beznak (1952) observed that coarctation of the aorta in rats with bilateral adrenalectomy failed to demonstrate significant increases in heart weight. She did not measure arterial blood pressures so it was not known whether adrenalectomy directly influenced the degree of pressure overload to the heart. In similar experiments, Nichols et al. (1983) demonstrated that the hemodynamic load was elevated, but in the

absence of the adrenal gland, there was a marked reduction in the extent of myocardial hypertrophy after pressure overload. In these studies, adrenal steroids or catecholamines were not added back to adrenalectomized animals to ascertain which specific adrenal hormones were required for the hypertrophy process.

Consistent with these findings, Schreiber et al. (1980) found a significant increase in the weight of rat adrenals following either experimental hyperthyroidism or after banding of the abdominal aorta to induce cardiac hypertrophy. There was a signficiant positive correlation between heart weight and adrenal weight which provided indirect evidence that an adrenal hormone(s) may play a role in modulating cardiac mass.

To distinguish between the role of the adrenal cortex and the adrenal medulla in the hormonal modulation of cardiac mass, the function of each region was hormonally or surgically manipulated to selectively suppress function. To selectively modulate adrenal cortical function, Tepperman (1980) described hypophysectomy-produced atrophy of the adrenal cortices as a result of deficiency of the corticotrophin (ACTH) essential for the maintenance of the zona fasuculota. The region of the cortex which produces the salt-retaining mineralcorticoids, zona glomerulosa, has some degree of independence from ACTH stimulation. It is for this reason that untreated adrenalectomy is fatal whereas hypophysectomy is not.

Effects of Hypophysectomy on Cardiac Hypertrophy

We demonstrated that hypophysectomy in the hemodynamically overloaded rat: 1) demonstrated that hormones serve an intermediate role between hemodynamic stimulus and heart growth, and 2) that adding back pituitary hormones one at a time in hypophysectomized animals delineated the pituitary hormones essential for hypertrophy. Hypophysectomized Sprague-Dawley rats failed to develop cardiac hypertrophy following banding of the proximal abdominal aorta (Table 2). Also, in the non-hemodynamically overloaded hypophysectomized animals, the heart atrophied significantly compared to the non-hypophysectomized controls. Furthermore, direct catecholamine-induced hypertrophy also was dependent upon an intact endocrine system (Table 3). Low-dose isoproterenol (0.5 mg/kg/day) produced a 152% growth in heart mass in 5 days compared to control but no increase in mass was seen in isoproterenol-treated hypophysectomized rats. These data demonstrate an endocrine mechanism in heart growth and show that hemodynamic or catecholamine stimuli alone are not totally responsible for development of cardiac hypertrophy.

In similar studies, Beznak (1963, 1969) advanced the hypothesis that myocardial growth is modulated by endocrine mechanisms and found that the treatment of hypophysectomized rats with thyroxin and growth hormone restored the ability to maintain cardiac mass. In these long-term studies, the untreated hypophysectomized rats failed to thrive in body development; therefore, pituitary hormones may act as permissive factors rather than possessing trophic roles.

Table 2: Effect of Hypophysectomy on Afterload-induced Cardiac Hypertrophy in Rats

Treatment Group[a]	Heart Dry Wt/Body Wt	% of Control
Sham + Sham (N = 6)	0.72 ± 0.03	---
Sham + Hypox (N = 6)	0.58 ± 0.03[b]	81
Aortic Band + Sham (N = 6)	0.88 ± 0.06[b]	124
Aortic Band + Hypox (N = 6)	0.76 ± 0.04	109

[a]Data are expressed as the mean ± S.E.M.

[b]Data differ from control ($p < 0.05$).

The percentage increase in mass over sham-operated controls of heart dry weight to body weight ratios resulting from banding the aorta were studied after 5 days.

Table 3: Effect of Hypophysectomy on Isoproterenol-induced Cardiac Hypertrophy in Rats

Treatment Group[a]	Heart Dry Wt/Body Wt	% of Control
Saline Sham (N = 6)	0.71 ± 0.05	---
Saline + Hypox (N = 6)	0.60 ± 0.02[b]	84
Isoproterenol Sham (N = 6)	1.08 ± 0.02[c]	152
Isoproterenol + Hypox (N = 6)	0.69 ± 0.01	97

[a]Data are expressed as the mean ± S.E.M.

[b]Data differ from control ($p < 0.05$).

[c]Data differ from control ($p < 0.01$).

The percentage increase in mass over saline-treated controls of heart dry weight to body weight ratios were assessed after treatment for 5 days with isoproterenol (0.5 mg/kg/day, s.c.).

These findings are supported not only in heart growth but appear to be a consistent generality in both growth and proliferative responses. Hypophysectomized/partially hepatectomized animals were markedly delayed in the proliferative index of liver compared to control (Weinbren, 1959). Hypophysectomy also abrogated the excursion of ODC at 4 h post partial hepatectomy; a normal ODC response could be completely reestablished with pretreatment of exogenous growth hormone and thyroxine (Russell and Snyder, 1969). Both in the liver and heart, thyroxine and growth hormone were felt to be necessary for growth; it is plausible that thyroxine restored the metabolic rate to normal and that the presence of growth hormone was necessary for development of an anabolic state.

Adrenal Medullary Effects on Models of Myocardial Hypertrophy

These hypophysectomy studies implied that the lack of ACTH and resulting lack of adrenal glucocorticoids did not prevent the growth of the heart and liver. Therefore, studies of the role of adrenal medullary catecholamines in heart growth were conducted in the dog.

We tested the role of the adrenal medulla in cardiac hypertrophy produced by increased left ventricular pressure overload in response to coarctation of the aorta (Womble et al., 1980; Larson et al., 1982b). We measured serial plasma epinephrine, norepinephrine and dopamine in parallel with the development of canine myocardial hypertrophy induced by inflation of an intraluminal balloon. The endogenous plasma catecholamine which increased concomitant with ventricular hypertrophy was epinephrine (Table 4). Circulating

Table 4: Left Ventricular and Septal Dry Weight to Body Weight Ratios and Corresponding Plasma Catecholamine Concentration in Dogs

Group (N)[a]	LV+S/Body Wt (g/kg)	Epinephrine (pg/ml)	Norepinephrine (pg/ml)
Control (10)	845 ± 29	110 ± 7	325 ± 52
Coarctation (10)	1032 ± 26[b]	336 ± 51[c]	343 ± 84
Propranolol (5)	881 ± 24	42 ± 5[c]	210 ± 82
Coarctation plus propranolol (10)	882 ± 22	135 ± 16[b]	172 ± 106
Adrenal medulla denervation (6)	792 ± 22	21 ± 1[c]	283 ± 101
Coarctation plus adrenal medulla denervation (6)	770 ± 15[c]	29 ± 9[c]	324 ± 00

[a]Each plasma value represents a minimum of 10 samples in duplicate. Data are expressed as the mean ± S.E.M.

[b]Data differ from control ($p < 0.05$).

[c]Data differ from control ($p < 0.01$).

A model of coarctation of the aorta was produced by an intraluminal aortic balloon chronically inflated to produce a 50% increase in left ventricular afterload for a period of 7 days. Propranolol (240 mg, p.o., BID) was initiated 3 days prior to inflation of the intraluminal balloon. Adrenal medulla denervation was accomplished through surgical sectioning of the splanich nerves. (from Larson et al., 1982b).

norepinephrine and dopamine levels were not different from the time- and weight-matched controls. The dogs treated with the nonspecific β-adrenoceptor antagonist propranolol failed to exhibit ventricular hypertrophy; plasma epinephrine levels were attenuated. Therefore, epinephrine via the β-receptor was implicated as being responsible for signaling the onset of macromolecular synthesis resulting in ventricular hypertrophy.

Having demonstrated that an increase in plasma epinephrine concentration was linked to cardiac enlargement, subsequent studies were designed to test the dog cardiac hypertrophy model after ablation of the major source of circulating epinephrine. Since the adrenal medulla is responsible for the majority of plasma epinephrine, interruption of cholinergic innervation to the medulla should result in decreased plasma levels. In addition, if epinephrine were directly responsible for ventricular hypertrophy, its elimination should alter the hypertrophic process. Adrenal medulla denervation in dogs prevented the hemodynamic overload-induced left ventricular hypertrophy with heart weights in both the denervation and denervation plus constriction groups 91-93% of control. Concurrent with the failure of constriction to induce hypertrophy after adrenal denervation, plasma epinephrine levels were essentially eliminated, whereas plasma norepinephrine concentrations remained in the same range as control. This suggested a specific adrenal medullary relation to cardiac hypertrophy in the dog model and supported the hypothesis that humoral epinephrine regulated cardiac enlargement.

Effects of Renin and Angiotensin on Cardiac Hypertrophy

In this coarctation dog model, renal perfusion pressure was reduced by 50% and, even though not measured, the plasma renin concentrations would be expected to be elevated. The renin-angiotensin humoral system has not been demonstrated to have a direct trophic role in any tissue; this is further supported in studies of heart. However, hypertensive rats treated with angiotensin converting enzyme inhibitors had a significant reduction in myocardial mass as well as in arterial blood pressure (Sen et al., 1980; Kentera et al., 1981). The administration of propranolol to rats with renal hypertension significantly lowered ventricular hypertrophy without an associated decrease in blood pressure or serum renin concentration (Fernandes et al., 1976). Additionally, in dogs after inferior vena caval constriction where the plasma renin concentration was demonstrated to increase from 4- to 8-fold above control, chronic treatment with propranolol did not alter plasma renin concentrations (Hanson et al., 1976). These findings demonstrate that renin may modulate arterial blood pressure but that myocardial mass was modulated by other substance(s) which were subject to β-adrenergic blockade.

Models of Thyrotoxic Cardiac Hypertrophy

As demonstrated by Beznak (1967) and Russell (1969), an euthyroid state appears necessary for compensatory or regenerative growth. As hormonal means to produce elevations in myocardial mass, thyrotoxic models have been used in rats (Bartosova et al., 1969; Beznak et al., 1969) and guinea pigs (Goodkin et al., 1974). The thyrotoxic model

in dogs is not very consistent in the development of hypertrophy in animals receiving identical dosages (1 mg/kg/day). No heart growth was seen compared to control in a report by Taylor et al. (1969), whereas in two other studies, significant hypertrophy was detected (Piatnik and Olson, 1961; Ito et al., 1981). In the human thyrotoxic state, cardiac hypertrophy was not seen unless there was accompanying cardiac disease (Friedberg and Sohval, 1937; Sadler and Wilson, 1959). In the calf, an induced thyrotoxic state was not followed by ventricular hypertrophy, but the left ventricular performance indices and dimensions were increased markedly (Goldman et al., 1982). Pressure overloaded, hypophysectomized rats failed to show evidence of myocardial hypertrophy with replacement of thyroxine alone (Beznak, 1947). These findings support the view that the pattern of hypertrophy in a thyrotoxic state is species-specific, dependent both upon other hormones and on the hemodynamic load produced by a thyrotoxic state.

Hormonal Modulation of β-Adrenergic Receptors

To understand the evolution of the hypertrophied state in thyrotoxic rat and dog models, β-receptor number and affinity were quantitated. Hyperthyroidism in the rat caused an increased affinity and number of β-adrenergic receptors as characterized by [^{3}H]dihydroalprenolol binding, and hypothyroidism produced only a decrease in β-receptor number (Stiles and Lefkowitz, 1981; Zitnik and Roth, 1981; Atkins et al., 1983). Heart mass increased in parallel to β-receptor number in thyrotoxic dogs, but chronic ventricular volume overload produced by a veno-arterial fistula caused significant hypertrophy

without an increased β-adrenergic receptor level (Ito et al., 1981). Inconsistencies seen in the thyrotoxic models make it very difficult to draw conclusions concerning this hormone's role in the regulation of myocardial mass, but one fact is most evident; that is, in animal species where thyrotoxicosis produced an hypertrophied state, there was a consistent increase in the number of β-adrenergic receptors. There is a possibility that the increased number of β-adrenergic receptors in response to a thyrotoxic state in these models may be related to a hypertrophied myocardium through the increased β-receptor number with normal levels of circulating epinephrine.

Other hormones have been shown to affect the adrenergic system; glucocorticoids have relatively specific effects on the β-adrenergic receptors through their ability to increase adenylate cyclase activity in response to epinephrine stimulation and to increase cyclic AMP by as much as 20-fold (Iizuka and Ohkawara, 1983). Also, a lack of glucocorticoids after adrenalectomy results in markedly increased clearance of [^{3}H]epinephrine from the heart (Parvez and Parvez, 1974) which most likely is mediated through enhanced activity of catecholamine-O-methyl transferase (COMT) and monoamine oxidase (MAO), comparable to the rate of reduced [^{3}H]epinephrine accumulation in the heart of the hypophysectomized rat (Landsberg and Axelrod, 1968a, 1968b). Parvez and Parvez (1974) demonstrated that adrenal cortical steroids were coupled to epinephrine accumulation, and that replacement of adrenal glucocorticoids following adrenalectomy significantly increased the accumulation time of [^{3}H]epinephrine. Glucocorticoids also are known to be intimately involved in catecholamine biosynthesis, specifically in the induction

of phenylethanolamine N-methyltransferase, the enzyme involved in the methylation of norepinephrine and the final step in epinephrine synthesis (Wurtman and Axelrod, 1965, 1966). These studies suggest that elevated glucocorticoid concentrations may cause accelerated synthesis of epinephrine, a reduction in the degradation of epinephrine, and an enhanced response by the myocardial β-receptor to epinephrine.

Catecholamines and Cardiac Hypertrophy

Sympathetic nerves innervating the heart have been suggested as causative in the induction of compensatory cardiac hypertrophy. The neurotransmitter of the sympathetic nerves, norepinephrine, has been postulated to be the "myocardial hypertrophy hormone" by Laks et al. (1973) who noted cardiac hypertrophy after infusion of subhypertensive doses of norepinephrine into the right atrium via the right jugular vein of dogs for 6 to 63 weeks. However, the study was not definitive since they did not measure the circulating catecholamine concentrations, they did only two sham-operated dogs, and the degree of hypertrophy was low (111% of control), which could be accounted for solely by psychological stress to the animals.

In models of exercise compensatory hypertrophy, sympathetic nerves via norepinephrine have been felt to play a direct trophic role. Evidence for this conclusion was obtained after peripheral sympathectomy of rats, which destroyed the sympathetic nervous system without affecting the central nervous system or the adrenal medulla and which prevented exercise-induced hypertrophy (Östman-Smith, 1976, 1979). Conversely, cardiac hypertrophy produced by deoxycorticosterone-induced

hypertension was not prevented by 6-hydroxydopamine treatment (Cohen, 1974). Sympathectomy did not prevent cardiac hypertrophy in spontaneously hypertensive rats (Cutilleta et al., 1977). Regular low doses of the β-adrenergic agonist, isoproterenol, caused generalized cardiac hypertrophy (Cohen, 1974; Stanton et al., 1969), and the isoproterenol treatment also produced cardiac hypertrophy in chemically sympathectomized rats (Östman-Smith, 1979). These later studies suggested that compensatory growth of the heart is mediated by the myocardial β-adrenergic receptor following increased pressure load stress to the heart, and leave open the possibility of adrenal medulla hormone(s) mediation of cardiac hypertrophy.

