CONGESTIVE HEART FAILURE SECONDARY TO MYOCARDIAL INFARCTION: ABNORMALITIES IN RAT CARDIAC SARCOLEMMA

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by

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ABSTRACT

Depressed cardiac pump function is the hallmark of congestive heart failure and it is suspected that decreased influx of Ca^{2+} into the cardiac cell is responsible for depressed contractile function. Since Ca^{2+} movements in the sarcolemmal membrane are known to involve Ca^{2+}-channels, Na^{+}-Ca^{2+} exchange, Ca^{2+}-pump, Na^{+}-K^{+} ATPase, ß-adrenoceptors and alpha-adrenoceptors directly or indirectly, the status of these mechanisms was examined by employing an experimental model of congestive heart failure. For this purpose the left coronary artery was ligated and hearts were examined 4, 8 and 16 weeks later; sham-operated animals served as controls. Hemodynamic assessment revealed decreased total mechanical energy (left ventricular systolic pressure x heart rate), increased left ventricular diastolic pressure, and decreased positive and negative dP/dt in experimental animals at 4, 8 and 16 weeks. Although accumulation of ascites in the abdominal cavity was evident at 4 weeks, other clinical signs of congestive heart failure in experimental rats were evident from the presence of lung congestion and cardiac dilatation at 8 and 16 weeks of inducing myocardial infarction. On the basis of these data, the experimental animals at 4, 8 and 16 weeks of myocardial infarction were considered to represent early, moderate and severe stages of congestive heart failure.

As Ca^{2+}-channels in the sarcolemmal membrane are considered to be an important route for the entry of Ca^{2+}, we examined the status of Ca^{2+}-receptors/channels in failing rat hearts subsequent to myocardial infarction of the left ventricular free wall. The density of Ca^{2+}-receptors/channels in crude membranes, as assessed by [^{3}H]-nitrendipine binding assay, was found to be decreased in the uninfarcted experimental left ventricle at 8 and

16 weeks; however, no change in the affinity of nitrendipine was evident. A similar depression in the specific binding of another dihydropyridine compound ([^{3}H]-PN200-110) was also evident in failing hearts. Brain and skeletal muscle crude membrane preparations, unlike right ventricle and liver revealed a decrease in Ca^{2+}-receptors/channels density in 16 weeks experimental animals. Reduction in the Ca^{2+}-channel number was also seen in heart homogenate as well as purified sarcolemmal preparations from 16 weeks failing animals. These data suggest that changes in Ca^{2+} channels in the sarcolemmal membrane occur as a consequence of events during the development of congestive heart failure.

Since the physiologic actions of norepinephrine in the heart are mediated by adrenoceptors located in the sarcolemmal membrane we examined the status of alpha - and ß-adrenoceptors in failing rat hearts subsequent to myocardial infarction. The density of ß-adrenoceptors in crude membranes, as assessed by [^{3}H]-dihydroalprenolol binding assay, was decreased in the viable left ventricle at 4, 8, and 16 weeks without any change in the affinity of ligand for binding. The density of cardiac alpha-adrenoceptors, as assessed by [^{3}H]-prazosin binding assay, was increased in membrane preparations from experimental animals at 8 and 16 weeks, without any change in the binding affinity. Reduction in the ß-adrenoceptor number was also seen in purified sarcolemmal preparations from 4 weeks failing animals and an increase in the alpha-adrenoceptor number was observed in these preparations from 16 weeks experimental animals. In isolated perfused heart, the positive inotropic action due to isoproterenol infusion was depressed in experimental animals at 4, 8, and 16 weeks whereas the responsiveness to phenylephrine infusion was unchanged in these groups. These data suggest that changes in the ß-adrenoceptors may be associated with early stages of failure whereas

those in alpha-adrenoceptors are secondary to the development of congestive heart failure.

As disturbances in Ca^{2+} metabolism in the cardiac cell can lead to depressed activation of the contractile apparatus, and since sarcolemmal Na^{+}-Ca^{2+} exchange and Ca^{2+}-pump are thought to play a role in transsarcolemmal Ca^{2+} movements, we examined the activities of both Na^{+}-dependent Ca^{2+} uptake and ATP-dependent Ca^{2+} uptake in failing rat heart after myocardial infarction. The sarcolemmal Na^{+}-dependent Ca^{2+} uptake was depressed in 8 weeks experimental animals when the activity was assayed either as a function of time or Ca^{2+} concentration. Lineweaver-Burk plot of the data on Na^{+}-dependent Ca^{2+} uptake revealed a depression of V_{max} with no change in the K_a for Ca^{2+} in experimental preparations. No change in the Ca^{2+} pump (ATP-dependent Ca^{2+} accumulation and Ca^{2+}-stimulated ATPase) activities was evident in the 8 weeks experimental group. Nonspecific and passive Ca^{2+} binding were similar at 8 weeks in experimental and control groups. The Na^{+}-dependent Ca^{2+} uptake, unlike the ATP-dependent Ca^{2+} uptake and Ca^{2+}-stimulated ATPase, was also depressed in sarcolemmal vesicles from 4 weeks failing hearts. These results indicate that changes in Na^{+}-Ca^{2+} exchange may be associated with depressing Ca^{2+} movements at the sarcolemmal membrane in early stages of congestive heart failure.

Since Na^{+} ions, which are controlled by Na^{+}-K^{+} ATPase in the cell membrane, are considered to modulate the contractile force development by the cardiac muscle, we characterized the sarcolemmal Na^{+}-K^{+} ATPase activity in failing rat hearts after myocardial infarction. The depression in Na^{+}-K^{+} ATPase in purified sarcolemmal membrane from the uninfarcted experimental left ventricle at 8 weeks was associated with depressed V_{max} without any changes in the affinities for MgATP, Na^{+} and K^{+} or the pH optimum for the enzyme.

The K_d values of both the high and low affinity binding sites for [^{3}H]-ouabain, which is considered to interact with Na^+-K^+ ATPase, were increased; however, no change in the density of either class of ouabain binding site was evident. The depression of Na^+-K^+ ATPase in failing hearts at 16 weeks of myocardial infarction was not different from that observed at 8 weeks but the enzyme activity was not altered at 4 weeks of coronary occlusion. These results suggest that changes in the sarcolemmal Na^+-K^+ ATPase may occur as a consequence of events during the development of congestive heart failure.

The data from this study indicate that Ca^{2+} channels which govern entry of Ca^{2+} in the myocardium may be depressed in moderate and severe stages of congestive heart failure. Furthermore, changes in sarcolemmal Na^+-Ca^{2+} exchange and ß-adrenoceptors may be associated with the development of early stages of congestive heart failure whereas alterations in sarcolemmal Na^+-K^+ ATPase and alpha-adrenoceptors may serve as adaptive mechanisms for compensating the depression in contractile force development at moderate and severe stages of congestive heart failure. These changes do not appear to be due to a generalized defect in the sarcolemmal membrane because the Ca^{2+}-pump activities were not altered in failing hearts. Thus it is evident that changes in Na^+-Ca^{2+} exchange as well as ß-adrenoceptors and Ca^{2+} channels may contribute towards decreasing Ca^{2+} influx at early and moderate stages of congestive heart failure, respectively. On the other hand, changes in alpha-adrenoceptors and Na^+-K^+ ATPase may act as compensatory mechanisms for maintaining Ca^{2+} influx at moderate and late stages of congestive heart failure. It is suggested that defects in the sarcolemmal membrane may cause an overall reduction in the availability of Ca^{2+} for the contractile apparatus and this may play a crucial role for the depression of contractile activity in this model of congestive heart failure.

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I. INTRODUCTION AND STATEMENT OF THE PROBLEM

Although congestive heart failure is one of the major medical problems facing mankind, the exact mechanisms associated with contractile abnormalities in this syndrome are poorly understood. Extensive studies over the past 20 years have revealed that disturbances in Ca^{2+} metabolism are suspected to be involved in a wide variety of experimental models of heart disease (1-3); however, the defects in mechanisms concerned with Ca^{2+} movements are not clearly defined in the myocardium. It should be noted that Ca^{2+} entry to the interior of the myocardium is believed to occur mainly via voltage- or receptor- operated calcium channels located in the sarcolemmal membrane (4) and it has been shown that the functional status of these Ca^{2+} channels in the cell can be monitored by determining the specific binding of Ca^{2+}-antagonists (5). In view of changes in Ca^{2+} channel density in different experimental models of contractile failure (5-9), it appears that the status of Ca^{2+}-channels in the myocardium may depend upon the type of heart disease. It should be noted that altered inotropic responsiveness of the myocardium to adrenergic stimulation has also been demonstrated in both clinical heart failure and in a variety of experimental models of heart disease (1,10-16). Furthermore, it has been shown that the functional status of ß-adrenoceptor, and alpha-adrenoceptor populations are altered in the failing hearts when monitored by determining the specific binding of appropriate adrenoceptor antagonists (11-13,17) and these changes have been suggested to result in derangement of Ca^{2+} homeostasis in the myocardial cell. Since no information concerning changes in the status of Ca^{2+} channels as well as alpha- and ß-adrenoceptors in the myocardium during the development of congestive heart failure subsequent to myocardial infarction is

available in the literature, we have undertaken a study concerning the density and affinity of Ca^{2+} channels as well as alpha- and ß-adrenoceptors in cardiac membranes by employing a rat model of congestive heart failure as a result of infarction of the left ventricular free wall. In order to test the physiological function of these receptor systems, we measured responsiveness of the isolated hearts from control and experimental animals to exogenously applied alpha- and ß-adrenoceptor agonist drugs during the development of heart failure.

Abnormalities in Ca^{2+} channels and adrenoceptors in the failing heart could be taken to suggest that there is a generalized defect in the sarcolemmal membrane. Accordingly, it is possible that other sarcolemmal functions such as Na^+-K^+ pump, Na^+-Ca^{2+} exchange and Ca^{2+}-pump may also become defective in congestive heart failure. In this regard, it should be mentioned that Na^+-K^+ ATPase has been localized in cardiac sarcolemma and is considered to be involved in the maintenance of intracellular Na^+ and K^+ concentrations in the myocardium (18,19). It is believed that this enzyme is a receptor for cardiac glycosides and that the glycoside-mediated positive inotropic effect on the myocardium may be an indirect effect of the inhibition of Na^+-K^+ ATPase activity (18,19). Earlier studies have revealed a wide variety of changes in Na^+-K^+ ATPase activity in different types of heart disease (20-28,292). Since the movements of Na^+ and Ca^{2+} ions across the sarcolemma are thought to be necessary for initiating the contractile phase of the cardiac cycle (29), membrane-bound sarcolemmal proteins such as the Na^+-Ca^{2+} exchanger and Ca^{2+}-pumping ATPase can play a critical role in this event. Recent studies have demonstrated defects of varying degree in the sarcolemmal Na^+-Ca^{2+} exchange and Ca^{2+}-pump activities due to myocardial ischemia (30,31), hypoxia-reoxygenation injury

(22), chronic diabetes (32,33) and hypertrophy due to hypertension or aortic banding (34). Therefore it is evident that the status of sarcolemmal Na^+-Ca^{2+} exchange and Ca^{2+}-pump like that of sarcolemmal Na^+-K^+ ATPase may depend upon the type and stage of heart disease. In view of the lack of information concerning the status of sarcolemmal Na^+-K^+ ATPase, Na^+-Ca^{2+} exchange and Ca^{2+}-pump activities during the development of congestive heart failure, we have undertaken a study of these parameters in cardiac sarcolemmal membranes of rats with congestive heart failure. It should be pointed out that the hallmark of congestive heart failure is the inability of the heart to maintain contractility and the pathophysiological events depend upon the progression of this syndrome. Thus it was considered important to assess the experimental animals for hemodynamic performance to seek a relationship between changes in sarcolemmal activities and cardiac function at different stages of congestive heart failure.

II. REVIEW OF THE LITERATURE

1. Introduction. The question of a fundamental mechanism responsible for the contractile abnormality associated with reduction of the work by the heart in common forms of cardiac failure has long intrigued cardiologists. However, no single common biochemical defect has yet been identified to explain the contractile abnormalities of the failing, hemodynamically overloaded heart. Moreover the resolution of a primary defect responsible for heart failure and secondary compensatory mechanisms that assist these hearts in coping with overload has yet to be achieved. A number of biochemical defects have been excluded for the explanation of depressed contractility in failing hearts. Defective mitochondrial ATP production as a mechanism for reduced contractile force development has been extensively studied for the past three decades. Early studies have shown that heart failure can occur in the presence of normal myocardial perfusion and oxygen availability (35-37). Considerable effort was also focussed on whether or not the mitochondrial oxidative phosphorylation process is abnormal in heart failure (38-41). From studies involving the measurement of oxidative phosphorylation and the high-energy phosphate content in failing heart, it is apparent that changes in mitochondrial function are not related to the development of heart failure because the contractility of these hearts is impaired before the occurrence of any defect in mitochondrial function (41,42). Secondly, myocardial energy utilization was postulated to play a role in the development of heart failure because the external efficiency of the heart, manifested as the ratio of work performed to oxygen utilized, is usually depressed in chronic myocardial failure. In other words, the possibility of a defect in the conversion of metabolic energy to contractile work was implied and in this respect, much

evidence has accumulated to indicate that myosin heavy chains are differentially expressed and are associated with altered myofibrillar ATPase activity in heart failure (43-47). However, it has been suggested that this remodelling of the myocardium may increase the efficiency of the myocardium and thus represents a beneficial alteration rather than a cause leading to the development of heart failure (42,47).

When the critical role of Ca^{2+} ion in the excitation-contraction cycle was established, it became apparent that abnormalities in intracellular Ca^{2+} metabolism may be the basis of depressed contractility in heart failure. Specifically, it was suggested that either intracellular Ca^{2+}-overload or intracellular Ca^{2+}-deficiency was responsible for defective myocardial contractility as these events may initiate the disruption of energy-generating processes or abnormal activation of the contractile machinery (48). Since cardiac sarcolemma and sarcoplasmic reticular membranes are known to participate in the beat-to-beat regulation of myoplasmic Ca^{2+} levels (1,2,49,50), a great deal of research has been focussed on abnormal sarcoplasmic reticular function in failing myocardium and some work has been carried out to identify sarcolemmal defects in heart failure. Evidence for rapid regulation of myoplasmic Ca^{2+} levels by mitochondria is lacking, but these organelles are known to be capable of accumulating a large quantity of Ca^{2+} in vitro as well as under certain pathological conditions (49,51,52). Thus in light of the importance of Ca^{2+} ion in excitation-contraction coupling, it is intended to summarize the interaction of Ca^{2+} with myofibrils, sarcoplasmic reticulum, mitochondria, and sarcolemma in cardiac contraction and relaxation processes in health and disease. Since the term heart failure is frequently used in a loose manner, it is considered necessary to define this disease state before discussing abnormalities of Ca^{2+}

metabolism in heart disease. Acccordingly, heart failure has been defined as the pathophysiologic state in which cardiac dysfunction is responsible for failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues (53). Congestive heart failure may be defined as heart failure with accumulation of extracellular fluid in the lungs and extremities brought about by factors contributing to pressure and volume overload of the venous system, which are manifest clinically as dyspnea and are used to gauge the severity of left ventricular dysfunction (54). The presence of defective contractile function always results in heart failure, however heart failure is not always accompanied by an abnormality in intrinsic cardiac contractility. In this regard, it may be pointed out that heart failure may be brought about by abnormalities of the circulation, such as changes in blood volume or abnormalities in cardiac filling (53).

2. The function of contractile proteins in normal and failing hearts. Myofibrils are the contractile machinery of the cardiac cell and these form sarcomeres which occupy about 50 % mass of the cell (55). The length of cardiac sarcomere varies between 1.6 to 2.2 u and is dependent upon the state of contracture (56,57). Myofibrils are arranged end to end to lend a striated appearance to the cardiac myocyte (57,58). The thin filaments surround the thick filaments and interact by overlapping (57); two strands of actin form a double helix to comprise a thin filament (59). Although troponin and tropomyosin are known to play a regulatory role for the interaction of actin and myosin, an additional protein with a molecular weight of 150,000 (C-protein) has been identified in cardiac muscle (60). This C-protein has been shown to serve as a substrate for phosphorylation by cAMP-dependent protein kinase, however, the role of the interaction of this protein with other proteins in the contraction process is speculative (61). While actin and

myosin form cross-bridges and shorten against mechanical load at the expense of the chemical energy contained in the high-energy phosphate bonds of ATP, the tropomyosin and troponin proteins confer Ca^{2+} sensitivity on the contractile machinery (62). Troponin C is the subunit which binds Ca^{2+} and troponin I is known to inhibit Mg^{2+}-stimulated actomyosin ATPase, whereas troponin T serves to anchor the trimer to actin and tropomyosin (62). Binding of Ca^{2+} to the troponin C subunit derepresses the inhibition on binding of troponin I to actin, and thus actin and myosin are allowed to interact for contraction.

Cardiac muscle can respond to increases in preload (resting muscle length and sarcomere length) with increased muscle force over the physiologic range which is observed clinically as graded improvements in cardiac performance when diastolic volume of the heart is increased (63,64). Recently, a study of the effects of length on Ca^{2+} sensitivity during tension development on isolated demembranated cardiac muscle was made, in which the cardiac troponin C was substituted with skeletal troponin C (65). These results suggest that intrinsic molecular properties of the cardiac troponin C subunit confer the length-induced autoregulation of the troponin complex which in turn regulates the activation of the actin filaments. In vitro evidence indicates that phosphorylation of serine, threonine, or tyrosine residues of the troponin I subunit by cAMP-dependent protein kinase occurs in the myocardium (66). Since the phosphorylation of troponin I in isolated troponin complex was shown to reduce Ca^{2+} affinity, it has been suggested that the role of this catecholamine-specific phosphorylation process is associated with an increase in the rate of relaxation of the heart (67). The dark central A band of the sarcomere (thick filament), is about 1.5 u in

length, and is composed of myosin proteins (58,63). Myosin consists of a rodlike tail section each with a globular head made up of two heavy-chain subunits which are the site of ATPase activity (68). The heavy-chain composition of the myosin head is related to the intrinsic ATPase activity of the myofibrils and speed of contration of the myocardium. Three myosin isozymes have been described - V_1, V_2, and V_3- composed of alpha-alpha, alpha-beta, and beta-beta subunits, respectively (46,47). Myosin ATPase activity is highest in the V_1 and lowest in the V_3 isoforms. It is known that the presence of a given myosin isoform is not associated with any alteration in Ca^{2+} sensitivity of cardiac myofibrils (47).