The ventricular myocardium possesses α_1-, β_1-, and β_2-adrenoceptors (Rabinowitz et al., 1975; Minneman et al., 1979; Stiles et al., 1983) and infusion of agonists possessing α and/or β characteristics produced a marked excursion of myocardial ODC (Warnica et al., 1975; Johnson et al., 1983). It is well established that trophic hormones induce ODC in physiological growth systems to produce increased RNA and protein synthesis (Russell, 1983). Norepinephrine as a single agent has been shown in vivo to cause a 200% increase in cardiac ODC activity (Johnson, 1983). As discussed previously, propranolol has been demonstrated to inhibit myocardial growth through β-adrenergic blockade without reducing the pressure overload of the heart (Fernandes et al., 1976). It follows that if norepinephrine were acting directly on the myocardial β-receptors to induce cardiac ODC, then propranolol should block the norepinephrine induction of ODC. Johnson et al. (1983)

demonstrated that propranolol does not inhibit the ability of norepinephrine to induce ODC. Therefore, norepinephrine may have caused an increased arterial vascular resistance (not measured by Johnson, 1983) which may in turn have caused the induction of myocardial ODC through a release of the myocardial trohpic hormone(s), possibly epinephrine, from the adrenal medulla.

There is further, albeit indirect, evidence of adrenal medullary hormone(s) in the mediation of cardiac hypertrophy. The concept of junctional, preferentially norepinephrine-sensitive β_1-adrenergic receptors and extrajunctional preferentially epinephrine-sensitive β_2-adrenergic receptors is gradually being substantiated in the literature (Baker et al., 1980). Ariëns (1981) postulated that β_1- and α_1-receptors are postsynaptic junctional receptors for the neurotransmitter norepinephrine release at the sympathetic nerve endings, and the β_2- and α_2-receptors are extrajunctional receptors particularly sensitive to epinephrine. This implies that α_1- and β_1-receptors are neuronal receptors mediating function, and α_2- and β_2-receptors are adrenergic hormonal receptors modulating metabolism. There is good evidence that α_2-adrenergic receptors also are presynaptic and act to inhibit norepinephrine release whereas β_2-receptors also are presynaptic and "facilitate" norepinephrine release (Rand et al., 1980). This circumstantial evidence suggests that β_1-receptors respond to norepinephrine as a neurotransmitter and that β_2-receptors more likely would be the trophic receptors responding to the hormone epinephrine.

The β_2-adrenoceptor has been shown to be coupled to growth in numerous tissues as reviewed in detail by Womble and Russell (1983). We have demonstrated that myocardial β-receptors may be coupled to myocardial growth prior to heart rate in fetal heart (Haddox et al., 1981), and myocardial β_2-receptors are coupled to cardiac hypertrophy in both fetal and adult animals (Copeland et al., 1982; Womble and Russell, 1983).

In these studies, cardiac mass increased to 150% of control following chronic treatment with the β_2-specific agonist terbutaline in mice, rats and dogs. At a biochemical level using ODC as a marker, the trophic effects of terbutaline were totally blocked by antagonists possessing β_2 properties. The implication of these studies was that β_2-receptors may be coupled to a trophic response not only in heart but also in other tissues possessing β_2-receptors. There appears to be a direct link between β_2-specific catecholamine agonists and increased RNA, DNA and protein synthesis in lymphocytes (Whitfield, 1980), parotid gland, kidney and duodenum (MacManus et al., 1971). Since epinephrine is the only endogenous catecholamine possessing β_2-agonist properties, this suggests that epinephrine may have a generalized trophic effect in a variety of tissues during times of increased release from the adrenal medulla.

Circulating plasma catecholamines are known to be markedly increased during stress conditions (Kvetňanský, 1973; Matlina, 1976; Mikulaj et al., 1976; Petrovíc et al., 1976; Kopin et al., 1978; Womble et al., 1978, 1980; Larson et al., 1982b; Adams and Thist, 1983; Brisson et al., 1983; Dulac et al., 1983). In humans, prolonged

exercise stress, known to cause cardiac hypertrophy, caused a 4-fold increase in circulating epinephrine and norepinephrine concentrations plus a 4-fold increase in serum cortisol and prolactin concentrations whereas insulin and growth hormone remained unchanged (Brisson et al., 1983; Dulac et al., 1983). In terms of sustained stress, the adrenal secretory patterns changed and glucocorticoid release gradually declined within 24 h; however, epinephrine levels remained significantly elevated throughout the 10-day study (Petrovíc et al., 1976). There appears to be a close association between physical stress and hemodynamic stress since it has been repeatedly observed in benign essential hypertension (Thomas and Marks, 1978; Nezu et al., 1983) and during heart failure states in humans that the circulating catecholamine concentrations are markedly elevated (Franco-Morselli et al., 1977).

In addition, we have described in dog models of hemodynamic overload stress that circualting epinephrine concentrations remain significantly elevated during the 5-day studies (Womble et al., 1978, 1980) and that epinephrine concentrations significnatly correlated with the dry heart weight to body weight ratios (Larson et al., 1982b). Thus, there is substantial evidence that epinephrine may be a trophic hormone for the heart both in terms of its known release during conditions where heart growth is understood to occur and it is the only endogenous hormone possessing potent β_2-adrenergic agonist properties.

Epinephrine probably does not work as a single agent in adaptive cardiac hypertrophy. As illustrated by Brisson et al. (1983) and Dulac et al. (1983), there are at least four circulating hormones which

increase in concentration in response to stress. In other growth systems where hormonal concentrations are tightly regulated, there is a need for tissue exposure to a precise sequence of hormones to elicit growth and differentiation in vitro (Oka et al., 1982). If a hormone is exposed to the target tissue at an inappropriate time of cell cycle, a trophic hormone may become inhibitory based on studies from Radley and Hodgson (1971, 1973). Thus, the hormonal regulation of adaptive cardiac hypertrophy could be argued to be a result of a "hormonal cascade" following a hemodynamic overload stress.

In summary, there appears to be much circumstantial evidence to support the hypothesis that adrenal hormones are the direct physiological link initiating cardiac hypertrophy. More direct evidence will be presented by answering the following questions:

(1) In models of right ventricular pressure or volume overload where perfusion of the endocrine organs is unaltered, does the extent of cardiac hypertrophy correlate to circulating plasma catecholamine concentrations?

(2) If a circulating hormone(s) regulates myocardial mass, do both ventricles hypertrophy in response to a selective hemodynamic stress to one ventricle?

(3) Can an essentially nonworking denervated heart be caused to hypertrophy in parallel to a working innervated heart by altering either β-receptor number or circulating catecholamine concentration?

(4) Will endogenous hormone(s) release after hemodynamic stress cause a nonworking denervated heart to hypertrophy, and is there a temporal relationship to hemodynamic stress and myocardial growth?

CHAPTER 3

RIGHT VENTRICULAR HYPERTROPHY IN DOGS

This study was designed to test the generality of increased circulating plasma epinephrine as a result of pressure or volume overload models of right ventricular hypertrophy. Models were selected to gradually produce increased pressure or acutely produce increased volume overload to the right ventricles without altering the renal perfusion or arterial pressure to the carotid baroreceptors. The catecholamine concentrations were correlated to the extent of right and left ventricular hypertrophy and to the hemodynamic parameters. Myocardial mass was characterized by the ventricular dry weight to body weight ratios, and ventricular wall dimensions were expressed as the planimetered longitudinal area to body weight ratios. The findings of these studies suggested that increased hemodynamic overload to the right ventricle induced compensatory cardiac hypertrophy mediated hormonally by increased plasma epinephrine and not directly by the hemodynamic indices.

Methods

A total of 57 adult dogs weighing 15 ± 1.3 kg, S.E.M., were preconditioned to the kennel environment while being maintained on an 0700-1900 h photoperiod and fed standard dog chow and water ad libitum. A Cordis Introducer Catheter (7 Fr., #501-608, Cordis Corp., Miami, FL)

was permanently implanted in the external jugular vein of each dog after pentobarbital sodium anesthesia (30 mg/kg, i.v., Harvey Laboratories, Philadelphia, PA). Three days after catheter implantation, a Swan-Ganz thermodilution catheter (8 Fr., #93-132-8F, Edwards Laboratories, Santa Ana, CA) was inserted aseptically through the Cordis introducer to a distance which positioned the distal catheter tip in the pulmonary artery. Hemodynamic parameters measured with the Swan-Ganz catheter included: cardiac output, heart rate, right atrial, right ventricular, and pulmonary artery pressures. Cardiac outputs were determined utilizing the Edwards cardiac output computer (9520A, American Hospital Supply Corp., Santa Ana, CA). Hemodynamic measurements and blood samples for catecholamine, Na^+, K, Cl^-, CO_2, BUN, creatinine, glucose, T_4, T_3 and cortisol analyses were obtained at 1600 h on days zero and 7 for one group and zero, 15 and 30 for a second group. Studies were conducted in a dimly illuminated, quiet room with the dogs standing in a Pavlov harness. Catecholamine blood samples were drawn from the Swan-Ganz distal lumen located in the pulmonary artery in evacuated tubes containing 20 µl of a solution of EDTA (90 mg/ml) and glutatione (60 mg/ml), pH 6.0-7.4, and maintained at 4°C. At the completion of the 7- or 30-day study period, animals were sacrificed by i.v. pentobarbital sodium, and the hearts removed and dissected free of atria, valves and great vessels. Ventricular volumes were determined by volume of saline held in by the ventricles. The right and left ventricles were dissected from the septum, and the wet tissue weights were obtained (Fig. 1). In anatomical terms, the

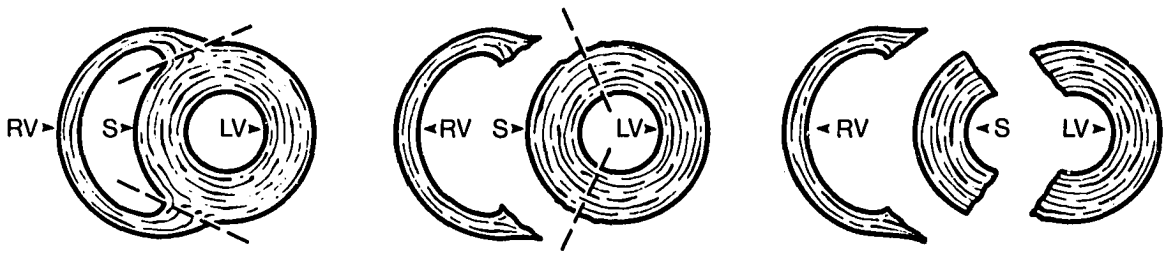


Figure 1: Dissection of Heart Tissue. Through a left thoracotomy the heart was excised and trimmed of the great vessels. The heart was further trimmed of atrial tissue and valvular tissue prior to measurement of the ventricular volumes with saline. Hearts were halved on a longitudinal-coronal axis through the midportion of the right ventricular free wall, ventricular septum and left ventricular free wall. A blot of the myocardial wall thickness was made, and the heart was further dissected as diagrammed. Wet heart component weights were measured prior to drying in a vacuum oven at 60°F for 48 h for measurement of dry weights.

ventricular wall thickness varies greatly from base to apex. To determine changes in wall thickness as a result of the hemodynamic overload, longitudinal wall thickness areas were determined by sectioning each ventricular wall in a coronal plane and defining thickness as mm^2 per kg body weight by the planimetered area of the sectioned ventricular impression using a computer digitizing system. Each tissue was dried for 48 h at 60°C and -20 psi and the myocardial mass was characterized by ventricular dry tissue weight to body weight ratios and expressed as mg/kg. The body surface area was determined as described by Smith (1956) for normalization of hemodynamic data.

Radioenzymatic Assay of Catecholamines

The Upjohn Cat-A-Kit® (Upjohn, Kalamazoo, MI) was used for the determination of circulating plasma catecholamine concentrations in dogs. The kit is based on methods described by Passon and Peuler (1975) where a single isotope assay measured catecholamines with less than one ml of plasma. This method utilizes the enzyme catechol-O-methyl-transferase (COMT) to catalyze the transfer of a [^{3}H]methyl group from S-adenosyl-L-methionine-[^{3}H-methyl] (^{3}H-SAM^3) to epinephrine and norepinephrine. Isolation of the resulting products, [^{3}H]metanephrine, [^{3}H]normetanephrine and [^{3}H]-3-methoxytryramine was accomplished by thin-layer chromatography with a solvent of tertiary amyl alcohol/toluene/methylamine, 6:2:3 (v/v/v). Each labeled derivative was converted by periodate oxidation to [^{3}H]vanillin and then extracted. The radioactivity was determined by scintillation counting and was proportionate to the amounts of norepinephrine or epinephrine in each

50-µl sample. Assay sensitivity was in the range of 2-5 picograms for epinephrine and norepinephrine and 15-20 picograms for dopamine.

Experimental Groups

Fifteen dogs were subjected to right ventricular pressure overload induced by daily x 7, i.v. injections of silica particles (diatomaceous earth, grade 1, Sigma Chemical Co., St. Louis, MO) in a concentration of 12.5 mg/kg in 50 ml 0.9% NaCl with 2 units/ml sodium heparin (Upjohn Co., Kalamazoo, MI). The silica suspension was delivered through a sidearm injection tube on the Cordis Introducer Catheter. Five silica-treated dogs also were given propranolol hydrochloride, 15 mg/kg/day orally (Ayerst Laboratories, New York, NY). For a 7-day study in a group of 10 dogs, volume overload of the right ventricle was accomplished by surgically implanting a right ventricular outflow tract to right atrial shunt graft (16 mm ID by 8 cm; Microvel Double Velour #081608, Meadox Medical, Oakland, NJ). Quantitation of shunt flow to cardiac output ratio was measured in the open chest dog on days zero and 30 with a Statham Electromagnetic Flow Meter, Model 2204 (Statham Instruments, Inc., Oxnard, CA). In the second 30-day study, pressure overload was induced by injecting the plant pyrrolizidine alkaloid, monocrotaline, into 6 dogs in 3 i.p. doses of 10 mg/kg on consecutive days. Monocrotaline has been described in rodents to cause progressive pulmonary arterial hypertension secondary to medial hyperplasia of the pulmonary arteries and resulting right ventricular hypertrophy (Huxtable et al., 1977, 1978; Ghodsi and Will, 1981).

Results

Right and Left Ventricular Hypertrophy in Response to Selective Right Ventricular Overload

The models of right ventricular volume or pressure overload studied led to essentially similar results (Table 5). These models produced significant increases in right ventricular weight to body weight ratios ($p < 0.001$) in addition to increased left ventricular weight to body weight ratios ($p < 0.05$) compared to the respective control group. The ventricular septum tended to increase in response to the overload state but with less significance. Increased ventricular weight was not due to water accumulation, as the water content of ventricles in the untreated and treated groups was not different (77.1% vs. 77.8%, $p <$ ns). Propranolol administration caused an inhibition of significant hypertrophy in response to pressure overload.