Remodelling of the failing heart as a shift in myocardial isozyme content from V_1 to V_3 has been demonstrated in many types of experimental heart failure (69-71,314). This isoenzymatic shift is believed to occur at the transcriptional level and has been demonstrated in a wide variety of mammalian hearts subjected to overload (72). Support for the transcription regulation of these protein changes is evidenced in response to stimulation of $alpha_1$-adrenoceptors in neonatal rat cardiomyocytes and it has been suggested that hypertrophy in these cells is characterized by selective up-regulation of early developmental contractile protein isogenes, including those for skeletal alpha-actin and ß-myosin heavy chain (73). Alteration of contractile proteins is not confined to the myosin heavy-chains, as changes in human light-chain myosin isoforms have also been demonstrated in human hearts subjected to increased mechanical stress (74). Increased synthesis of V_3 myosin isozyme could explain many of the functional changes present in the failing heart, such as the characteristic depression of the force-velocity curve. With regard to energy utilization, it has been suggested that the increase in mechanical energy derived from ATP used at the expense of

depressed rate of myocardial contraction, may actually be beneficial in heart failure (75). The shift of myosin isozyme content is known to occur in the atria of failing human heart, but has not been observed in human ventricle (76). Since the majority of isozyme myosin content of myocardium from ventricles of large mammals is V_3 (77), the shift in isozymes in response to hemodynamic overload may be precluded in these species. In this regard, autopsy samples from patients with hypertrophic cardiomyopathy were assayed for myosin Ca^{2+}-stimulated ATPase and isoenzyme content and the results indicated that similar ATPase activities existed in control and cardiomyopathic heart, and no evidence for the presence of different isozymes was found (78). Therefore this mechanism may not be held solely responsible for cardiac pump dysfunction observed in heart failure. The possibility of structural changes in actin, troponin, and tropomyosin in the failing heart requires detailed investigation for any meaningful conclusion. Since it is possible to vary the maximum shortening velocity of failing hearts by pharmacologic intervention that enhances intracellular Ca^{2+} movements, it can be argued that altered sarcoplasmic reticular, mitochondrial, or sarcolemmal function rather than contractile proteins may play a role in pathogenesis of contractile failure.

3. Sarcoplasmic reticular control of intracellular Ca^{2+} movements in normal and failing hearts. The cardiac sarcoplasmic reticular network is an important rapid intracellular Ca^{2+} sequestration and release site in the myocardium and is critical in delivery of Ca^{2+} (activator Ca^{2+} measured as the Ca^{2+} transient) for activation of the contractile machinery (49,79,80) in excitation-contraction coupling. It is evident that a spectrum in the relative contribution of the sarcoplasmic reticulum to the Ca^{2+} transient exists with respect to species, as rat myocardium receives the largest

contribution from the sarcoplasmic reticulum, and rabbit the least (81). The sarcoplasmic reticulum borders the myofibrillar contractile apparatus and sarcolemma and sarcolemmal T-tubule system. The energy-dependent Ca^{2+} uptake process of the sarcoplasmic reticular network depends on the function of the Ca^{2+}-stimulated ATPase which is believed to represent 50 - 90 % of the total protein content of the sarcoplasmic reticulum (49). The Ca^{2+} affinity (K_m of 0.5 uM) and V_{max} of Ca^{2+}-ATPase is sufficiently high to support the concept that the activity of this enzyme is the primary determinant of the rate of fall of the Ca^{2+} transient (82) ushering the relaxation phase of cardiac contraction.

Alteration in the activity of the sarcoplasmic reticular Ca^{2+}-stimulated ATPase may affect the excitation-contraction cycle (1,2). Regulation of contractile function is, in part, accomplished by hormonally controlled processes, and it is well established that cAMP-dependent as well as Ca^{2+} and calmodulin-dependent protein kinases phosphorylate phospholamban, a sarcoplasmic reticulum-bound protein (83) and thus were shown to increase the Ca^{2+}-stimulated ATPase activity. $ß_1$-adrenergic activation has been associated with an accelerated decline of the aequorin signal for cytoplasmic Ca^{2+} in rat ventricular muscle and in rat heart cells (50,84). Biochemical evidence suggests that phosphorylation of phospholamban allows an increase in the activity of the Ca^{2+}-stimulated ATPase and thus augmenting the Ca^{2+} sequestering ability of the sarcoplasmic reticulum (85). A different mode of regulation of sarcoplasmic reticular Ca^{2+}-stimulated ATPase wherein protein kinase C-mediated phosphorylation of sarcoplasmic reticular phospholamban has been demonstrated (86). Recent evidence for an inhibition (about 45 %) of Ca^{2+} accumulation by the sarcoplasmic reticulum upon treatment of cultured permeabilized heart myocytes with phorbol esters,

12-O-tetradecanoylphorbol-13-acetate (TPA), and phorbol 12,13-dibutyrate suggest that phosphorylation of phospholamban by this mechanism may cause inhibition of the sarcoplasmic reticular Ca^{2+}-stimulated ATPase (87). Muscarinic agonists have been shown to reduce hormone stimulation of adenylate cyclase, which has been associated with reduced tissue levels of cAMP and diminished phosphorylation of the sarcoplasmic reticular phospholamban (88).

The beat-to-beat presence of the intracellular Ca^{2+} transient in the myocardium is linked to cardiac sarcoplasmic reticular Ca^{2+}-release, and is mediated by distinct protein channels located in the membrane which can be blocked with ryanodine (50,89-91). Evidence is now available to suggest that ryanodine may lock the cardiac Ca^{2+}-release channel in a conduction state, resulting in more rapid loss of Ca^{2+} from the sarcoplasmic reticulum and thereby interfering with normal Ca^{2+}-release (82,92,93). The ryanodine receptor from sarcoplasmic reticulum of skeletal muscle has been shown to be the Ca^{2+}-permeable pore of the Ca^{2+}-release channel, with a molecular weight of about 450 kDa (94). The positive inotropic effect of caffeine, which is associated with prolonged Ca^{2+} transient (95), was previously believed to be due to inhibition of Ca^{2+}sequestration by the sarcoplasmic reticulum; however, this agent is now known to interact directly with the Ca^{2+}-release channels by increasing the open time and sensitivity of the channels to activation by Ca^{2+} (96). These actions of caffeine culmunating in excessive Ca^{2+}-release may effectively deplete the sarcoplasmic reticulum of stored Ca^{2+} (97). Although the mechanism of release of Ca^{2+} from the cardiac sarcoplasmic reticulum is somewhat controversial, much data are available to support the concept of Ca^{2+}-induced Ca^{2+} release as a pivotal component of cardiac excitation-contraction coupling (50,98-104).

Calsequestrin is a high capacity, moderate affinity, Ca^{2+}-binding protein localized in the lumen of junctional sarcoplasmic reticulum in cardiac

and skeletal muscle (105,106). Recent anatomical evidence from rat myocardium indicates that the Ca^{2+} accumulated across the lumen of the sarcoplasmic reticulum is then bound to calsequestrin stored in the junctional and corbular sarcoplasmic reticulum (107), and these authors suggest that both the corbular sarcoplasmic reticulum and the junctional sarcoplasmic reticulum are potential sources of releasable Ca^{2+}. Although the exact role of calsequestrin in cardiac muscle is not clear, results from a recent study using skeletal muscle suggest that Ca^{2+}-dependent conformational changes of calsequestrin affected the junctional face of the membrane-bound proteins including the 400 kDa Ca^{2+}-release channel and that calsequestrin may then regulate Ca^{2+}-release into the cytoplasm (108). It is known that cardiac and skeletal calsequestrin bind similar amounts of Ca^{2+}, even though structural differences exist between the cardiac and skeletal types (109,111), presumably by acting as a charged surface rather than by presenting multiple discrete Ca^{2+}-binding sites (109). Experiments with skinned cardiac cells have shown that the inactivation of sarcoplasmic reticular Ca^{2+}-release is Ca^{2+}-dependent (100) and thus there may be a negative feedback system for the release of Ca^{2+} from the sarcoplasmic reticulum.

Among the first experiments designed to assess sarcoplasmic reticular function were those carried out over twenty years ago in microsomal preparations from spontaneously failing canine heart-lung preparations which showed that Ca^{2+}-uptake and Ca^{2+}-stimulated ATPase activities were depressed (110). Since depressed activity of the sarcoplasmic reticular Ca^{2+}-stimulated ATPase could cause reduced sequestration and reduction of the amount of activator Ca^{2+} available for contraction, these changes could conceivably alter function of the cardiac pump, by slowing the normal relaxation phase (2,112). A number of biochemical studies have clearly

demonstrated abnormalities of the sarcoplasmic reticular function in vitro in various animal models of heart failure as well as in failing human heart (1). Specifically, early observations of depressed rate of sarcoplasmic reticular Ca^{2+}-uptake were made in failing heart muscle obtained from humans (113), rabbits (114), and hamsters (115,116). In rabbits with hypertrophied hearts secondary to aortic insufficiency, alterations in sarcoplasmic reticular Ca^{2+}-uptake did not occur until late stages, but were further depressed with the development of overt left ventricular failure (117). In studies of heart failure due to bacterial infection in rabbits, and cardiomyopathic hamsters (BIO 14.6), depressed calcium uptake activities of the microsomal fraction was noted with no accompanying change in the sarcoplasmic reticular Ca^{2+}-stimulated ATPase activity in either species model (115,118). It was suggested that changes in sarcoplasmic reticular function may be depend on the type and stage of heart failure (1). In this regard, a recent study of experimental congestive heart failure secondary to myocardial infarction in rat has demonstrated depressed sarcoplasmic reticular Ca^{2+}-uptake and Ca^{2+}-stimulated ATPase activity in sarcoplasmic reticulum isolated from hearts of animals in severe stage of heart failure when compared to control values (119). Data from a recent biochemical study of isolated sarcoplasmic reticular vesicles isolated from left ventricles of human hearts with idiopathic dilated cardiomyopathy failed to observe any change in Ca^{2+}-uptake properties from this organelle, and it was suggested that abnormal Ca^{2+}-handling in the myocardium of these patients was not due to altered sarcoplasmic reticular Ca^{2+}-handling properties (120). Other reports have indicated that alterations observed in sarcoplasmic reticular function in heart failure are at the transcriptional level, insofar as hypertrophy of rat and rabbit hearts is associated with a decreased myocardial level of

sarcoplasmic reticular Ca^{2+}-ATPase mRNA and protein concentration (121,122). Similar changes have been reported in a pooled group of patients with idiopathic cardiomyopathy, coronary artery disease with attendent myocardial infarction, or valvular insufficiency when compared to hearts of patients without heart failure (123). The possibility of a specific defect in the stimulation of Ca^{2+}-uptake by phospholamban has been studied with the use of phospholamban-specific monoclonal antibodies, whereupon binding of this antibody to isolated sarcoplasmic reticulum prepared from normal and failing hearts was taken as simulation of phosphorylation of phospholamban by cAMP-dependent protein kinase (124). Since the observed stimulation of sarcoplasmic reticular Ca^{2+}-stimulated ATPase was similar among failing and normal hearts, these investigators suggested that the pathogenesis of heart failure in idiopathic dilated cardiomyopathy is independent of intrinsic alteration of this mechanism. As the Ca^{2+} transient is released from the sarcoplasmic reticulum in a graded response to permeation of extracellular Ca^{2+} across the sarcolemma (50), studies of sarcolemmal function in the pathogenesis of contractile failure in patients with idiopathic dilated cardiomyopathy are warranted. Nevertheless, it appears that defective sarcoplasmic reticular function may represent a cause of contractile failure as many of the abnormalities associated with this membrane occur concomitantly with loss of cardiac contractile function.