Effects of Volume or Pressure Overload on Plasma Catecholamine Levels

In unsedated dogs, a marked elevation of plasma epinephrine concentration was detected throughout the volume or pressure overload study period (Table 6). Plasma epinephrine concentration was consistently elevated by 3-fold compared to control in the silica and shunt groups at 15 and 30 days following overload, but norepinephrine displayed significant changes only after 30 days of volume overload. Propranolol attenuated the release of epinephrine from pressure overloaded, silica-treated dogs. Monocrotaline progressively increased pulmonary vascular resistance to a maximal effect at 15 to 20 days, and plasma epinephrine concentrations increased coincident with the progressive increase in the pulmonary vascular resistance.

Table 5: Ventricular Dry Weight to Body Weight Ratios Following Right Ventricular Pressure or Volume Overload in the Dog

7-Day Study	Right Ventricle	Septum	Left Ventricle	Total
Control (N = 20)	296 ± 9	265 ± 6	582 ± 13	1143 ± 24
Silica (N = 10)	381 ± 15[a]	317 ± 21[b]	646 ± 33[b]	1343 ± 61[a]
Silica plus Propranolol (N = 5)	341 ± 15	285 ± 44	631 ± 16	1257 ± 18
30-Day Study				
Control (N = 6)	310 ± 10	263 ± 9	578 ± 29	1151 ± 33
Monocrotaline (N = 6)	416 ± 16[a]	306 ± 17	684 ± 28[c]	1406 ± 45[a]
Shunt (N = 9)	398 ± 12[a]	311 ± 14[c]	715 ± 39[b]	1424 ± 53[a]

Dry weight/body weight (mg/kg) ratio, mean ± S.E.M.

[a]Data differ from controls ($p < 0.001$)

[b]Data differ from controls ($p < 0.01$)

[c]Data differ from controls ($p < 0.05$)

Table 6: Plasma Epinephrine and Norepinephrine Concentrations following Ventricular Pressure or Volume Overload in the Dog

	Epinephrine (pg/ml)	Norepinephrine (pg/ml)
Day 0		
Baseline (N = 23)	104 ± 16	246 ± 66
Day 7		
Control (N = 6)	102 ± 6	182 ± 14
Silica (N = 5)	301 ± 57[a]	332 ± 83
Silica plus Propranolol (N = 5)	117 ± 28	301 ± 108
Day 15		
Control (N = 5)	139 ± 18	292 ± 69
Monocrotaline (N = 5)	159 ± 29	179 ± 42
Shunt (N = 5)	321 ± 48[b]	279 ± 52
Day 30		
Control (N = 6)	85 ± 35	230 ± 46
Monocrotaline (N = 6)	343 ± 51[a]	365 ± 85
Shunt (N = 10)	476 ± 83[a]	494 ± 56[a]

Data are expressed as the mean ± S.E.M.

[a]Data differ from controls ($p < 0.001$)

[b]Data differ from controls ($p < 0.01$)

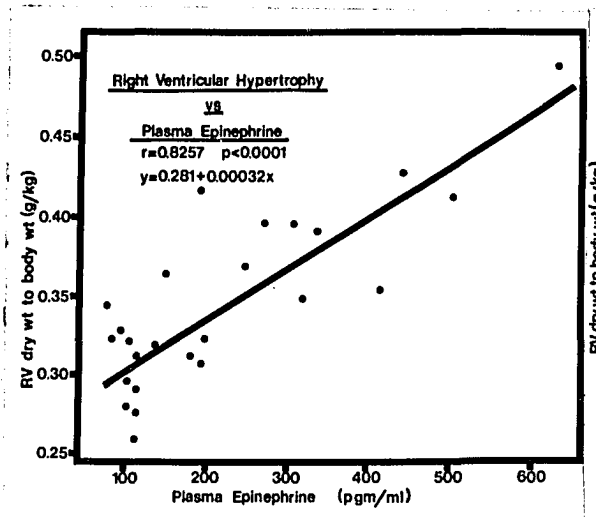
Correlations between Plasma Catecholamine Concentrations and Ventricular Mass

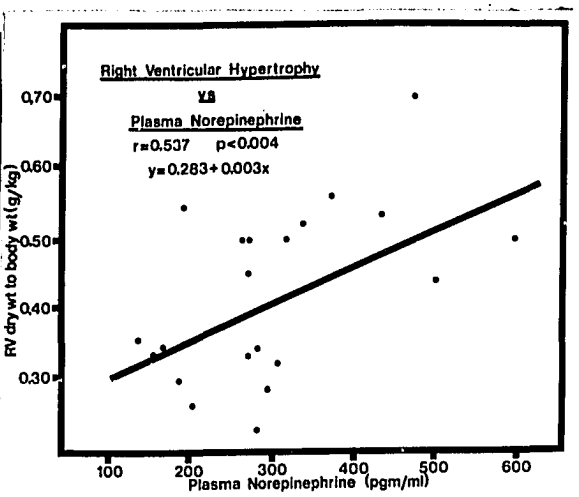
Following volume or pressure overload to the right ventricle, there was a significant positive correlation between plasma epinephrine concentration and the right and left ventricular mass ratios (Fig. 2). Plasma norepinephrine concentrations were not as predictive of the ventricular mass ratios as epinephrine concentrations. Multiple linear regression analysis comparing ventricular mass ratios to epinephrine with norepinephrine provided evidence that the predominant trophic effect was contributed by epinephrine (Table 7).

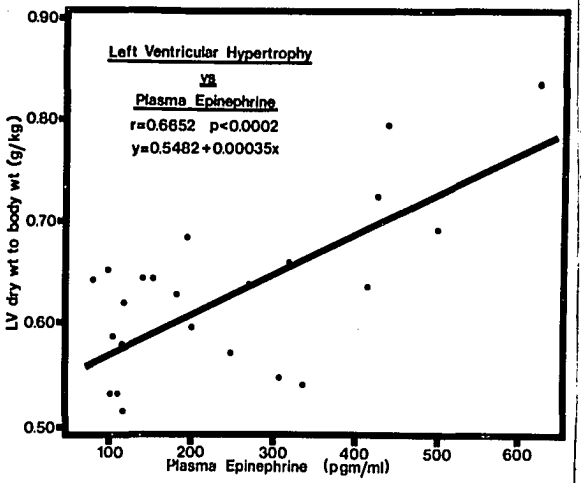
Measurement of Hemodynamic Parameters during Right Ventricular Overload

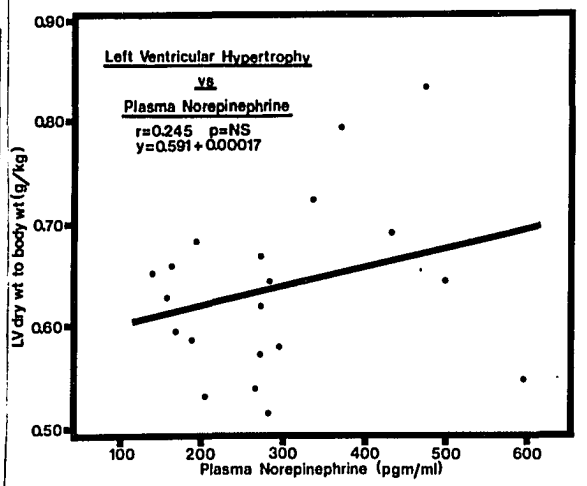
Hemodynamic parameters measured at the time of blood collection are shown in Table 8. Hemodynamic measurements obtained in each dog model as well as calculated right ventricular work indices are shown in Table 9. There was no consistent correlation among groups with right ventricular stroke work index (RVSWI), pulmonary vascular resistance (PVR), and cardiac index (CI) or stroke volume index (SVI), and either total dry heart weight ratios or RV dry weight ratios or plasma epinephrine concentrations. However, the right ventricular diastolic pressure (RVd) correlated closely with elevated plasma epinephrine ($r = 0.8502$; $p < 0.001$) and the total ventricular dry weight ratios ($r = 0.9057$; $p < 0.001$). Notably, monocrotaline treatment failed to produce increased PVR in dogs, yet RVd and circulating epinephrine levels were significantly elevated. Dogs in the graft-shunt group demonstrated an increased RVd to 550% of control while RVSWI increased to only 140% of control. Silica

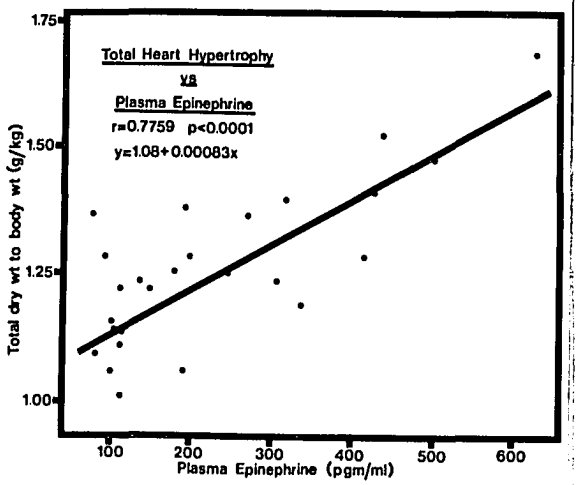
Figure 2: Correlations between ventricular dry weight to body weight ratios and concentrations of epinephrine and norepinephrine in plasma in 30-day study dogs.


Right Ventricular Hypertrophy
vs
Plasma Epinephrine
r=0.8257 p<0.0001
y=0.281+0.00032x
RV dry wt to body wt (g/kg)
Plasma Epinephrine (pgm/ml)


Right Ventricular Hypertrophy
vs
Plasma Norepinephrine
r=0.537 p<0.004
y=0.283+0.003x
RV dry wt to body wt (g/kg)
Plasma Norepinephrine (pgm/ml)


Left Ventricular Hypertrophy
vs
Plasma Epinephrine
r=0.6652 p<0.0002
y=0.5482+0.00035x
LV dry wt to body wt (g/kg)
Plasma Epinephrine (pgm/ml)


Left Ventricular Hypertrophy
vs
Plasma Norepinephrine
r=0.245 p=NS
y=0.591+0.00017
LV dry wt to body wt (g/kg)
Plasma Norepinephrine (pgm/ml)


Total Heart Hypertrophy
vs
Plasma Epinephrine
r=0.7759 p<0.0001
y=1.08+0.00083x
Total dry wt to body wt (g/kg)
Plasma Epinephrine (pgm/ml)

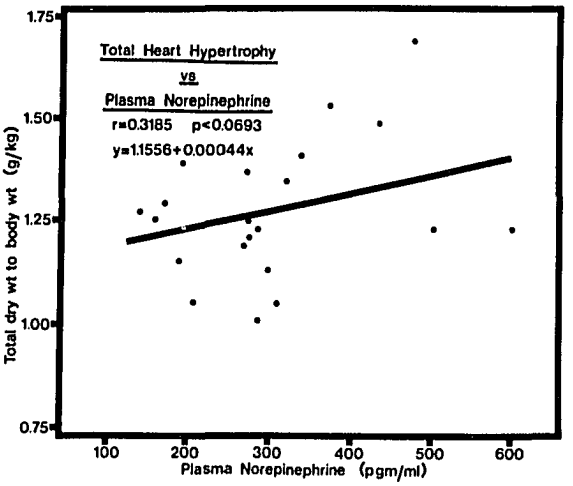

Total Heart Hypertrophy
vs
Plasma Norepinephrine
r=0.3185 p<0.0693
y=1.1556+0.00044x
Total dry wt to body wt (g/kg)
Plasma Norepinephrine (pgm/ml)

Table 7: Multiple Linear Regression of Ventricular Dry Weight to Body Weight Ratios and Plasma Catecholamine Concentrations (pg/ml) during Right Ventricular Overload

	r Value	p Value
Right Ventricle vs. Epinephrine and Norepinephrine	r = 0.511	p < 0.001
Left Ventricle vs. Epinephrine and Norepinephrine	r = 0.505	p < 0.001
Total vs. Epinephrine and Norepinephrine	r = 0.555	p < 0.0001

Blood Electrolytes and Hormone Concentrations

	Na	K	CL	CO_2	BUN	Cr	GLU	T_4	T_3	Cortisol
Control (n = 3)	146 ± 2	4.5 ± 0.3	114 ± 3	22 ± 2	15 ± 2	0.35 ± 0.13	111 ± 9	2.7 ± 0.4	54 ± 8	2.9 ± 1.0
Shunt (n = 3)	145 ± 5	3.9 ± 0.3	111 ± 5	25 ± 1	21 ± 2	0.85 ± 0.12	115 ± 6	1.7 ± 0.3	74 ± 19	13.4 ± 2.1
Monocrotaline (n = 3)	141 ± 5	4.2 ± 0.2	113 ± 2	19 ± 1	12 ± 2	0.68 ± 0.10	93 ± 2	1.0 ± 0.2	36 ± 23	—

Values expressed mean ± SD

Table 8: Blood Electrolytes and Hormone Concentrations

Na = serum sodium

K = serum potassium

CL = serum chloride

CO_2 = serum carbon dioxide

BUN = serum blood urea nitrogen

Cr = serum creatinine

GLU = serum glucose

T_4 = serum thyroxine

T_3 = serum triiodothyronine

Cortisol = serum cortisol

Table 9: Comparison of Right Ventricular Hemodynamic Parameters in a 7-day Study and a 30-day Study in Dog Models of Right Ventricular Pressure or Volume Overload

[a]HR	Heart rate (beats/min)
Temp	Temperature, pulmonary artery (°C)
PAs	Pulmonary artery systolic (mmHg)
PAd	Pulmonary artery diastolic (mmHg)
PAx	Pulmonary artery mean (mmHg)
PAW	Pulmonary artery Wedge (mmHg)
RVs	Right ventricular systolic (mmHg)
RVd	Right ventricular diastolic (mmHg)
RVx	Right ventricular mean (mmHg)
CO	Cardiac output (liters/min)
PVR	Pulmonary vascular resistance (dyne·sec·cm^{-5})
SV	Stroke volume (ml/beat)
PVSW	Right ventricular stroke work (gm·m)
BSA	Body surface area (M^2)
CI	Cardiac Index (liters/min/M^2)
PVRI	Pulmonary vascular resistance index (dyne·sec·cm^{-5}/M^2)
SI	Stroke Index (ml/beat/M^2)
RVSWI	Right ventricular stroke work index (gm·m/M^2)

[b]Data differ from controls ($p < 0.05$)

[c]Data differ from controls ($p < 0.01$)

[d]Data differ from controls ($p < 0.001$)

Comparison of Right Ventricular Hemodynamic Parameters in a 7 Day Study and a 30 Day Study in Dog Models of Right Ventricular Pressure or Volume Overload