Since changes in intracellular Ca^{2+} handling are not identical in every type or stage of heart failure (1), consideration of the specific abnormalities of cellular membrane function in the pathogenesis of any given type of heart failure is necessary. Thus it behooves the investigator to take note of the particular etiology of heart failure when studies on subcellular organelle function are carried out in humans. With regard to animal models of

heart failure, the occurrence of intracellular Ca^{2+}-overload is believed to exist in experimental models of genetic cardiomyopathy, hypertrophy due to pressure overload, diabetic cardiomyopathy, and acute ischemia-reperfusion injury (1,2,3,125). The intracellular Ca^{2+} transients measured with aequorin from right ventricular tissue of patients with idiopathic dilated cardiomyopathy, ischemic heart disease, valvular insufficiency, or hypertrophic cardiomyopathy were both markedly diminished and prolonged (126,127). These muscles were characterized by a concomitant prolongation of isometric tension development with a delay of relaxation and is consistent with a reduced rate of sequestration and release of intracellular stores of Ca^{2+} (127). Accordingly, it was concluded that impaired uptake of calcium by the sarcoplasmic reticulum results in a rise in the cytoplasmic concentration of Ca^{2+} and resting tension. Although it was speculated that changes in myofibrillar sensitivity to Ca^{2+} was the cause of abnormalities in cardiac function in these patients, it should be pointed out that the sensitivity of the myofibrils to Ca^{2+} is known to be unaffected by the different structural elements of the thick filaments that occur as myosin isozymes (128).

There is controversy about the response of the failing myocardium to increases in extracellular Ca^{2+} (125,129,130). Furthermore, it has been shown that the efficacy of Ca^{2+}-antagonist (vasodilator) therapy of some patients suffering from congestive heart failure is questionable, whereas vasodilator therapy with nitrate drugs is beneficial to the condition of these patients (131). Therapy to increase the contractile force development in the failing heart with ouabain in patients with congestive heart failure, is associated with increased delivery of Ca^{2+} to the myocardium (19,133); this remains the most widely used mode for treatment of these patients (54). Thus

it is conceivable that an insufficient amount of Ca^{2+} may be delivered to the myofilaments in myocardium of patients with congestive heart failure. While some of the confusion in this field may result from the type and stage of congestive heart failure, it is also conceivable that the each type of failure may be associated with specific defects in intracellular Ca^{2+} metabolism ultimately resulting in either intracellular Ca^{2+}-overload or Ca^{2+} deficiency. The exact role of the sarcoplasmic reticulum in terms of Ca^{2+} release and Ca^{2+} uptake at different stages of congestive heart failure has not yet been clearly defined.

4. Ca^{2+} movements in mitochondria in normal and failing hearts. Although the ability of mitochondria to take up large amounts of calcium has been known for more than twenty-five years, relatively low affinity of mitochondria for Ca^{2+} (10 uM) and low rate of Ca^{2+} transport (0.6 umol per mg protein per min) have suggested that the importance of mitochondria as cytosolic buffers was overestimated (49). Furthermore mitochondrial Ca^{2+} content is now known to be much lower in situ (1 or 2 nmoles per mg protein) than was estimated from isolated mitochondrial preparations (132). Thus at submicromolar free cytosolic Ca^{2+} levels, the rate of Ca^{2+}-uptake by the low affinity system of the mitochondria is marginal, however, this uptake was postulated to be continuous due to the presence of a transmembrane potential and this could eventually lead to calcification of the organelle (49). Mitochondrial Ca^{2+}-uptake in normal myocardium is known to be balanced by electroneutral release of Ca^{2+} in exchange for transmembrane Na^{+} ion so that a slow dynamic equilibrium of Ca^{2+} movement is achieved (134). The suggestion that extramitochondrial Na^{+} may play a role in the release of Ca^{2+} from the mitochondrial stores has been made; however, a review of data from studies of this nature discount this mechanism and organelle as an important means of

delivery of Ca^{2+} in the normal myocardium (49). Nevertheless, it has been indicated that increases in intracellular Na^+ by pharmacologic means such as digitalis treatment may evoke release of intramitochondrial Ca^{2+} to the myoplasm (49). Evidence for existence of mitochondrial H^+/Ca^{2+} exchange and Na^+/H^+ exchange mechanisms have been put forward by several investigators (135-138) but their significance in terms of contractile function of the cardiac muscle is not clear at present.

A number of studies have been carried out concerning the Ca^{2+} handling capacity of mitochondria in experimental heart failure (1). In rabbits with heart failure due to aortic regurgitation, cellular Ca^{2+} content was assayed and found to be normal, however, a redistribution of Ca^{2+} to the mitochondria was noted (52). This redistribution of cellular Ca^{2+} from defective sarcoplasmic reticulum to the mitochondria may diminish availability of Ca^{2+} to the contractile machinery and thus can be seen to reduce the ability of cardiac muscle to generate contractile force. The rate and capacity of energy-linked Ca^{2+} uptake by mitochondria from hearts of cardiomyopathic hamsters have been demonstrated to be reduced (115) and this has also been suggested to impair contractile force development. Although cardiac mitochondria are not considered to participate in delivery of Ca^{2+} for the genesis of the Ca^{2+} transient, these organelles may play a role in the disruption of intracellular Ca^{2+} homeostasis in the failing myocardium. Nonetheless, it is commonly held that mitochondria serve as a buffer for the regulation of intracellular Ca^{2+} in diseased myocardium (1).

5. Sarcolemmal Ca^{2+} movements in normal and failing hearts. The cardiac sarcolemma functions in the generation and maintenance of transmembrane gradients of Na^+, K^+, and Ca^{2+} that are essential for membrane excitability and the regulation of myoplasmic Ca^{2+} levels. Operating

together, sarcolemmal membrane-bound ion channels, ion-exchange systems and ATPase pumps contribute to either the regulation of membrane potential or the delivery and removal of Ca^{2+} cardiac excitation-contraction coupling process.

a. Voltage-sensitive Ca^{2+} channel mediated Ca^{2+}-influx. The main components which determine the shape of cardiac ventricular action potential are the rapidly activating and inactivating Na^+ current (I_{Na}), the more slowly activating and inactivating inward Ca^{2+} current (I_{Ca}), and the time-independent and time-dependent outward currents carried by K^+ (I_K) (139). Evidence of graded sarcolemmal Ca^{2+} current (I_{Ca})-regulated Ca^{2+} release from the sarcoplasmic reticulum in rat myocytes points to the Ca^{2+}-channel in the sarcolemmal membrane as an important mechanism for excitation-contraction coupling in the myocardium (50). The voltage-sensitive slow Ca^{2+} channel or L-type Ca^{2+} channel is labelled by the dihydropyridine class of the Ca^{2+}-antagonist drugs (140). Although the precise function of the T-type Ca^{2+} channel is not clear, it has been demonstrated that these channels activate and deactivate at more negative transmembrane potentials than the L-type and may participate in very rapid Ca^{2+} permeation to the myocyte (141). Ca^{2+} passes through the time-dependent gated slow Ca^{2+} channel down its concentration gradient with a conductance of 15 - 25 pS, and each channel may allow passage of about 3×10^{-6} Ca^{2+} ions per sec (49,139,142, 143) in response to a conformational change in the protein channel gate which is sensitive to membrane electrical potential (144). Thus the Ca^{2+} channels must have, in addition to a selectivity filter for Ca^{2+}, voltage sensors which are suggested to be charged regions within the protein for the regulation of open or closed states of the channel gates (144). Consideration of the density and conductance properties of Ca^{2+}-channels have allowed

calculation of unbuffered rise in myoplasmic Ca^{2+} concentration in the myocardium (49) and it was estimated that transsarcolemmal Ca^{2+} influx would result in a rise in the intracellular concentration of free Ca^{2+} to about 1 uM, assuming uniform distribution within the cell. This scenario is unlikely to be accurate when one considers the limits of ion diffusion and buffering action of internal Ca^{2+} binding sites (49,145). It was hypothesized that sarcolemmal Ca^{2+} concentration upon excitation may reach relatively high levels in the narrow zone just below the plasma membrane (49).

Since the density of L-type slow Ca^{2+}-channels in the cardiac sarcolemma is an order of magnitude lower than that present in skeletal muscle sarcolemma, most investigators have used the latter tissue as a source for research directed at elucidation of the structure of this channel. The interpretation of the results from skeletal muscle has led investigators to suggest a theoretical model of dihydropyridine-sensitive Ca^{2+}-channels (146). The subunit which is believed to form the channel ($alpha_1$) is known to contain Ca^{2+}-antagonist binding sites, cAMP-dependent phosphorylation sites, and hydrophobic domains which are homologous to transmembrane domains of the rat brain sodium channel alpha subunit. Located on the cytoplasmic side of the membrane and closely associated with the $alpha_1$ subunit, the smaller ß-subunit is also known to be phosphorylated by cAMP-dependent protein kinase. The Ca^{2+}-antagonist drug binding sites are associated with the $alpha_2$ and delta subunits which are located at the extracellular side of the membrane. Finally the gamma subunit is localized at the extracellular side, contains a transmembrane segment, and exists as a glycoprotein. Although differences undoubtably exist between cardiac and skeletal muscle dihydropyridine Ca^{2+}-channels, which may be manifested functionally as voltage-dependent activation of contraction in skeletal muscle and Ca^{2+}-

dependent activation of contraction in cardiac muscle, a model of skeletal muscle Ca^{2+}-channel may yield some insight into the structure and function of cardiac Ca^{2+}-channels. Recent work utilizing complementary DNA strands for the skeletal muscle dihydropyridine-sensitive sensitive Ca^{2+}-channels have confirmed the findings that ß-subunit is a peripheral membrane protein and is without homology to any known protein sequences (147). It was also suggested that this protein contains sites which are preferentially phosphorylated by protein kinase C and GMP-dependent protein kinase. The complementary DNA strands for $alpha_1$ and $alpha_2$ subunits were also used to deduce the structure of these subunits (148) from skeletal muscle. It was confirmed that $alpha_1$ and $alpha_2$ are hydrophobic and hydrophillic proteins, respectively, and it was suggested that $alpha_1$ and $alpha_2$ are expressed differentially from gene families in a tissue specific manner.

Inactivation of the open state of the cardiac cell slow Ca^{2+}-channel is voltage- and Ca^{2+} ion-dependent (142). The dynamics of channel operation has been explored with the use of voltage- and patch-clamping technologies and it was found that opening occurred in bursts interspersed with relatively long quiescent periods (4). Biochemical studies of passive Ca^{2+} movement through Ca^{2+}-channels in isolated sarcolemmal vesicles indicated that a fraction of Ca^{2+} movement into vesicles upon artificial polarization-depolarization cycling could be ascribed to these channels; however, these experiments were confounded by Ca^{2+} movements via the sarcolemmal Na^{+}-Ca^{2+} exchange mechanism (149,150). The possibility that the sarcolemmal membrane isolation process may alter the structure of the slow Ca^{2+}-channel was raised by experiments demonstrating a diminution of time-dependent properties and sensitivity to Ca^{2+}-blockers (151). The discrepancy in the binding affinity of Ca^{2+}-antagonist drugs to cardiac membranes in the intact cell and to

sarcolemmal preparations in vitro was postulated to be due to binding affinity dependency on the inactivation state in the whole cell membrane (152). Recently, modulation of smooth muscle L-type calcium channels by ATP was reported wherein application of ATP (1-5 mM) to a patch-clamp preparation was associated with an increase in the number of channels available for opening and/or the probability of their being in an open state, with no change in current amplitude or mean open time (153). Further results from this study indicated that Bay-K-8644 may change the nature of channel activation by making it resistant to deterioration with time. Ca^{2+}-channels may also be involved in the positive inotropic effects of digitalis in the myocardium as it has been shown with the patch-clamp technique that amplitudes of low-threshold T-type and high-threshold L-type Ca^{2+}-currents were significantly increased in the presence of 1 uM ouabain (154).

Biochemical ligand-binding characteristics of L-type Ca^{2+}-channels in the sarcolemmal membrane has been described in some experimental models of heart failure. Data from several reports indicate that increased sarcolemmal Ca^{2+}-channel density is present in hearts of genetically cardiomyopathic hamsters (5,6,155). However, in older cardiomyopathic hamsters suffering extensive cardiac hypertrophy and congestive heart failure, no change in Ca^{2+}-channel density was observed (7). Increased sarcolemmal Ca^{2+}-current has recently been described in diabetic rat heart wherein increased action potential duration was evident and the Co^{2+}-induced action potential shortening effect was more pronounced when compared to control values; these changes were not accompanied by a concomitant alteration Na^{+}-Ca^{2+} exchange current or transient outward current (156). Thus two different experimental models of cardiomyopathic disease, which are associated with the occurence of intracellular Ca^{2+}-overload (2), are associated with either increased

Ca^{2+}-channel density or increased sarcolemmal Ca^{2+} current. Although intracellular Ca^{2+}-overload is also associated with ischemic heart disease, it has been shown that [^{3}H]-nitrendipine and [^{3}H]-verapamil binding site density is reduced in hearts subjected to global ischemia or hypoxia-reoxygenation injury (8,9). The number of [^{3}H]-nitrendipine binding sites in the myocardium does not seem to be linked to the incidence of myocardial hypertrophy as [^{3}H]-nitrendipine binding sites were shown to be increased and decreased in hearts of 22 week old spontaneously hypertensive rats and 28 day aortic banded rats, respectively (34). Furthermore, the number of Ca^{2+}-channels in hypertrophied right ventricle of rats with congestive heart failure secondary to large myocardial infarction of the left ventricular free wall was comparable to control values (157). Thus the status of Ca^{2+} channels may depend on the type of heart failure.

b. Beta-adrenergic receptor mediated Ca^{2+}-influx. Sympathetic stimulation of the heart produces an increase in the strength and rate of cardiac contraction through cAMP-dependent protein kinase mediated phosphorylation of slow Ca^{2+}-channels for increased transsarcolemmal Ca^{2+}-influx (4,139,142,158-160). Injection of an inhibitor of the endogenous cAMP-dependent protein kinase into myocardial cells showed a diminution of slow inward current (159,161). Biochemical experiments indicate that cAMP-dependent phosphorylation in the isolated sarcolemmal preparations was associated with an increase in the intravesicular uptake of Ca^{2+} (162). This study was criticized with respect to the significance of the measured Ca^{2+}-transport activity as well as the role of a sarcolemmal-bound phospholamban in this process (163); some investigators failed to reproduce these results (164). Although the existence of phosholamban in sarcoplasmic reticulum is well documented, evidence for the localization of phospholamban

in the sarcolemmal membrane is not clear cut. Nonetheless, it has been suggested that phosphorylation of phospholamban may be a mechanism for the increase of the slow Ca^{2+} current due to catecholamines (165). Recent investigation of the mechanisms of calcium channel modulation by ß-adrenergic agents has revealed that two different factors underlie the enhancement of Ca^{2+}-channel activity and these include an increase in the number of functional channels and an increase in the proportion of open channels (166). These patterns of modulation are remarkably similar to those observed in other experiments of ATP modulation of L-type Ca^{2+}-channels (153).

Guanine nucleotide binding G proteins are known to function as couplers of receptors to enzyme effectors, and thereby play a critical role in the response to hormonal signalling (167,168). In the myocardium there exists two mechanisms for the activation of Ca^{2+}-channels; the first is circuitous involving G_S activation of the adenylate cyclase enzyme in response to the activation of ß-adrenoceptor which then catalyzes the formation of cAMP from ATP to activate the cAMP-dependent protein kinase whereas the second mechanism is the direct activation of Ca^{2+}-channel by a G proteins (168). The presence of G_i and G_S proteins in the sarcolemmal membrane has been demonstrated and their role was initially believed to be limited to the inactivation and activation of the adenylate cyclase enzyme, respectively. It is also known that the alpha-subunt of G_i may inhibit the adenylate cyclase enzyme directly or via the beta and gamma subunits (169-171). Since then, the alpha-subunit of G_S has been shown to increase sarcolemmal Ca^{2+}-channel activity (172) whereas the activation of ß-adrenoceptor is necessary for operation of this pathway. The physiological significance for the existance of the two pathways for modulation of the sarcolemmal Ca^{2+}-channel is not clear; however it has been suggested that G proteins may function as both

integrators and signal transducers in the myocardium (172).

Since the sympathetic regulation of cardiac contractility is crucial in adjusting blood flow to meet the metabolic requirements of the body, considerable effort has been directed at elucidating the status of the cardiac sarcolemmal β_1-adrenoceptor and associated protein systems in experimental and clinical heart failure (10,11,13-16). A review of early studies which focussed on the adenylate cyclase activity from hearts of different strains of cardiomyopathic hamsters, rabbits with bacterial endocarditis and aortic constriction, and of rats with substrate-lack, Ca^{2+}-lack, or Ca^{2+}-overload observed that this activity was decreased, and that this depression was associated with the degree of failure in some experiments (1). Deficient cAMP production has also been shown in the failing human heart (173). The first to document a defect in beta receptor density in heart failure was Bristow et al (11) wherein a decrease in these receptors were described in failing human heart; this finding has been confirmed recently (174). The cardiac G_i protein (associated with the adenylate cyclase enzyme) has been shown to increase in hearts of patients with idiopathic dilated cardiomyopathy, and more recent data indicates that this change occurs concomitant with increased cellular levels of mRNA encoding for G_i and G_s alpha-subunits in failing human hearts compared to nonfailing human hearts (175,176). Since the time of discovery of altered ß-adrenergic number in humans, many similar studies have been carried out on experimental animal models of heart failure. A loss of high affinity cardiac ß-adrenergic receptors and G_s protein content has been observed in pressure-overloaded dog hearts compared to controls (14,177). A decrease of ß-adrenergic receptor number was reflected by a decrease in cell surface binding sites with no change in the total cellular content of these receptors in diabetic rat heart (178); insulin treatment was shown to reverse

the internalization of these receptors. Aortic banding in rats was associated with a decrease in myocardial responsiveness to ß-adrenoceptor agonist stimulation and decreased ß-adrenoceptor density (179). The viable myocardium of rat hearts 3 weeks following induction of myocardial infarction was tested for ß-adrenoceptor density, and no significant difference was found when compared to controls (180). Similar results were obtained from animals of the same model after either 2 or 7 days following coronary occlusion (181). On the other hand, a loss of sensitivity to adrenergic stimulation and a depression of ß-adrenoceptor density and affinity was shown in uninfarcted left ventricle 3 days following coronary occlusion (15,16). Hearts from genetically cardiomyopathic hamsters and from thyroxine-treated rats were associated with increased density of ß-adrenoceptors when compared to controls (13,182). A study of spontaneously hypertensive rats revealed that the number of myocardial ß-adrenoceptors were decreased, and this change was subsequently shown to be due to cellular internalization of these receptor proteins, and that the decrease in apparent receptor numbers may be a shift in the receptors between two cellular pools (183,184).