Hemodynamic Parameter	7 Day Control n = 6	Silica n = 5	Silica Plus Propranolol n = 5	30 Day Control n = 6	Monocrotaline n = 6	Shunt n = 5
HR	110 ± 5	116 ± 2	81 ± 1	112 ± 3	112 ± 12	189 ± 6
Temp	38.9 ± 0.1	39.0 ± 0.3	40.2 ± 0.3[b]	38.8 ± 0.2[b]	38.6 ± 0.3	39.2 ± 0.2
PAs	24 ± 2	44 ± 6[d]	47 ± 4[d]	25 ± 2	24 ± 1	33 ± 2[c]
PAd	11 ± 1	20 ± 4[d]	20 ± 6[c]	10 ± 1	11 ± 2	10 ± 1
PAx	16 ± 1	29 ± 4[d]	31 ± 4[d]	15 ± 2	15 ± 1	17 ± 2
PAW	4 ± 1	4 ± 1	3 ± 2	3 ± 1	4 ± 1	2 ± 1
RVs	25 ± 4	46 ± 4[d]	48 ± 4[d]	26 ± 3	25 ± 2	35 ± 3[c]
RVd	1.5 ± 0.5	3.8 ± 0.7[c]	6.3 ± 0.9[d]	2.5 ± 0.5	6.8 ± 0.8[d]	8.3 ± 1.1[d]
RVx	10 ± 3	17 ± 2[c]	19 ± 3[d]	11 ± 2	13 ± 1	17 ± 2[c]
CO	3.6 ± 0.3	4.2 ± 0.5	5.1 ± 1.0[c]	3.3 ± 0.4	3.0 ± 0.7	5.1 ± 0.3[c]
PVR	251 ± 24	495 ± 82[c]	505 ± 155[b]	291 ± 31	293 ± 104	272 ± 19
SV	33 ± 2	37 ± 5	63 ± 11[d]	30 ± 3	28 ± 7	37 ± 4
RVSW	0.542 ± 0.58	1.286 ± 0.232[d]	2.245 ± 0.096[d]	0.566 ± 0.057	0.436 ± 0.127	0.772 ± 0.107[b]
BSA	0.81 ± 0.03	0.81 ± 0.05	0.89 ± 0.02	0.83 ± 0.04	0.83 ± 0.09	0.81 ± 0.02
CI	4.5 ± 0.3	5.3 ± 0.8	5.6 ± 1.0	4.1 ± 0.7	3.4 ± 0.5	6.2 ± 0.4[b]
PVRI	310 ± 30	611 ± 101[d]	567 ± 174[d]	351 ± 37	353 ± 125	335 ± 11
SI	40.3 ± 1.7	45.9 ± 6.5	69.4 ± 11.6[d]	42.2 ± 5.6	32.9 ± 7.6	45.7 ± 4.6
RVSWI	0.659 ± .060	1.625 ± 0.0356[d]	2.510 ± 0.044[d]	0.682 ± 0.060	0.510 ± 0.140	0.954 ± 0.133[c]

infusion, which resulted in right ventricular hypertrophy, also elevated the RVSWI to 250% of control. Conversely, propranolol treatment in the silica infusion group attenuated hypertrophy and plasma epinephrine levels while the RVSWI was elevated to 380% of control. In addition, the propranololol-treated dogs demonstrated RVd elevations to 420% of control pressure even though the extent of hypertrophy and epinephrine levels were attenuated. The heart rate was increased in the graft-shunt group, presumably due to increased catecholamine concentrations, and was diminished with silica plus propranolol treatment due to β-adrenergic receptor blockade.

Measurement of Concentrations of Blood Electrolytes and Hormones

Due to costs involved in the analysis of these parameters, a limited number of samples was analyzed. Most notably, creatinine and cortisol were increased following hemodynamic overload. The elevated creatinine concentrations may have been due to increased catecholamine concentrations and to decreased renal function in the face of normal or elevated cardiac outputs. The cortisol elevation is consistent with that noted in other models of stress and may be an important factor affecting epinephrine's trophic effect on the heart.

Ventricular Diastolic Volumes/Body Weight Ratios in Volume Overloaded Dogs

Table 10 illustrates that right ventricular volume in the shunt group increased to 200% of control ($p < 0.001$) and the left ventricular volume increased to 124% of control ($p < 0.05$). The volumes in the pressure overloaded monocrotaline group did not significantly increase.

Table 10: Ventricular Diastolic Volume in Volume or Pressure Overload in Dogs

	Right Ventricle	Left Ventricle
Control (N = 6)	1.50 ± 0.14	1.49 ± 0.16
Shunt (N = 5)	3.02 ± 0.27[a]	1.85 ± 0.17[b]
Monocrotaline (N = 5)	1.44 ± 0.22	1.49 ± 0.19

Volume/body weight (ml/kg) ratio, mean ± S.E.M.

[a]Data differ from control ($p < 0.001$)

[b]Data differ from control ($p < 0.05$)

Myocardial Area/Body Weight Ratios in Volume Overloaded Dogs

Measurement of myocardial area (Table 11) in response to right ventricular volume load demonstrated not only a significant increase in right ventricular area ($p < 0.001$) but also a highly significant increase in left ventricular wall area ($p < 0.001$). In the monocrotaline group, myocardial area increased to 127% of control, consistent with the measured increase in heart weight/body weight ratios.

Discussion

These data, together with the demonstrated ability to produce marked hypertrophy by administration of catecholamines with β-agonist properties, suggested that epinephrine is a major circulating hormone with β-receptor activity that regulates myocardial macromolecular synthesis, i.e., hypertrophy. The extent of cardiac hypertrophy in response to pressure or volume overload to the right ventricle of dogs was directly related to the increase in circulating epinephrine concentrations. Also, this study demonstrated that significant left ventricular hypertrophy occurs in models designed to selectively overload the right ventricle. This one essential finding possibly excludes neuronal or mechanical stretch as causative factors in adaptive hypertrophy and is an essential criterion supporting circulating trophic factors.

To add further substance to this interpretation, we found that in models of right ventricular hypertrophy, right ventricular hemodynamic indices, although statistically correlated with right ventricular hypertrophy, did not correlate at all with a lack of hypertrophy in

Table 11: Myocardial Area Dimensions to Body Weight Ratios following Right Ventricular Overload in 30-day Study Dogs

	Right Ventricle	Ventricular Septum	Left Ventricle	Total
Control (N = 6)	141 ± 21	260 ± 44	367 ± 39	767 ± 94
Shunt (N = 6)	272 ± 30[a]	307 ± 24	521 ± 42[a]	1100 ± 90[a]
Monocrotaline (N = 5)	179 ± 12[b]	241 ± 43	392 ± 26	801 ± 35

Myocardial area/body weight (mm^2/kg) ratio, mean ± S.E.M.

[a]Data differ from control ($p < 0.01$)

[b]Data differ from control ($p < 0.05$)

propranolol-treated dogs. These findings are similar to observations other have made demonstrating a lack of consistent correlation of hemodynamic parameters to cardiac mass (Sen et al., 1974, 1977).

Preliminary evidence from this study supports the concept that epinephrine may be one of several hormones acting as the intermediate link between hemodynamic stress and the chain of biochemical events associated with cardiac hypertrophy. We failed to find a dose association between norepinephrine and cardiac hypertrophy in these dog studies because circulating norepinephrine concentrations are a result of neuronal spillover due to facilitated norepinephrine synaptic release stimulated by epinephrine, sympathetic neurotransmission, and adrenomedullary secretion, but we certainly cannot imply that norepinephrine does not have a role in cardiac hypertrophy

The optimal sampling site for measurement of these circulating catecholamines was determined to be the pulmonary artery. The pulmonary circulation extracts circulating norepinephrine (Hughes et al., 1969; Nicholas et al., 1974) making pulmonary venous norepinephrine levels 75% lower than the pulmonary artery (Sole et al., 1979). Further, pulmonary sympathetic activity due to hemodynamic stress has been shown to alter pulmonary norepinephrine extraction and spillover (Blomberg and Heinzow, 1983), and pulmonary hypertension has been shown to decrease norepinephrine extractions significantly (Sole et al., 1979). Epinephrine concentrations are unaltered across the lung; therefore, the mixed venous sample taken from the pulmonary artery has the same epinephrine concentration as that measured in the aorta (Sole et al., 1979). Thus, mixed venous catecholamine samples were felt to

best reflect both sympathetic spillover of norepinephrine into the venous blood measured prior to variable extraction of the pulmonary circulation and epinephrine concentration which is unaffected by passage through the pulmonary circulation.

The results of these studies and others also suggest that the stress-mediated release of other adrenal hormones as well as prolactin may play a role in hormonal regulation of heart mass. Additional studies are clearly needed in the understanding of the role(s) of glucocorticoids and prolactin in cardiac hypertrophy.

Regarding thyroxine concentrations in these models of right ventricular hypertrophy, the results of plasma T_3 and T_4 were not sufficiently significant to relate these hormones as physiologic signals for cardiac hypertrophy. Thyroxine-induced cardiac hypertrophy probably can be considered a pathological, developmental hypertrophy rather than a true compensatory cardiac hypertrophy.

Models of silica injection pressure overload and pulmonary artery to right atrium vascular shunt volume overload were designed in this laboratory for selective right ventricular overload. The advantages of these models were that silica injections caused a slowly progressive mechanical obstruction in the pulmonary vasculature without any toxic effects, and the shunt permitted quantitation of shunt flow to cardiac output ratios. These models affected a single organ -- the heart -- without associated systemic effects as seen with many models of cardiac hypertrophy such as experimental hyperthyroidism (Beznak et al., 1969), aortic constriction (Beznak et al., 1954, 1969),

nephrogenic hypertension (Sen, 1980; Kentera et al., 1981; Fernandes et al., 1976), administration of exogenous catecholamines (Stanton et al., 1969; Byus et al., 1970; Gans and Cater, 1970), chronic ethanol administration (Rossi, 1980; Adams and Hist, 1983), and monocrotaline (Huxtable et al., 1977; Ghodsi and Will, 1981).

Monocrotaline intoxication has been associated with pulmonary arterial hypertension and right ventricular hypertrophy in rodents (Huxtable et al., 1977; Ghodsi and Will, 1981). This study presented data that failed to demonstrate increased pulmonary artery pressures or resistances but increased myocardial mass occurred with concomitant increased circulating plasma epinephrine concentrations in dogs. The only other study using a monocrotaline dog model for right ventricular hypertrophy used the metabolite of monocrotaline, dehydromonocrotaline, to induce toxic lung injury (Raczniak et al., 1979). These authors did not describe the reason the metabolite was used rather than the parent compound; it could be presumed that the parent compound does not produce structural damage to the dog lung. In the present study, the possibility cannot be ruled out that monocrotaline produced the myocardial toxicity as evidenced by markedly increased RVd pressures, reduced cardiac output, reduced pulmonary artery pressures, and increased myocardial mass. Also, Raczniak et al., (1979) dosed dogs with a single pulmonary injection of 3 mg/kg whereas we dosed the dog intraperitoneally with 3 doses of 10 mg/kg/day on 3 consecutive days. It is likely that the dosing schedule as well as the metabolite may explain differences between our study and that of Raczniak.

In conclusion, therefore, a consistent circulating catecholamine pattern was observed in these dogs which led to adaptive cardiac hypertrophy. Unexpectedly, significant left ventricular hypertrophy paralleled the development of right ventricular hypertrophy, which implies that hemodynamic parameters alone may not directly initiate the chain of biochemical events leading to cardiac hypertrophy. It is hypothesized that the activation of baro-, chemo- or stretch receptors of the heart or proximal arteries may signal, via afferent cardiac nerves and the central nervous system, the release of epinephrine from the adrenal medulla.

CHAPTER 4

CONCURRENT LEFT AND RIGHT VENTRICULAR HYPERTROPHY

Previous studies reported in this dissertation have demonstrated that a significant degree of left ventricular hypertrophy occurs in models of selective overload of the right ventricle. It is essential to characterize these findings further because of their implication that circulating hormonal factors regulate myocardial mass. In other words, a physiological stress condition evoking cardiac hypertrophy mediated by circulating hormone(s) should lead to biochemical events and changes in heart mass both in the right and left ventricle.

We studied, therefore, effects of prolonged volume or pressure overload on the right and left ventricular performance during right ventricular overload using methods of two-dimensional and M-Mode echocardiography. Importantly, we tested the generality of these findings by determining whether right ventricular hypertrophy occurs following left ventricular pressure overload, and we examined ODC activity in right and left ventricles following left ventricular pressure overload.

Methods

Surgical Technique for Two-dimensional and M-Mode Echocardiography

Three mongrel dogs weighing 15-18 kg were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and ventilated with a standard volume pump. A median sternotomy was performed and the pericardium opened. The main pulmonary artery was dissected to allow the placement

of a constricting pulmonary artery band. Right and left ventricular performance were studied following the implantation of high-fidelity pressure micromanometers (Milar, 5 FR) and ultrasound imaging studies were performed with an E for M/Honeywell system. A two-dimensional image of the left ventricular outflow tract was obtained by positioning the transducer over the left ventricular body aiming superiorly. As the pulmonary band was made more occlusive, pull back pressure gradients were documented across the pulmonary band, left ventricular outflow tract and imaged with two-dimensional and M-Mode echocardiography. Measurements were made with pulmonary artery gradients of 0, 20, 75, and 125 mmHg with duplicate measurements at each point.

Technique for Tracheal Tranplant

In an attempt to develop a prosthetic trachea, a consistent length of trachea (5 tracheal rings) was removed from 5 recipient dogs; a detergent-treated homograft of an equivalent length was sutured into its place. In the 5 controls, a nondetergent autograft was implanted. At 14 days posttransplantation, homograft tracheas became constrictive leading to stridor and upper tracheal obstruction. Five days after the first indication of stridor, catecholamine blood samples were drawn and the dogs were terminated by pentobarbital overdose. Dry ventricular weight to body weight ratios were determined as described previously.

Aortic or Pulmonary Artery Banding in Rats

Male Sprague-Dawley rats, 100-150 g, from the Division of Animal Resources (University of Arizona, Tucson) were housed in temperature- and light-controlled rooms and fed standard rodent chow

and water ad libitum. While being anesthetized with ethrane anesthesia and ventilated with a pressure-limited ventilator, a median sternotomy was performed to expose the aorta and the pulmonary artery. The main pulmonary artery was dissected free of the ascending aorta, and a pre-calibrated Weck® clip placed around either one of these great vessels. The sternum and tissues were closed and allowed to recover. Ten days after the banding procedure, rats were killed by cervical dislocation and hearts removed for weight determinations.

Ornithine Decarboxylase Activity following Aortic Banding

Male Sprague-Dawley rats, 100-150 g, (48 from the Division of Animal Resources, University of Arizona, Tucson) were housed in light- and temperature-controlled rooms and fed standard rodent chow and water ad libitum following aortic banding (described in the previous section). The rats were injected with isoproterenol (10 mg/kg, s.c.) at 0700 h on days zero through 7 post banding. ODC activity was assayed in the right ventricle, septum and left ventricle 4 h after isoproterenol.

Ornithine Decarboxylase Assay

Hearts were homogenized in 5 vol chilled 50 mM Na_2HPO_4-KH_2PO_4 buffer, pH 7.2, containing 60 μM pyridoxal phosphate, 1 mM dithiothreitol, and 100 μM phenylmethylsulfonylfluoride. The homogenates were centrifuged for 10 min at 48,000 x g. Fifty to 100 μl of the supernatant fraction was assayed for ODC activity according to the method of Russell and Snyder (1968). The assay was conducted in 15-ml tapered centrifuge tubes fitted with rubber stoppers and center wells (Kontes Company, Vineland, NJ) at a final volume of 200 μl. The buffer used

in the assay was the same as the homogenization buffer and included 1 μCi 1-[^{14}C]ornithine and sufficient unlabeled ornithine to adjust the assay concentration to 0.5 mM ornithine. The assay was stopped with 0.5 ml 1 M citric acid, and the $^{14}CO_2$ released was collected on Whatman 3 MM filter papers prespotted with 20 μl of 2 N NaOH. Filter papers were counted in toluene/Omnifluor scintillant. All enzyme activities were corrected for blanks which were stopped at time zero by addition of citric acid. Enzyme activity was linear with respect to incubation time and enzyme concentration and was expressed as pmol CO_2 evolved/min/mg protein. Protein concentration was assessed by the Bradford (1976) dyebinding technique.

Results

Concurrent Left and Right Ventricular Hypertrophy in Models of Right Ventricular Overload

As described in the previous chapter, significant hypertrophy of the left ventricle occurred concomitantly with hypertrophy of the right ventricle. In addition, the mass of each ventricle correlated significantly with the circulating plasma epinephrine concentrations. Figure 3 illustrates a significant correlation between right ventricular mass and left ventricular dry weight/body weight ratio.

Two-dimensional and M-Mode Echocardiographic Studies of the Left Ventricular Outflow Tract

Figure 4 shows representative two-dimensional and M-Mode echocardiographic recordings from a dog with acute right ventricular pressure overload imposed by pulmonary artery constriction. With increased pulmonary occlusion there was an immediate rise in right

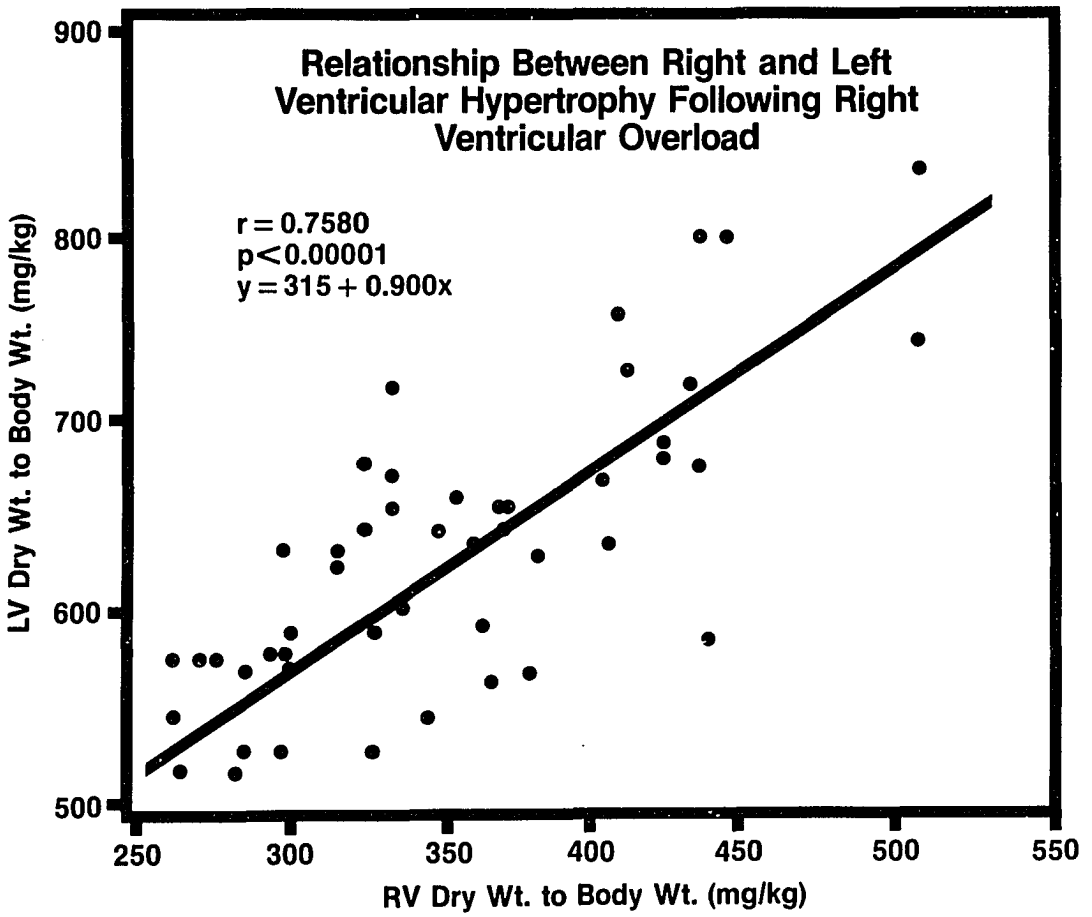
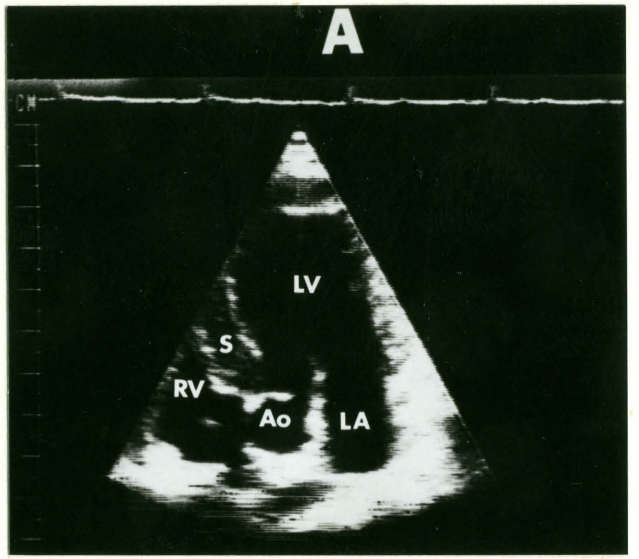


Figure 3: Relationship between Right and Left Ventricular Hypertrophy following Right Ventricular Overload. The 30-day study dogs demonstrated a high positive correlation between left and right ventricular dry weight/body weight ratio ($r = 0.758$; $p < 0.00001$).

Figure 4. Two-dimensional and M-Mode Echocardiographikc Studies Demonstrate that Pressure Overload of the Right Ventricle (RV) Does not Product Obstruction of the Left Ventricular Outflow Tract (LVOT) in Dogs. In anesthetized, open chest dogs, the ventricular septum (S) and LVOT were imaged with echocardiographic techniques during selective constriction of the pulmonary artery. In addition, pressures were measured with Milar catheters across the LVOT and in the RV. Panel A shows a two-dimensional echocardiographic view of the LVOT at mid-systole with a mean RV pressure of 20 mmHg. Panel B shows a two-dimensional echocardiographic view of the LVOT at mid-systolc with a mean RV pressure of 75 mmHg. Panel C shows an M-Mode image of the RV, S. and LVOT with a mean RV pressure of 75 mmHg. Panel D illustrates, at a mean RV pressure equal to that of the chronic pressure overload dogs, no pressure gradient occurs across the LVOT.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S
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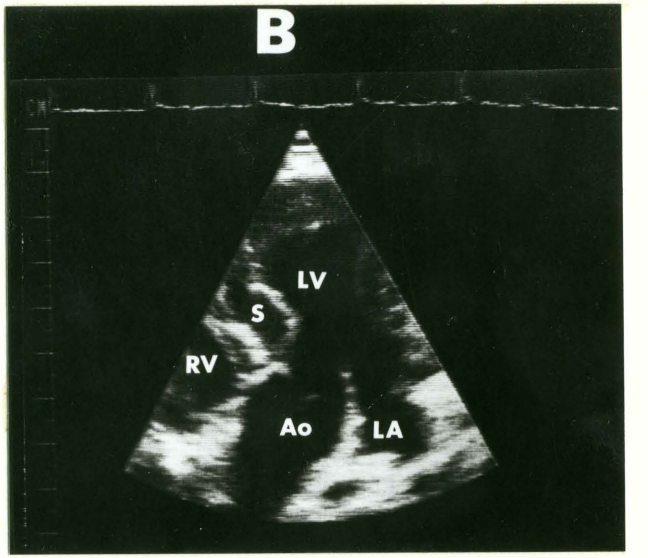
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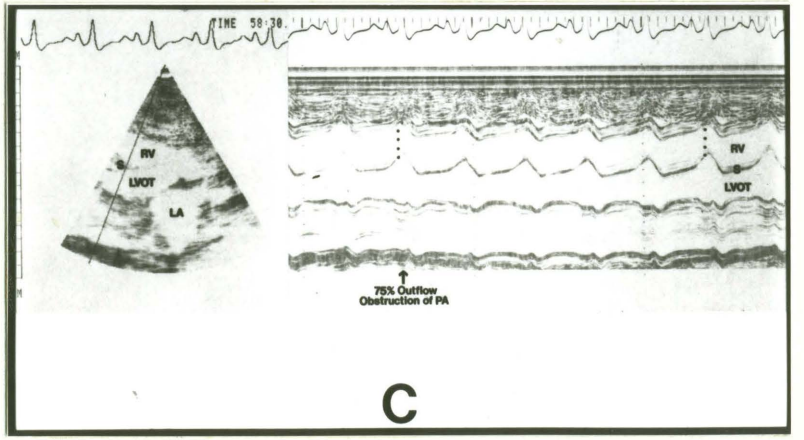
TIME 58:30
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LVOT
LA
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75% Outflow
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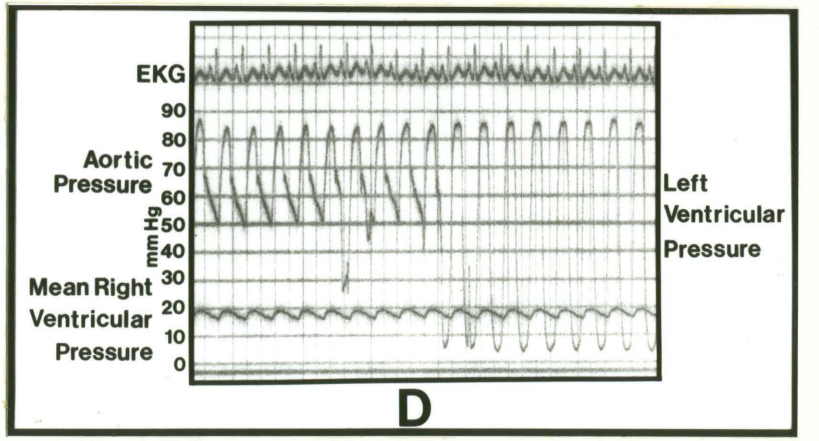
C


EKG
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ventricular systolic and diastolic pressure but no change in hemodynamic parameters in the left ventricle except when the pulmonary artery gradient exceeded 125 mmHg when both the left ventricular systolic and diastolic pressures fell. Further, two-dimensional echo and M-Mode cardiographic analyses of the left ventricular outflow tract demonstrated no outflow obstruction; but at the 125-mmHg gradient, the left ventricle was depleted of volume causing a septal leftward shift. These methods did not localize a possible anatomical alteration which could create increased left ventricular dynamics to account for the left ventricular hypertrophy.

Rat Model of Single Ventricular Pressure Overload Demonstrates Bilateral Ventricular Hypertrophy

Table 12 shows that banding of the pulmonary artery caused significantly increased right ventricular dry weight/body weight ratios. In addition, banding of the ascending aorta produced significant right ventricular and left ventricular hypertrophy.

Isoproterenol-induced Ornithine Decarboxylase Activity following Aortic Banding in Both the Right and Left Ventricles and Ventricular Septum

The ability of isoproterenol to induce ODC increased to 10-fold of control by day 5 post aortic banding in both the right and the left ventricles and ventricular septum (Fig. 5). At day 7 the isoproterenol induction of ODC returned to the level at 1 day post aortic banding.

Upper Airway Obstruction Stress Increased Circulating Plasma Epinephrine Concomitant with Increased Ventricular Mass

After 5 days of upper airway obstruction, there was significantly increased plasma epinephrine and significantly decreased plasma

Table 12: Concurrent Bilateral Ventricular Hypertrophy in Rat Models of Pulmonary Artery Banding or Aortic Banding

	RV	LV	Total + Septum
Control Sham (N = 6)	145 ± 8	364 ± 19	638 ± 34
Pulmonary Artery Band (N = 6)	265 ± 23[a]	450 ± 13[b]	833 ± 26[a]
Aortic Band (N = 6)	196 ± 10[b]	519 ± 35[a]	889 ± 52[a]

Dry weight/body weight (mg/kg) ratio, mean ± S.E.M.

Calibrated clips were fixed on either the ascending aorta or the proximal pulmonary artery to produce pressure overload of the respective ventricle in a 10-day study.

[a]Data differ from control ($p < 0.00001$)

[b]Data differ from control ($p < 0.01$)

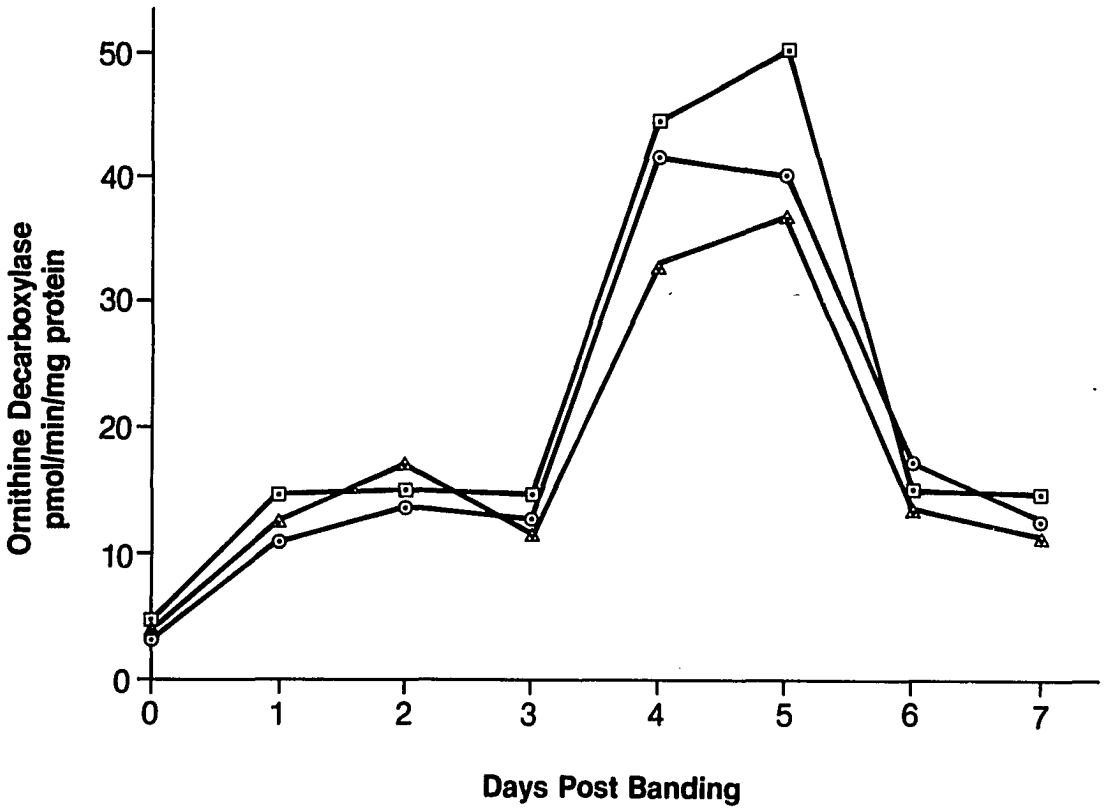

Figure 5: Following Aortic Banding, the Inducibility of Ornithine Decarboxylase by Isoproterenol Stimulation Increases Significantly in Both Ventricles of the Rat Heart. Isoproterenol (10 mg/kg, s.c.) was administered at the indicated days post aortic banding 4 h prior to the assay of heart ODC activity. Circles represent the right ventricle, triangles the left ventricle, and squares represent the ventricular septum.

norepinephrine (Table 13). Additionally, there was significant cardiac hypertrophy (Table 14). Increases in myocardial mass were proportionate in all three ventricular regions.