Given the variation of results within the experimental models studied, a unifying explanation for the mechanism of alterations in adrenergic receptor has yet to be put forward; however, two possibilities exist. First, ischemic or hypoxic injury results in decreased ß-adrenoceptor density in the myocardium (185,186) because low-output cardiac failure, which is associated with decreased delivery of oxygen to tissues including the heart (187), could cause a reduction in the number of receptors. Secondly, there is a body of evidence to suggest that cardiac-derived norepinephrine, by binding to the receptor, may initiate receptor down-regulation and thus provide an autoregulatory mechanism (188-190). An increase in ß-adrenoceptor number has

been reported in patients with idiopathic dilated cardiomyopathy receiving selective β_1-adrenoceptor antagonist therapy (188). A correlation between coronary sinus norepinephrine levels and ß-adrenoceptor down-regulation has been made in the failing human hearts (189) and it is known that norepinephrine is a powerful stimulus for decreasing the density of these receptors in some heart cell systems (190). Although the affinity of β_1-adrenoceptors for norepinephrine is higher than that of cardiac β_2- or alpha-adrenoceptors (190), there is reason not to accept this mechanism for explaining the observed changes in ß-adrenoceptor density. A weak correlation ($r = 0.6$) of coronary sinus norepinephrine to total ß-adrenoceptor density in the failing human hearts has been shown and it is known that 30 - 50 % of β_1-adrenoceptors are resistant to norepinephrine-induced down-regulation in myocyte model systems (190). Thus defects in ß-adrenoceptor number, G_i or G_s number, and adenylate cyclase function may be present in heart failure, depending on the stage and type of failure and these changes may be mediated in part at the transcriptional level.

c. Alpha adrenergic receptor mediated Ca^{2+}-influx and intracellular mobilization. While the positive inotropic effects of catecholamines in the heart are mainly due to the activation of ß-adrenoceptors, $alpha_1$-adrenoceptors have also been shown to exist in the heart and the inotropic effect of selective alpha-agonists (achieved in most cases in the presence of a ß-blocking agent) has been described (190,191). The magnitude of the positive inotropic response to $alpha_1$-adrenoceptor stimulus has been shown to vary with species (193). The density of $alpha_1$-adrenoceptors in human myocardium has been found to be marginal as the effects of noradrenaline and adrenaline in human ventricular heart muscle were not affected by prazosin (194,195). Furthermore, the maximal inotropic effect of $alpha_1$-adrenoceptor

stimulation was about 10 % of that seen in response to ß-adrenoceptor stimulation (195). Thus the physiologic significance of these receptor systems in modulation of normal heart function is held in question while a greater functional importance of $alpha_1$-adrenoceptors may exist in pathophysiologic states wherein the response of the myocardium to ß-adrenergic stimulation is attenuated.

The mechanism of $alpha_1$-adrenoceptor mediated effects in the myocardium are likely independent of the adenylate cyclase cAMP-dependent protein kinase system. Adenylate cyclase activity was not decreased in rat cardiac myocytes when $alpha_1$-agonist drugs were applied to this preparation (196). Stimulation of $alpha_1$-adrenoceptors in the rat heart was associated with an increase in the Ca^{2+}-channel mediated slow inward current, and it was speculated that the concomitant phosphorylation of a 15 kDa protein may be involved (197). The lack of a substantial rise in the intracellular aequorin signal for Ca^{2+} in experiments concerning the effects of phenylephrine on the myocardium led to the suggestion that an increase in the responsiveness of the contractile proteins may be responsible for the $alpha_1$-adrenoceptor stimulation (198).

The nature of the signal transfer from $alpha_1$-adrenoceptor to the contractile machinery is postulated to involve the activation of a specific G-protein (G_p) for the activation of phospholipase C, which cleaves phosphatidylinositol-bisphosphate (PIP_2) for the subsequent production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol, both believed to function as second messengers (199). The role of IP_3 in the myocardium is controversial as evidence exists for both the release of Ca^{2+} from sarcoplasmic reticular stores in skinned myocyte preparation (200) and lack of effect on cardiac myocytes (313). Intracellular diacylglycerol remains

localized in sarcolemma to activate protein kinase C which may be involved in phosphorylation of sarcolemmal proteins (199,202) including sarcolemmal phospholamban (203). Since the tumor-promoting phorbol esters were able to mimic the endogenously produced diacylglycerol, considerable attention has been directed to the effects of phorbol esters and synthetic diacylglycerol analogues on the myocardium. Application of phorbol esters to neonatal rat heart myocytes was associated with an increase in the slow inward Ca^{2+} current (204) whereas phorbol myristate acetate (PMA) was observed to reduce the amplitude of this current in cultured chick heart cells (201). Although the results are somewhat conflicting, the possibility remains that protein kinase C may play a role in signal transduction initiated due to the activation of $alpha_1$-adrenoceptors. Lately, in experiments which focussed on phosphoinositol second messenger mechanisms, a synergism of IP_3 and IP_4 in the activation of calcium-dependent potassium channels has been demonstrated (205). It was suggested that IP_4 may operate to promote the extracellular entry of Ca^{2+} into cardiac cells.

The status of cardiac sarcolemmal alpha-adrenoceptor has not been extensively studied in heart failure, presumably due to the lack of knowledge with regard to the functional significance of this system in normal human cardiac function. Chronic infusion of either isoproterenol or norepinephrine failed to cause a change in the myocardial $alpha_1$-adrenoceptor density or affinity of binding in rats (206). $Alpha_1$-adrenoceptor density was observed to be significantly decreased and increased in ventricles from hyperthyroid and hypothyroid rats, respectively (207). Studies of hearts from guinea-pigs with pressure-overload, an increase in $alpha_1$-adrenoceptor density was observed in comparison to control (208). In regions of cat heart subjected to ischemia as well as in preparations of hypoxic adult canine myocytes, alpha-

adrenoceptor binding sites were shown to be increased (209,210). In contrast to these results, no change of $alpha_1$-adrenoceptor density was observed in failing human hearts compared to non-failing control, probably because these receptors only represent a small fraction of the total adrenoceptor population in the human myocardium (174,211). These results are in contrast to a recent report of an increase in alpha-adrenoceptor density in hearts of patients with idiopathic dilated cardiomyopathy (17). It is noted that even if no change could be seen in the absolute density of these receptors, $alpha_1$-receptors represent a greater proportion of the total adrenergic receptor population in heart failure (212). Thus the relative contribution of alpha-adrenoceptors to Ca^{2+} sarcolemmal membrane mediated adrenergic response may be augmented in the failing heart.

d. Na^+-Ca^{2+} exchange and Ca^{2+} pump mediated Ca^{2+}-fluxes. Since the discovery of Na^+-dependent Ca^{2+} transport in over twenty years ago, efforts have been made by many investigators to elucidate the kinetic properties, regulation, physiologic and pathophysiologic role of sarcolemmal Na^+-Ca^{2+} exhange system in excitable tissues including the heart (2,139,213). Mullins (214) was the first to show that electrogenic Na^+-Ca^{2+} exchange could both bring Ca^{2+} into the cell during the action potential making a direct contribution to the Ca^{2+} transient and extrude Ca^{2+} from the cell when intracellular Ca^{2+} was high. The cardiac Na^+-Ca^{2+} exhange system has been localized in the sarcolemmal membrane since the co-purification of this activity occurred with sarcolemmal markers (215). The Na^+-Ca^{2+} exchanger is able move 3 Na^+ ions for a single Ca^{2+}, thereby imparting a charge movement across the membrane (214,216). Charge imbalance occurs in sarcolemmal preparations which is proportional to the activity of Na^+-Ca^{2+} exchange and is known to inhibit further activity;

this inhibitory effect is prevented by increasing potassium-diffusion with valinomycin (217). A review of the biochemical literature indicates K_a values of Na^+-Ca^{2+} exchange for Ca^{2+} at 15-40 uM and the $K_{(1/2)(Na^+)}$ values at 7-32 mM (218). A recent report of Na^+-Ca^{2+} exchange velocity in cardiac sarcolemmal vesicles has indicated that the initial velocity of the exchange process is about 40.0 nmol/mg protein/sec (219). This finding suggests that the sarcolemmal Na^+-Ca^{2+} exchange system is large and may be capable of rapid movements of Ca^{2+} in and out of the myocardial cell.

The regulation of sodium-calcium exchange has been reported to be mediated by phosphorylation/dephosphorylation reactions which are modulated by Ca^{2+}-calmodulin dependent kinase and phosphatase (220). Phospholipase D and C treatment of isolated cardiac sarcolemma vesicles has been associated with increased Na^+-Ca^{2+} exchange activity. This change was suggested to be due to increased content of negative charge in the membrane which could increase the Ca^{2+} bound to the membrane and thus enhance Na^+-Ca^{2+} exchange activity (218). Data from a recent report of specific phosphatidylinositol cleavage-mediated stimulation of Na^+-Ca^{2+} exchange activity in sarcolemmal vesicles suggest that phosphatidylinositol itself may operate as an inhibitor to Na^+-Ca^{2+} exchange or this phospholipid may anchor an inhibitory protein to the membrane (221). It has also been demonstrated that Na^+-Ca^{2+} exchange in the sarcolemmal vesicle preparations was stimulated by cholesterol (222). On the other hand, phospholipid N-methylation of the sarcolemmal vesicles was reported to inhibit the Na^+-Ca^{2+} exchange activity (223).