Discussion

Clinical studies suggested that left ventricular hypertrophy occurs in patients with selective right ventricular pressure overload caused by chronic pulmonary disease (Kountz et al., 1936; Michelson, 1960; Altschule, 1962; Fluck et al., 1966; Murphy et al., 1974), but the cause has remained unknown. Studies have reported that up to 86% of patients with cor pulmonary secondary to chronic obstructive pulmonary disease have hypertrophy of both right and left ventricles (Zimmerman and Ryan, 1951). Other studies demonstrated that patients with nonobstructive pulmonary disease such as emphysema or chronic bronchitis have both right and left ventricular hypertrophy (Kountz et al., 1936; Fluck et al., 1966; Rao et al., 1968). In hemodynamic terms, these clinical studies have not been able to associate alterations in the hemodynamic state of the left ventricle in the generation of left ventricular hypertrophy (Williams et al., 1968; Baum et al., 1971; Burrows et al., 1972; Bahler, 1975; Steele et al., 1975; Unger et al., 1975; Kline et al., 1977; Christianson et al., 1979).

Further, studies of pulmonary artery banding in animals reported similar morphologic and biochemical changes in both the right and left ventricles; although, again, as in cor pulmonale, no altered hemodynamic parameters of the left ventricle were detected (Chidsey et al., 1964; Pool et al., 1967; Buccino et al., 1969). In an attempt to

Table 13: Plasma Epinephrine and Norepinephrine Levels in Dogs with Chronic Obstructive Tracheal Disease

	Epinephrine (pg/ml)	Norepinephrine (pg/ml)
Control (N = 15)	103 ± 5	273 ± 30
Tracheal Obstruction (N = 5)	206 ± 34[a]	188 ± 12[a]

[a]Data differ from controls ($p < 0.01$)

Table 14: Ventricular Dry Weight to Body Weight Ratios following Chronic Obstructive Tracheal Disease in Dogs

	Right Ventricle	Septum	Left Ventricle	Total
Control (N = 5)	300 ± 8	269 ± 6	566 ± 19	1135 ± 28
Tracheal Obstruction (N = 5)	355 ± 13[a]	347 ± 12[a]	666 ± 17[a]	1368 ± 31[a]

Dry weight/body weight (mg/kg) ratio, mean ± S.E.M.

[a]Data differ from controls ($p < 0.01$)

examine the hormonal factor initiating cardiac hypertrophy, Kira et al., (1982) and Schreiber et al. (1975) demonstrated an increased rate of protein synthesis in the left ventricle following pressure overload of the right ventricle. In addition, similar biochemical alterations were noted in the right ventricle of hearts subjected to increased pressure overload to the left ventricle (Ito et al., 1980). Moreover, molecules that initiate cardiac hypertrophy are not species-specific in that extracts from hypertrophying canine heart will induce isolated rodent hearts to initiate the chain of biochemical events characteristic of hypertrophy (Hammond et al., 1982).

The most direct experimental evidence characterizing the effects of prolonged right ventricular overload on left ventricular performance demonstrated a maintenance of normal left ventricular function (Badke, 1982). A study in which dogs were instrumented with high-fidelity left ventricular pressure micrometers and ultrasonic crystals to measure left ventricular dimensions was unable to document significant abnormalities in global or regional to left ventricular contraction during chronic right ventricular pressure overload. In this 6-week study, right ventricular pressures increased to 220% of control, right ventricular mass significantly increased to 223% of control, and left ventricular mass increased to 125% of control. These results, and those from previous studies, suggest that increased left ventricular mass noted during right ventricular overload may be due to circulating trophic hormones rather than to mechanical stretch.

We studied the effects of prolonged volume or pressure overload on the mass of the right and left ventricles, and the results were consistent with the pattern of ventricular hypertrophy described clinically in cor pulmonale. In addition, we validated studies of Badke (1982) in the overloaded right ventricle with two-dimensional and M-Mode echocardiographic techniques. For our hypothesis to be correct, left ventricular pressure overload should be accompanied, in fact, with left and right ventricular hypertrophy. We demonstrated that dry ventricular weight/body weight ratios increased significantly in both ventricles with pressure overload of either the right or the left ventricle. Secondly, biochemically, both ventricles respond equally to selective pressure overload to one ventricle, and these studies are evidence that increased β-receptor activity parallels myocardial growth. Finally, in a dog model of upper airway obstruction, there was significant biventricular hypertrophy similar to the hypertrophy patterns seen clinically. This condition produced an elevated plasma epinephrine concentration which could account for increased biventricular hypertrophy.

CHAPTER 5

HYPERTROPHY OF THE DENERVATED, NONWORKING HEART

The process of cell growth is hormonally controlled (Jimenez de Asua, 1980; Sato, 1980) and we have demonstrated that circulating hormone(s) regulate macromolecular synthesis in myocardial tissue. The chronic administration of exogenous catecholamines resulted in biventricular hypertrophy in adult and developing animals (Byus et al., 1976). In view of these observations and our previous studies, we proposed that a denervated, essentially nonworking heart would hypertrophy in a manner similar to an innervated working heart following chronic administration of exogenous catecholamines. Secondly, we proposed that an alteration in the myocardial β-receptor number after myocardial denervation, along with a consistent concentration of catecholamine, would cause a change in myocardial mass.

We are concerned with causative factor(s) initiating the chain of biochemical events leading to cardiac growth. Therefore, we examined a heterotopic heart-lung transplantation model in the rat in which a transplanted-denervated, nonhemodynamically stressed heart was anastomosed to the abdominal aorta of the rat which retained its native innervated working heart. The transplanted heart was essentially nonworking; cardiac output was limited to the coronary artery perfusion. The present study was undertaken to determine the role of catecholamine β-receptor stimulation in cardiac hypertrophy in working and nonworking

heart. In addition, we studied whether pulmonary artery banding of the recipient heart would produce the necessary hormonal cascade to cause hypertrophy of a denervated, nonworking heterotopically transplanted heart. In these studies, ODC activity following isoproterenol stimulation was used as a biochemical marker of β-receptor responsiveness in both the recipient and donor hearts.

Materials and Methods

Animal Procedures

Male Sprague-Dawley rats, 100-150 g (the Division of Animal Resources, University of Arizona, Tucson) were housed in temperature- and light-controlled rooms and fed standard rodent chow and water ad libitum. Heart-lung transplantation was performed using a method described by Lee et al. (1970) with minor modifications. Recipient rats were weight-matched to the isogeneic heart-lung donor. Following ligation of the superior and inferior vena cavae and transection of the ascending aorta, the graft was chilled to 4° by perfusion with 10 ml of Kreb's solution through the inferior vena cava. The graft was removed from the donor and immediately sutured to the recipient's abdominal aorta with 8-0 prolene by a side-to-end, aortic-to-aortic anastomosis as shown in Figure 6. At various times after the abdominal transplant procedure, rats were killed by cervical dislocation and hearts removed either for ODC assays or weight determinations. For the pulmonary artery band study, the surgical procedure was identical except that the donor pulmonary veins were ligated and the pulmonary artery was anastomosed to the inferior vena cava.

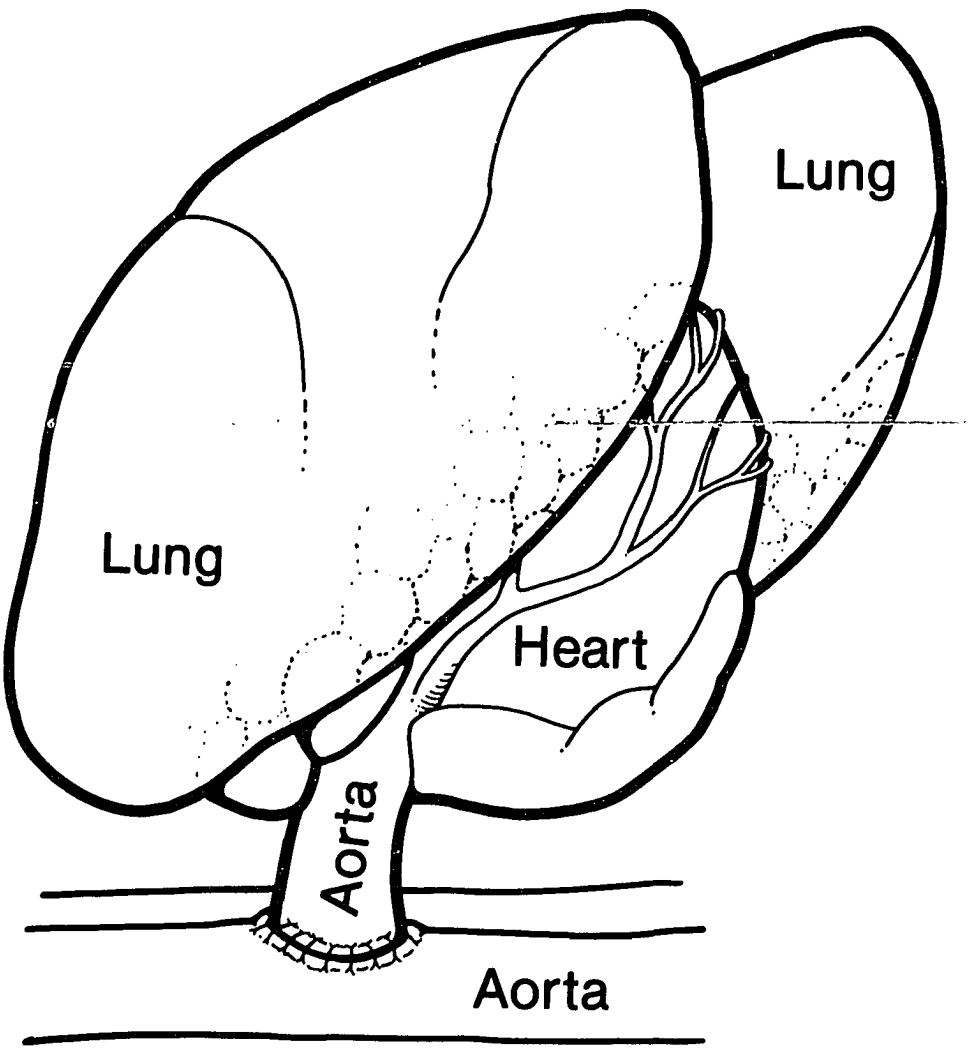

Figure 6: Heart-Lung Transplant Model. The donor heart-lung graft was sutured to the recipient abdominal aorta providing a single conduit for graft perfusion and removal of venous blood. Following perfusion of the coronary arteries, the venous blood enters the lungs from the right side of the heart and exits the aorta from the left side of the heart. The cardiac output of this heart-lung graft is limited to that of coronary artery perfusion.

Left ventricular pressure and stroke volume were measured in donor and recipient hearts 10 days posttransplantation. Simultaneous left ventricular pressure measurements of donor and recipient hearts were accomplished by insertion of a PE-50 polytheylene catheter into the apex of each left ventricle while the recipient rat was being ventilated with a pressure limit ventilator and anesthetized with ethrane anesthesia. The catheters were connected via a transducer to a photo-recording physiograph, and baseline pressures were measured. Pressures were measured also 2 min after a 15 mg/kg s.c. dose of isoproterenol, a time demonstrated to produce maximal alterations in hemodynamic parameters. The stroke volumes were measured by placing an electromagnetic flow prove (1.5 mm) around the ascending aorta of the hearts. Measurements were recorded at baseline as well as 2 min after 5 mg/kg or 15 mg/kg isoproterenol, s.c.

Tissue Dry Weight Procedure

Ventricular dry weight to pretreatment body weight was characterized by removal of the atria, valves and great vessels, drying for 48 h at 50°C at -20 psi and expressed as ventricular dry weight to body weight ratios (mg/kg).

Histology

Routine tissue samples for histology were fixed in 10% neutral formalin. Sections were stained with Masson's trichrome and evaluated for the occurrence and extent of possible tissue fibrosis.

Results

Time Course of Ornithine Decarboxylase Induction in Response to Isoproterenol in Recipient and Donor Hearts

ODC activity was assayed in both recipient and donor heart tissue 5 days posttransplantation (Fig. 7). After a single injection of isoproterenol (15 mg/kg, s.c.), peak ODC activity was detected at 4 h and was increased over 10-fold in both recipient working hearts and donor nonworking hearts. When compared to recipient hearts, donor hearts had lower unstimulated ODC activity and a lower total excursion (1-12 pmol/min/mg protein vs. 2.2-23 pmol/min/mg protein). As a result of this first experiment, all subsequent determinations of ODC activity were measured at 4 h after a single dose of isoproterenol.

Alteration in Isoproterenol-stimulated Ornithine Decarboxylase Activity in Recipient and Donor Hearts as a Function of Time after Transplant

Isoproterenol-stimulated ODC activity was measured in recipient and donor hearts at 1, 5 and 10 days posttransplantation. One day posttransplantation, the ODC excursion 4 h postinjection in the donor heart was 36% of that detectable in the recipient heart and 22% that of the isoproterenol-stimulated, nontransplanted heart (Fig. 8). At 5 days posttransplantation, donor heart isoproterenol-stimulated ODC activity increased to 64% that of the recipient heart and at 10 days was 245% of that detectable in recipient heart. Recipient heart isoproterenol-stimulated ODC activity was similar to nontransplanted, isoproterenol-stimulated heart at 5 and 10 days posttransplantation. Decreased β-receptor coupling to ODC activity occurred in the recipient heart only at day 1 posttransplantation.