The question surrounding the physiologic significance of sarcolemmal Na^+-Ca^{2+} exchange is not easily met, however, some insight to this matter has been provided in the form of two recent electrophysiologic studies (29,224). Release of sarcoplasmic reticular Ca^{2+} stores (activator Ca^{2+})

has been demonstrated occur in response to graded sarcolemmal Ca^{2+}-influx (50), and the Na^{+}-Ca^{2+} exchange mechanism has been suggested to be activated to cause transsarcolemmal Ca^{2+}-influx subsequent to a rapid influx of Na^{+} through the fast Na^{+} channels; this Ca^{2+} movement is associated with release of activator Ca^{2+} from the sarcoplasmic reticulum (29). On the other hand, to support the argument that the exchange mechanism may function to mediate sarcolemmal Ca^{2+}-efflux, Bridge et al (224) provided evidence to suggest that Ca^{2+} entering the heart cell during excitation is extruded by this system. Thus the sarcolemmal Na^{+}-Ca^{2+} exchange may be important in regulation of both influx and efflux of Ca^{2+} and thus can be seen to be implicated in contractile function of the heart.

The Ca^{2+}-stimulated ATPase localized in the cardiac sarcolemma and described biochemically as Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase, is generally believed to be involved in the transsarcolemmal Ca^{2+}-extrusion (2,49,225). Much information has been gathered from biochemical studies of ATP-dependent Ca^{2+} transport in isolated sarcolemmal vesicles and it has been shown that this activity co-purifies with other sarcolemmal activities such as Na^{+}-K^{+} ATPase enzyme and Na^{+}-Ca^{2+} exchange (225-227). Furthermore, sarcolemmal ATP-dependent Ca^{2+}-transport was shown not to be artifactual contamination of the sarcoplasmic reticulum (228). The presence of a Ca^{2+}-transporting system which hydrolyzes ATP to expel Ca^{2+} from the myocardium against a concentration gradient exhibited a K_m value for Ca^{2+} of 10.9 ± 1.9 uM in calmodulin-depleted sarcolemma, and 0.3 ± 0.2 uM in normal sarcolemma (49,228). The V_{max} of the Ca^{2+}-pump in sarcolemmal vesicles was reported to be 0.5 nmoles/mg protein/sec with high sensitivity to inhibition by vanadate, having a I_{50} of < 1 uM (49). These results indicate that the sarcolemmal Ca^{2+} ATPase enzyme is a low-capacity, high-affinity

Ca^{2+}-pumping system. The electrophysiologic measurement of the intracellular Ca^{2+} decline in guinea pig ventricular myocytes indicated that the half-time of the fall from 0.7 uM to 0.3 uM intracellular Ca^{2+} due to sarcolemmal Ca^{2+}-pump was too slow to be important in the process of relaxation of the myocardium (97). These results should be treated with some caution as the experimental design was quite complex and unphysiological. It should be pointed out that the sarcolemmal Ca^{2+}-pump is known to be under the control of phosphorylation/dephosphorylation reactions, mediated by both Ca^{2+}-calmodulin dependent and cAMP-dependent protein kinases (228,229). This mechanism of regulation may be important in balancing the increased influx of Ca^{2+} due to ß-adrenoceptor activation of the slow Ca^{2+}-channels. In this respect, data from a recent study of sarcolemmal Ca^{2+}-pump activated by calmodulin plus cAMP protein kinase indicates a 21-fold increase in V_{max} and a 28-fold decrease in K_m under these conditions (232). It was suggested that the behavior of the pump was more than sufficient to accomplish the transport of Ca^{2+} against the concentration gradient of 5000-10000 fold and thus it may fulfill its putative physiologic function.

Alterations in sarcolemmal Na^+-Ca^{2+} exchange and Ca^{2+} pump (measured biochemically as Ca^{2+}-stimulated ATPase and ATP-dependent Ca^{2+} uptake or accumulation) activities have been assessed in several experimental animal models of heart failure. Recent studies have demonstrated defects varying in magnitude and direction in these sarcolemmal activities due to myocardial ischemia (30,31), hypoxia-reoxygenation injury (22), chronic diabetes (32,33) and hypertrophy resulting from either hypertension or aortic banding (34). These findings reiterate the possibility that the type and stage of heart failure is related to alterations in these sarcolemmal activities. The most extensively studied model of heart failure with respect

to Na^+-Ca^{2+} exchange and Ca^{2+} pump activities is the genetic cardiomyopathic hamster. Decreased Na^+-Ca^{2+} exchange and Ca^{2+} pump activities in 120- and 280-day old (UM.X7.1 strain) as well as depressed Na^+-Ca^{2+} exchange activity in 360-day old (BIO 14.6) cardiomyopathic hamsters was observed (7,231). The state of failure of these animals was a factor in some of the aforementioned activities as a marked increase in the Na^+-Ca^{2+} exchange activity was observed in early stage of the BIO 14.6 variety (7). ATP-dependent uptake has been observed to be decreased in early failure stage of the UM.X7.1 variety of hamsters. The behavior of these sarcolemmal systems in the pathogenesis of these disease states has led investigators to suggest that depression of their activity may lead to reduced Ca^{2+}-efflux from the myocardium and this may contribute to the occurrence of intracellular Ca^{2+}- overload. These activities have not been carefully scrutinized in a non-cardiomyopathic model of heart failure with accompanying volume and pressure overload as intrinsic etiological factors with respect to graded stages of failure.

e. Role of Na^+-K^+ ATPase in transsarcolemmal Ca^{2+} influx. The Na^+-K^+ pump, measured biochemically as Na^+-K^+ ATPase activity, is well known to be localized in the cardiac sarcolemma and carries out an uphill transport of sodium and potassium ions at the expense of ATP hydrolysis at a ratio of three sodium ions outward to two potassium ions inward (18,19,230). From electrophysiologic experiments, it was concluded that cardiac sarcolemmal Na^+-K^+-pump could be influenced by alterations of intracellular sodium and extracellular potassium concentration, and that the reversal potential at which point the Na^+-K^+ pump would cease to operate was estimated at -160 mV (230). Recent evidence indicates that strongly activated forward Na^+-K^+ pump current is sigmoidally related to membrane potentials between -140

and +60 mV and that the pump current is steeply voltage-dependent over the physiologic range of diastolic membrane potentials (233). Since a decline of Na^+-K^+ pump current toward zero was postulated to accompany hyperpolarization of the membrane to -160 mV (230), the pump current must be smaller at resting membrane potential than when the membrane is in a depolarized state (233). Lowering of intracellular Na^+ ion concentration has been attributed to increased Na^+-K^+ pump activity due to modulation by norepinephrine and cAMP in canine cardiac purkinje fibres (234,235).

Regulation of intracellular homeostasis of Na^+ and K^+ ion concentration by Na^+-K^+ ATPase (18,19) is vital to normal cardiac cell function as homeostasis of both of these ions is necessary in the maintenance of electrical properties of the myocardium. Furthermore, it has been demonstrated that intracellular Na^+ ion concentration is involved in the regulation of myocardial contractile function (236). The concept that a rise in intracellular Na^+ concentration via inhibition of the Na^+-K^+ pump enzyme contributes to sarcolemmal Ca^{2+}-influx by activating sarcolemmal Na^+-Ca^{2+} exchange system was emphasized by Schwartz et al (19). Digitalis is believed to inhibit the Na^+-K^+ pump current at both high affinity (0.05 uM) and low affinity (64.5 uM) binding sites on the enzyme, although the relevance of the high affinity sight is disputed (237,238). Uptake of Ca^{2+} by Na^+ loaded neonatal rat myocytes has been shown to be increased due to augmented Na^+-Ca^{2+} exchange activity in the presence of ouabain when compared to myocytes in a ouabain-free medium (133). Thus the inotropic effects of digitalis treatment of the myocardium may be effected by this putative mechanism; however, recent data indicates that activation of sarcolemmal slow Ca^{2+}-channels by cardiac steroids may also be involved in

increased sarcolemmal Ca^{2+}-influx (154). Nevertheless, any change in the operation of sarcolemmal Na^+-K^+ ATPase activity could conceivably alter Ca^{2+} movements in the cell and thus may be seen to modify the cardiac contractile function.

Many early studies were directed at the status of the sarcolemmal Na^+-K^+ ATPase enzyme in both human and experimental heart failure as it was the first cardiac enzyme to be localized at the cardiac sarcolemmal membrane and to be kinetically charcterized. Decreased Na^+-K^+ ATPase activity has been observed in isolated rat heart subjected to ischemia-reperfusion or hypoxia-reoxygenation injury (21,22), failing human heart (23), UM.X7.1 cardiomyopathic hamsters (24), rabbits with left ventricular hypertrophy and failure due to pressure overload (25). However, increased Na^+-K^+ ATPase activity was observed in BIO 14.6 strain cardiomyopathic hamsters (26) and canine hearts with volume or pressure overload due to mitral valve insufficiency or aortic banding, respectively (27,28). Electrophysiologic evidence for defective Na^+-K^+ ATPase functioning in heart failure has been put forward by Houser et al (239) wherein cooling and rewarming of normal and pressure-overloaded failing feline papillary muscles was characterized by reduced ability of the failing preparations to generate an electric potential across the membrane. The bidirectional changes observed in different types of failure once again support the hypothesis that these changes are dependent on etiology of the disease. Such a view implies that this system may operate in parallel to more complex mechanisms in the compensation or decompenation of cardiac pump function in heart failure. Given the variation of results, it is unlikely that a change in function of this enzyme is a primary cause for contractile failure.