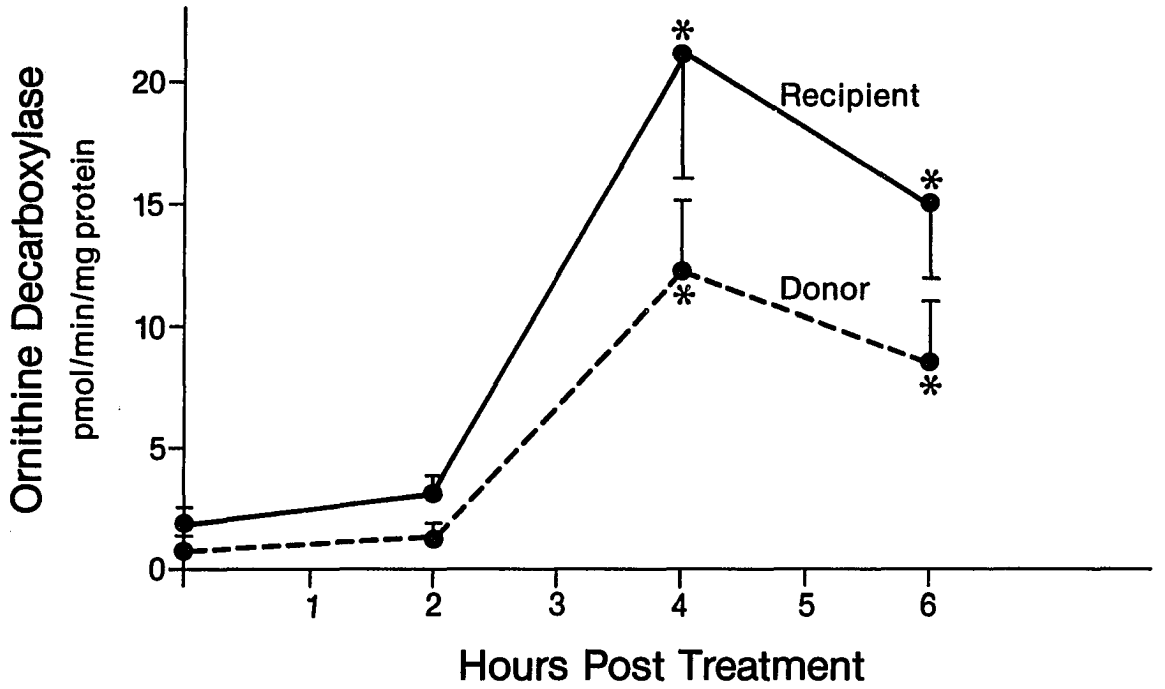

Figure 7: Isoproterenol stimulation of ODC induction in the recipient and donor heart tissue 5 days posttransplantation. Isoproterenol (15 mg/kg) was administered s.c. to recipient rats and ODC activity in the recipient and donor heart tissue was determined at 0, 2, 4, and 6 h. Each data point represents the mean ± standard deviation of 4 hearts assayed in duplicate. *P < 0.01.

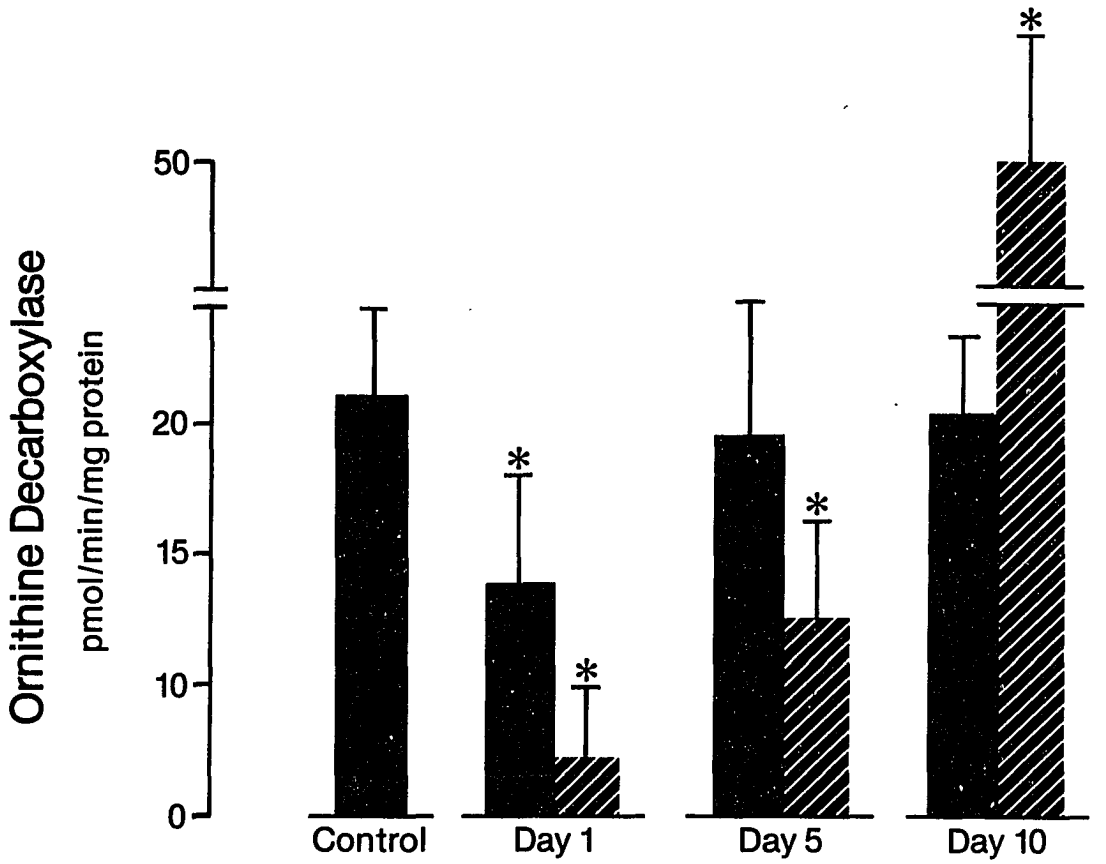

Figure 8: Alterations in isoproterenol-stimulated ODC activity in recipient and donor hearts as a function of time after transplant. Recipient and donor myocardial ODC activity was measured 4 h following a single dose of isoproterenol (15 mg/kg, s.c.) at 1, 5 and 10 days post heart-lung transplantation. The control measurements were obtained from nonoperated, isoproterenol-treated rats. Bars represent the mean ± standard deviation of measurements of 4 hearts assayed in duplicate. *P < 0.01.

Alterations in Ventricular Dry Weight to Body Weight Ratios in the Recipient and Donor Heart as a Function of Time after Transplant

The temporal growth increments of the normal and transplanted hearts paralleled the extent of ODC response to isoproterenol injection (Fig. 9). Ventricular dry weights in transplanted and recipient hearts were assessed at 0, 5, 10 and 15 days posttransplantation in the absence of isoproterenol treatment. The recipient heart did not significantly change in dry weight/body weight ratio throughout the study period, whereas the donor heart exhibited significant atrophy at both 5 and 10 days posttransplantation. By day 15, the donor heart dry weight/body weight ratio was significantly elevated ($p < 0.01$) compared to the day zero recipient heart weight/body weight ratio. The recipient body weights, posttransplantation, continued to increase at the same rate as controls.

Isoproterenol-stimulated Dose- and Time-dependent Increments in Ventricular Mass

In order to further characterize the role of β-adrenergic receptors in the regulation of myocardial hypertrophy, isoproterenol was administered daily x 2 in doses ranging from 0.1 to 15 mg/kg/day for 5 days (Fig. 10). A dose-dependent increase in ventricular dry weight/body weight was detected in both donor and recipient hearts, although the magnitude of the donor weight ratio was consistently 73% that of the recipient. In addition, in an experiment measuring the 10-day temperal response to chronic administration of isoproterenol (0.5 mg/kg/day), there was a continued increase in myocardial mass in the donor heart whereas recipient heart mass increased significantly

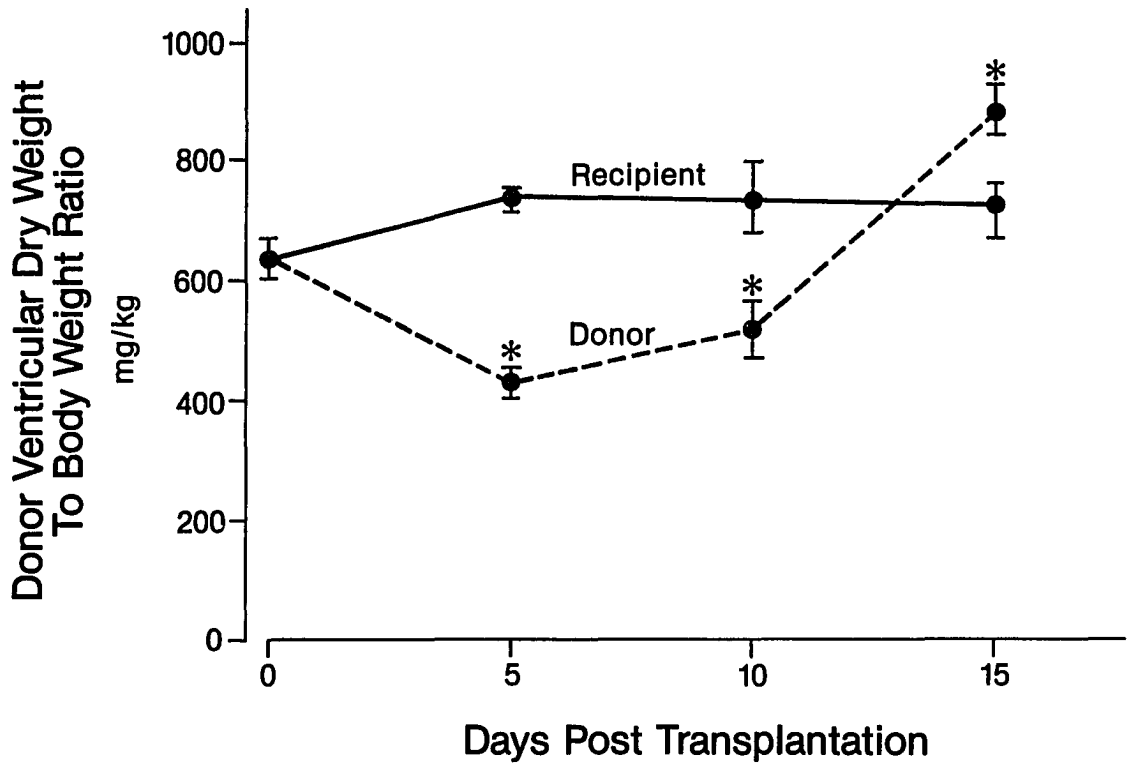

Figure 9: Changes in ventricular mass in recipient and donor hearts as a function of time after transplantation. Recipient and donor dry weight/body weight ratios were measured following transplantation. Each data point represents the mean ± S.E. of measurements of 6 hearts. *P < 0.01.

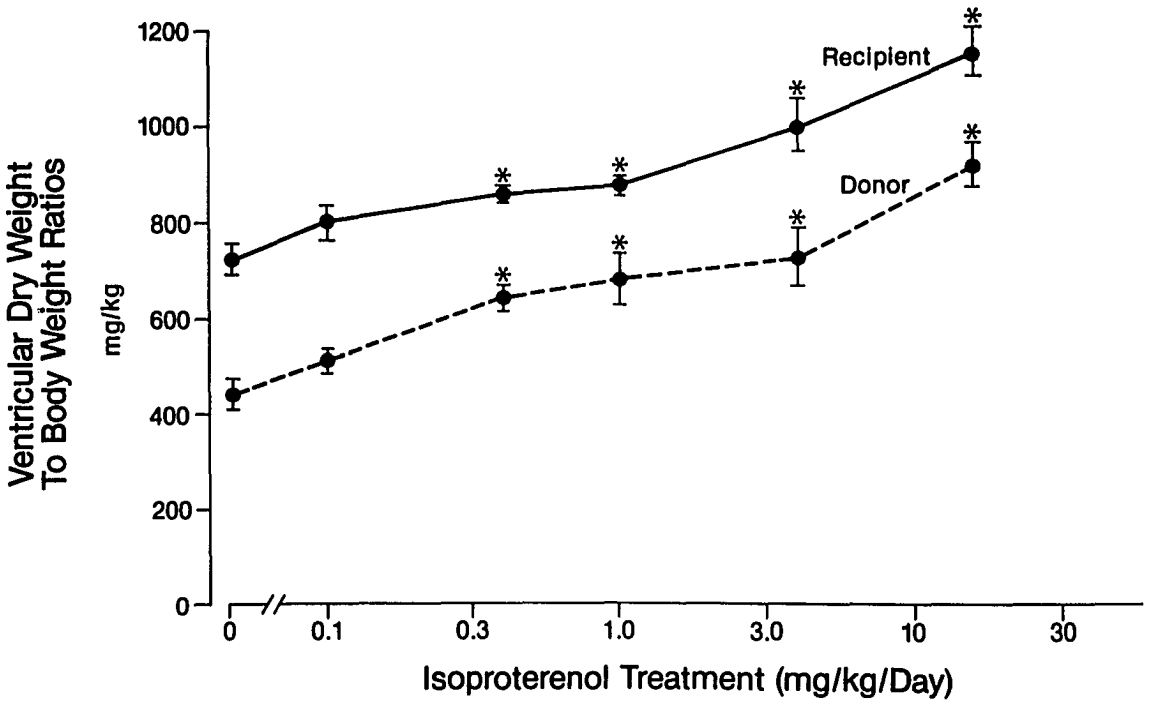

Figure 10: Dose response in recipient and donor heart mass following the chronic administration of isoproterenol for 5 days. Isoproterenol was administered daily x 2 at doses ranging from 0.1 to 15 mg/kg/day, s.c., for 5 days followed by the measurement of ventricular dry weight/body weight ratios. Each data point represents the mean ± standard error of measurements of 6 hearts. *Data differ from appropriate controls ($P < 0.01$).

only between days zero and 5 (Fig. 11). This was the lowest dose of isoproterenol resulting in a significant elevation in ventricular mass of both recipient and donor heart as assessed from data in Figure 10. In the donor heart, ventricular mass at day 10 posttransplantation differed significantly from both the day 5 group and controls.

Alterations in Hemodynamic Parameters in Response to Isoproterenol

Basal hemodynamic measurements were performed in recipient and transplanted hearts 10 days posttransplantation prior to and 2 min following isoproterenol administration (15 mg/kg, s.c.). Donor heart basal left ventricular systolic pressure was not significantly different from recipient heart; following isoproterenol treatment, left ventricular systolic pressure in both hearts decreased by 85% of control. Left ventricular end-diastolic pressure changed only in the donor heart, decreasing from 8 to 2 mmHg (Fig. 12). The donor heart stroke volume was 16% that of the recipient heart basal stroke volume and did not change significantly following isoproterenol administration (Fig. 13).

Myocardial Hypertrophy Mediated by Endogenous Hormones

Ten days following transplantation, donor hearts were significantly reduced in heart weight to body weight ratios (Table 13) and banding the recipient pulmonary artery 48 h prior to transplantation did not prevent the significant donor heart atrophy. Whereas, after banding of the recipient pulmonary artery 48 h subsequent to heart transplantation, both the recipient and donor hearts hypertrophied to 170% of control.

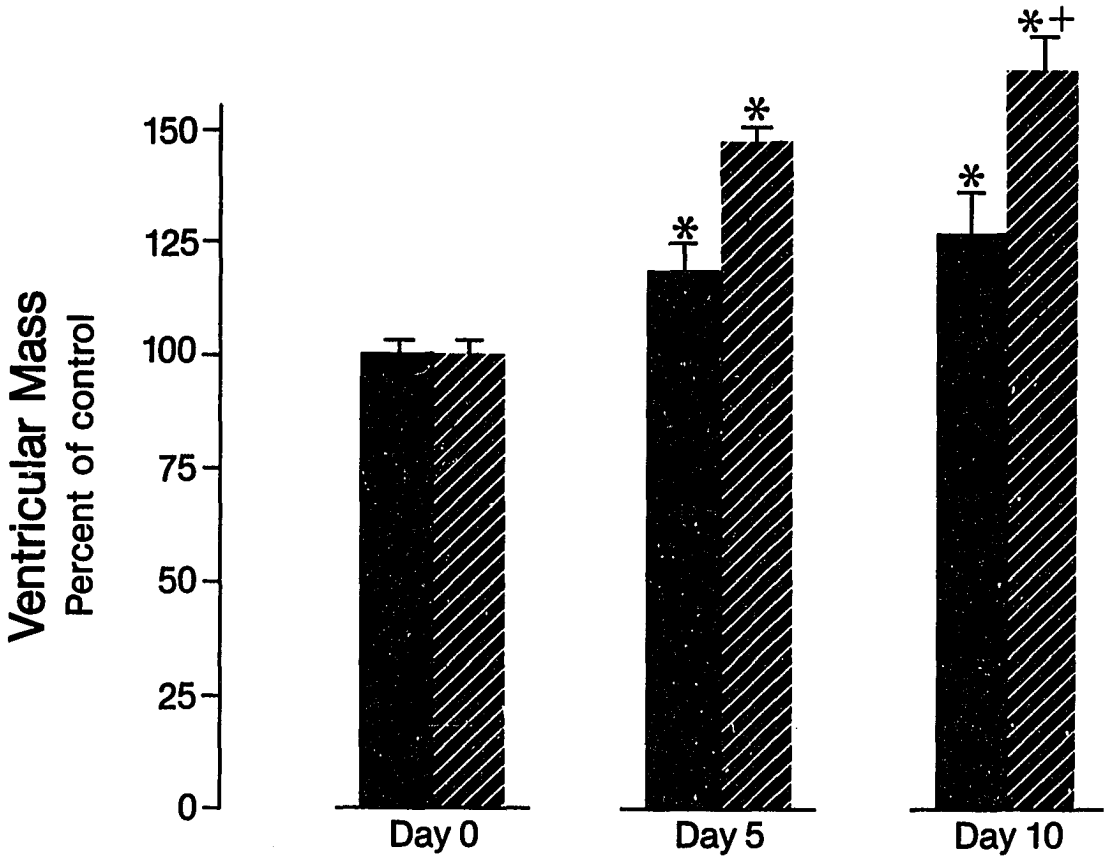

Figure 11: Temporal changes in ventricular mass after chronic isoproterenol administration. The percentage increase in mass over untreated controls of the recipient and donor heart dry weight to body weight ratios resulting from chronic stimulation with isoproterenol (0.5 mg/kg/day, s.c.) were studied for 0, 5, and 10 days posttransplantation. Bars represent the mean ± standard error of measurements of 6 hearts. *Data differ from controls ($P < 0.01$). **Data differ from both controls and Day 5 donor ventricular mass values ($P < 0.01$).

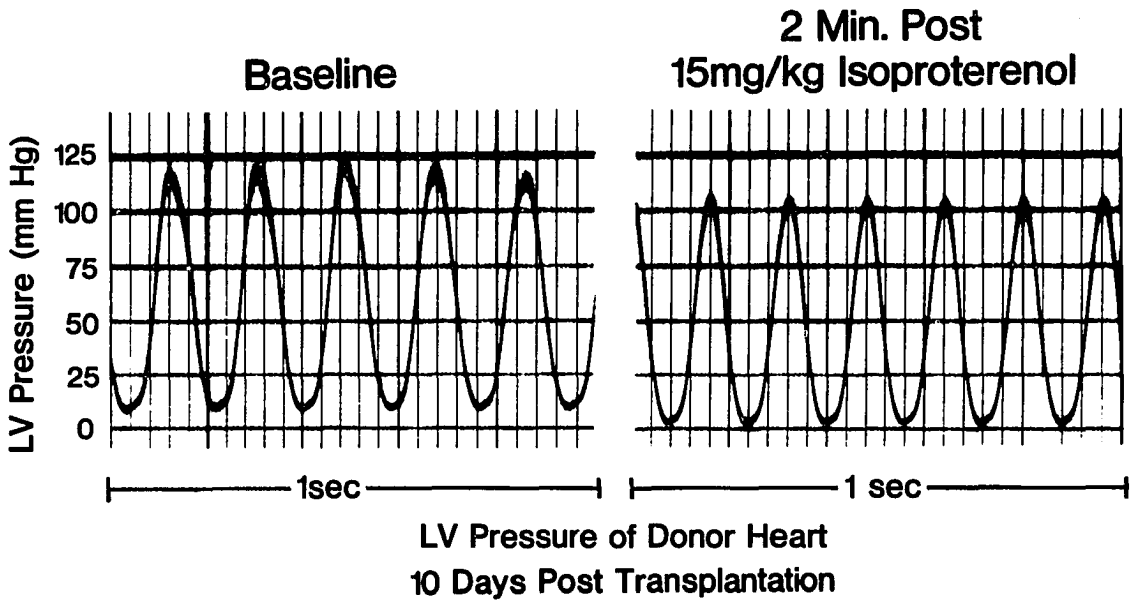

Figure 12: Left ventricular pressure measurements of the transplanted heart. Ten days following transplantation, left ventricular pressure was measured to assess hemodynamic alterations after isoproterenol (15 mg/kg, s.c.). Measurements were recorded prior to and 2 min after administration.

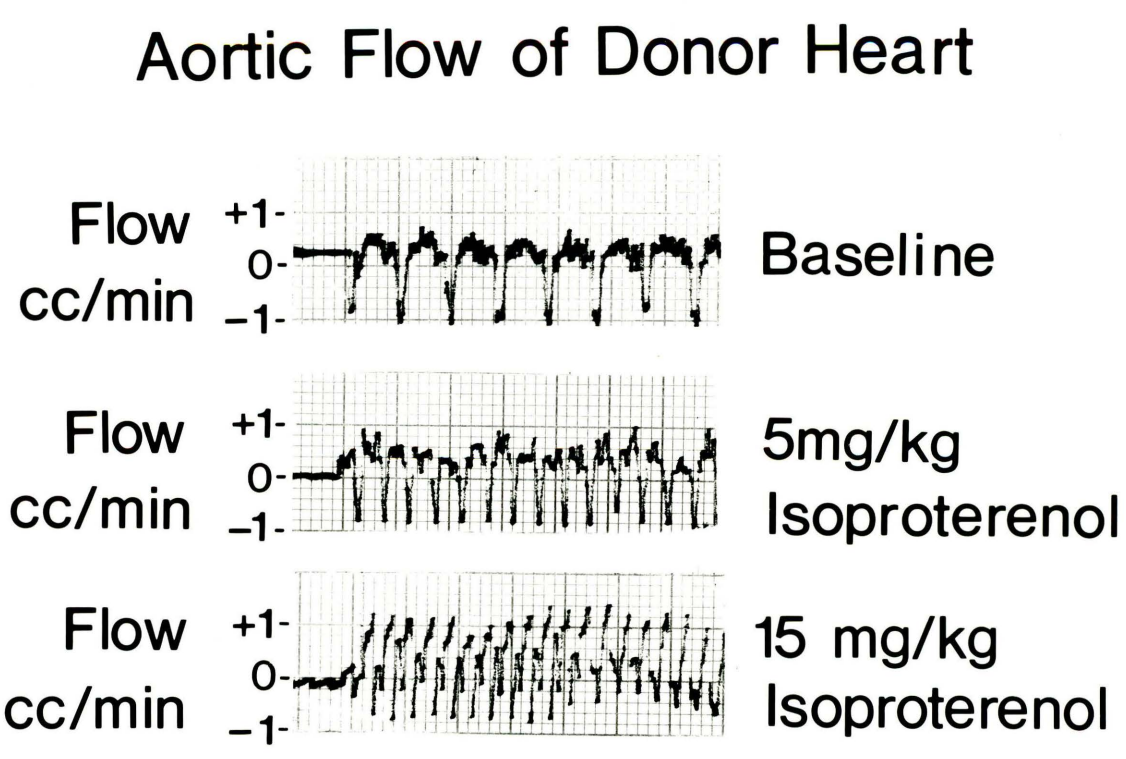

Figure 13: Stroke Volume Measurements of the Transplanted Heart. Ten days following transplantation, donor heart stroke volume was measured with an electromagnetic flow meter. Stroke volume was recorded prior to and 2 min after isoproterenol administration.

Table 15: Effect of Pressure Overload of the Recipient Right Ventricle on Hypertrophy of the Recipient and Donor Hearts in Rats

Groups	Recipient (mg/kg)	Donor (mg/kg)
Baseline (N = 9)	---	712 ± 45
Non-banded Control (N = 6)	751 ± 60	578 ± 51[b]
Heart transplant 48 h post pulmonary artery banding (N = 5)	1234 ± 114[a]	588 ± 12[b]
Pulmonary artery banding 48 h post heart transplantation (N = 5)	1204 ± 49[a]	1014 ± 80[a]

Data are expressed as the mean ± S.E.M.

Calibrated clips were fixed to the proximal pulmonary artery of the recipient heart 48 h prior to or 48 h following heteroropic heart transplantation. The ventricular dry weight to body weight ratios were determined 10 days posttransplantation.

[a]Data differ from controls ($p < 0.01$).

[b]Data differ from controls ($p < 0.05$).

Histological Evaluation

Masson't trichrome stain of the heart sections and routine histology performed on each group did not reveal any significant interstitial or subendocardial fibrosis or necrosis which would account for observed increases in heart mass.

Discussion

These results lend further support to our earlier observations that heart mass is regulated by circulating hormone(s) through catecholamine β-adrenergic receptor stimulation (Womble et al., 1978, 1980; Larson et al., 1982a, 1982b). In both recipient heart and the nonhemodynamically stressed donor heart, myocardial mass paralleled chronic exogenous catecholamine administration in an incremental dose-dependent manner, although the magnitude of the donor heart weight ratio was consistently less than the recipient. Secondly, there is substantial evidence that other hormone(s) may have a role in cardiac growth. We have in Chapter 3 documented a sustained increase in circulating plasma epinephrine concentrations during chronic ventricular overload. Indirect evidence suggests other hormones may have a role during the first 48 h following overload stress initiating myocardial growth. It also follows that long-term elevation of plasma epinephrine is required to maintain the hypertrophied state but that the initial growth stimulus to elevate cardiac mass to a new plateau requires more than epinephrine. This relationship also has been observed in other paired organ hypertrophy models; compensatory hypertrophy of one kidney also causes hypertrophy of the contralateral kidney (Morris, 1976).

Radioligand studies have demonstrated a defect in β-receptor coupling to adenylate cyclase activity in some models of cardiac hypertrophy and increased coupling in others (Upsler and Khairallah, 1982; Kumano et al., 1983; Wesslau, 1983). Furthermore, repeated doses of isoproterenol have been demonstrated to cause significantly decreased isoproterenol-induced ODC activity and [^{3}H]dihydroalprenol binding without a decrease in affinity of the β-receptors (Nomura et al., 1982). There appears to be a lack of quantitative fit between the response and β-receptor numbers. Therefore, β-receptor coupling to ODC activity may be employed to quantitate β-receptor density. In both the recipient heart and the nonhemodynamically stressed donor heart, the myocardial mass paralleled the ability of an isoproterenol bolus to stimulate ODC activity (β-receptor density coupled to ODC) in the respective heart. It was evidenced that both recipient and donor hearts were exposed to the same concentration of endogenous circulating hormones; therefore, the ability of donor heart mass to change independently of recipient appears to be due to denervation-induced fluctuations in β-receptor activity.

The ODC activity following isoproterenol stimulation in the denervated heart was consistent with myocardial β-receptor ligand studies in the denervated heart (Yamada et al., 1980; Lurie et al., 1983). Thus, β-receptor coupling in the donor heart was decreased 5 days after transplantation as assessed by the differential ability of a single dose of isoproterenol to stimulate ODC activity compared to the recipient. β-Receptor coupling to ODC activity in the donor

heart exceeded that of the recipient heart at 10 days posttransplantation. Myocardial mass of the donor fluctuated as did the β-receptor coupling to ODC activity, implying myocardial mass is regulated by: (1) endogenous circulating hormone levels or administered β-agonist concentrations, and/or (2) β-receptor activity.

These studies described a myocardial hypertrophy transplant model that does not require myocardial innervation and is independent of hemodynamic parameters. Alterations in myocardial mass paralleled β-adrenergic responsiveness as assessed by the ability of isoproterenol to elevate ODC activity and paralleled circulating concentrations of catecholamines. To extend these observations to other myocardial hypertrophy models, we studied the relationship of myocardial mass in donor and recipient hearts following pulmonary artery banding of the recipient heart. We found a specific temporal relationship of hypertrophy of the donor heart apparently resulting from a hormonal cascade leading to compensatory myocardial hypertrophy.

CHAPTER 6

CONCLUSIONS

(1) We have demonstrated that selective volume or pressure overload of the right ventricle produced a significant and sustained elevation of circulating plasma epinephrine concentrations, and these concentrations correlated positively with both right and left ventricular mass. The circulating norepinephrine concentrations did not correlate as highly to myocardial mass in these studies. Propranolol prevented myocardial hypertrophy during markedly elevated hemohynamic overloads and consistent with other studies of chronic propranolol administration, no evidence of cardiac decompensation was reported (Vaughan-Williams et al., 1975). Further, propranolol also attenuated the release of epinephrine from the adrenal medulla. These studies further support the view that epinephrine is involved in myocardial growth regulation mediated through myocardial β_2-receptors.

(2) The trophic effects of circulating hormones not only caused hypertrophy of the hemodynamically stressed ventricle but also caused hypertrophy of the contralateral nonhemodynamically stressed ventricle. Biochemical studies demonstrated that isoproterenol-stimulated ODC activity increased by 10-fold in both right and left ventricles during selective pressure overload of the left ventricle. An animal model of upper respiratory obstruction demonstrated bilateral ventricular hypertrophy with concomitant increased circulating plasma epinephrine levels.

(3) We further showed that cardiac hypertrophy is not dependent upon workload or innervation. Models of heterotopically transplanted hearts proved the hypothesis that myocardial growth was dependent upon β-receptor stimulation, and that altering either the circulating catecholamine concenrations or myocardial β-receptor number cause changes in myocardial mass.

(4) Finally, we provided definitive evidence that increased pressure overload to the right ventricle of the recipient heart will cause a hormonal cascade that leads to hypertrophy of a heterotopically transplanted denervated, nonworking heart.

In conclusion, therefore, it is suggested that hemodynamic stress-mediated hormonal activity induces compensatory growth of the heart and that the trophic action is mediated by epinephrine acting via a β_2-receptor. It is further suggested that, in most conditions leading to adaptive cardiac hypertrophy, circulating hormones are the pathway through which increased physiological or pathological demands for cardiac work initiate the biochemical events resulting in adaptive cardiac growth.

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