6. Subcellular basis of contractile function in heart failure. From the foregoing discussion it is clear that not only mechanisms for energy production and utilization become defective but derangements in the handling of different cations particularly Ca^{2+} by different structures in cardiac cell are associated with contractile failure. Some of these subcellular sites along with their putative functions are given in Table 1. It needs to be emphasized that alterations in subcellular mechanisms are dependent upon the type and stage of heart failure and thus it is important to investigate the time-course of changes in myocardium by employing different experimental models of heart disease. A close examination of the literature (1-3,240) reveals that a great deal of work has been carried out by employing a wide variety of failing hearts with contractile dysfunction; however, relatively little effort has been made to study biochemical events in congestive heart failure subsequent to myocardial infarction. It is therefore appropriate to describe an experimental model of congestive heart failure which can be employed for investigating subcellular defects following the induction of myocardial infarction.

7. Incidence of congestive heart failure subsequent to myocardial infarction. It is estimated that about 1.5 % of the total North American population have congestive heart failure but this figure escalates to > 10 % of the population over the age of 75 years (187,241,242). Approximately 500,000 people develop congestive heart failure every year and the five year mortality from the time of diagnosis is about 60 % in men and about 45 % in women. It is suspected that increasing prevalence of congestive heart failure since 1968 is due to the aging population as well as to improvements in therapy which have allowed patients with cardiovascular disease to live longer. Etiological studies of congestive heart failure reveal that the most prevalent cause of this

Table 1. Possible sites of subcellular defects for the occurrence of heart failure.

Subcellular Sites	Putative Function
A. Sarcolemmal mechanisms	
1. Voltage sensitive Ca^{2+} channels	Ca^{2+}-influx
2. ß-adrenergic receptors	Regulation of Ca^{2+} movements
3. Alpha-adrenergic receptors	Regulation of Ca^{2+} influx and mobilization
4. Na^{+}-K^{+} ATPase	Na^{+} and K^{+} movements and regulation of Ca^{2+} fluxes
5. Na^{+}-Ca^{2+} exchange	Ca^{2+} and Na^{+} movements
6. Ca^{2+}-pump	Ca^{2+}-efflux
B. Intracellular mechanisms	
1. Contractile proteins	Ca^{2+}-linked force generation and energy utilization
2. Sarcoplasmic reticulum	Ca^{2+} release and Ca^{2+} uptake from cytoplasm
3. Mitochondria	Ca^{2+} handling and energy production

pathophysiologic state is the presence of coronary artery disease followed by incidence of systemic hypertension, valvular heart disease, cardiomyopathy, and congenital heart disease (242). In other words, the best example of a patient with congestive heart failure is one with coronary artery disease who has had one or more infarctions with subsequent loss of cardiac muscle.

As congestive heart failure has become one of the most common serious disorders and is one of the most common causes of death, many different experimental models of heart failure have been developed to aid in the assessment of biochemical changes and to investigate various modes of treatment of the failing myocardium. However, there are very few models of congestive heart failure secondary to myocardial infarction (243) and one noted example in this regard is provided by Johns and Olson (244) who published the first comprehensive description of surgical ligation of the left coronary artery in rats. They initiated their study to find an alternative model of infarction from the canine model as the size of infarction and mortality in these animals due to infarction was "unpredictable" presumably due to collateral blood supply and differences amoung various breeds. By successfully mapping of the arterial network of hearts from these small animals, establishing the incidence of mortality as a result of coronary occlusion, and achieving a high incidence of infarction in rats, these investigators set the stage for further studies of experimentally produced myocardial infarction where congestive heart failure was noted by the presence of peripheral edema in some rats. Several years later a group lead by Selye found that with some modification of earlier techniques, larger myocardial infarct due to coronary occlusion and increased survivorship of the experimental animals was achieved (279). Earlier this rat model of myocardial infarction was used to study morphologic features of evolving infarcts, and to

assess the effect of various interventions on infarct size (245-247,280). This model of myocardial infarction was also used in the assessment of metabolic and mechanical adaptations in these hearts (248).

Although the rat heart lacks collateral capillaries, and is therefore dissimilar from human heart, the rat model of infarction was useful by virtue of the reproducibility of infarct size. The nature of the inflammatory response of the myocardium to chronic infarction was studied during a 21 day period following coronary occlusion (249). Following a brief inflammatory response at the margin of the necrotic myocardium, these investigators found chronic inflammation, vascular and collagenous proliferation, and resorption of necrotic tissue which progressed until 21 days whereupon scar formation was complete. Recently the process of scar reabsorption has been carefully assessed by morphometric techniques in rat heart and it was shown that these processes are responsible for a 59 % shrinkage of the necrotic myocardium in both small and large infarcts over the first 40 days (250). Although measurement of scar size is a valuable relative indicator of extent of damage to the myocardium, the first study to assess the left ventricular function in rats with large myocardial infarction appeared in 1979 wherein in vivo measurements of baseline and intravenous volume loaded hemodynamics were made in rats 21 days following coronary occlusion (251). Little change of baseline hemodynamics or peak indices of cardiac pumping or pressure generating ability was observed in experimental animals with small left ventricular infarct (4 - 30%) when compared to control. Experimental animals with moderate myocardial infarction (31 - 45%) had normal baseline hemodynamics but cardiac pump function was reduced upon volume overload. Rats with large infarcts (>45 %) had overt heart failure characterized by elevated filling pressures, reduced cardiac output, and low capacity to respond to pre- and afterload stress.

Subsequent work from this group indicated that coronary ligation in the rat provided an experimental model of graded left ventricular dysfunction, whose magnitude was closely related to the extent of the healed myocardial infarction (252,253).

A parameter used in forming the prognosis for survival of post-infarct patients is the presence and extent of left ventricular dilatation (254). Left ventricular distention and dilatation were illustrated by upward and rightward movement on the pressure-volume relationship of experimental animals when compared to control (252,253). It was suggested that increased ventricular volume aided maintenance of peak stroke volume despite the linear reduction of ejection fraction index with increasing infarction size in animals 3 - 4 weeks after the induction of myocardial infarction. A study of the time course (4, 7, 10, 20, and 35 weeks) of hemodynamic changes in rats with healed severe myocardial infarction revealed that at 4 weeks, peak left ventricular blood pressure, left ventricular maximum rate of contraction, diastolic blood pressure, and systemic vascular resistance were decreased when compared to control (255). Furthermore, the left ventricular end-diastolic pressure was increased by 313 % in these experimental animals. These investigators found no further significant deviation in diastolic blood pressure, left ventricular end diastolic pressure, peak left ventricular blood pressure, and maximum rate of left ventricular contraction after 4 weeks. The cardiac output and systemic vascular resistance progressively decreased and increased, respectively; these changes were accompanied by decreased blood flow to liver, stomach, brain, and kidney in the period from 7 - 35 weeks which suggested that progressive cardiac decompensation was present in these animals (255). Recent use of this model for investigation of pharmacological tolerance to nitrate therapy has revealed that the rapid reduction of left

ventricular end diastolic pressure caused by glyceryl trinitrate resembles that seen in man, and therefore could be used as a model for examining the mechanisms of nitrate action and tolerance (256). In humans, the most common cause of right-sided cardiac failure and pulmonary hypertension is the left-sided cardiac failure (242,257). Right ventricular hypertrophy was consistently present in experimental animals with elevated left ventricular filling pressures and a close relationship between left ventricular end-diastolic pressure and right ventricular systolic pressure was observed (251-253). Thus, left ventricular dysfunction may eventually cause severe right ventricular overload and dysfuction such that the right ventricle becomes a limiting factor in heart failure.

The incidence of myocardial hypertrophy after myocardial infarction has long been thought to be beneficial as a nonspecific compensatory mechanism for increasing mass and thereby functional capacity of these hearts (281). While the validity of this concept remains to be established, some research of cellular processes associated with the incidence of hypertrophy in infarcted rat heart has yielded some useful information (250,258). An early report of cardiac hypertrophy in experimental rat hearts 12 weeks after the induction of myocardial infarction suggested that overall cardiac hypertrophy was about 170 % when compared to control (259); no normalization for loss of the infarcted myocardium was incorporated in this estimate. Recent work has shown in hearts of rats sacrificed 5 weeks after coronary occlusion with so-called medium (> 15 - 30 %) or large (> 30%) infarct size that myocytes had undergone significant hypertrophy (34). In a study of the morphometry of the right ventricle in rat hearts 4 weeks after occlusion of the left coronary artery, the weight of the right ventricle had inceased by 30 %, with 17 % ventricular wall thickening and 13 % greater diameter of myocytes (258). Inadequate

growth of the microvasculature that supports tissue oxygenation was evidenced by relatives decreases in capillary luminal volume density (25 %), capillary luminal surface density (20 %), and by an increase in the average maximum distance from the capillary wall to the mitochondria of myocytes (20 %). These authors suggested that inadequate compensation of the coronary bed in the right ventricle may result in alterations in oxygen availability, diffusion and transport which may be detrimental to the myocardial tissue. A report of these parameters in viable left ventricular myocardium of rat hearts 5 weeks after the induction of myocardial infarction revealed about 80 % expansion of the spared myocardium which was found to be insufficient in restoration of ventricular tissue; infarcts affecting an average of 20 % of the left ventricle were characterized by a 25 % hypertrophic growth of the remaining myocardium (250). Hearts with large infarcts were shown to have 25 %, 30 %, and 30 % reduction in the absolute amounts of capillary lumen, surface, and length per ventricle, respectively; these changes indicated that the surviving ventricle may be vulnerable to additional ischemic episodes.

As the rat model of congestive heart failure became established, and because it is generally believed that the function of the scarred ventricle would depend not only on size and location of the infarct but also on evolving changes in neurohumoral influences, systemic vascular resistance and venous compliance, recent research has been directed toward description of these parameters in an attempt to clarify whether these animals are in a compensated or decompensated state. For example, marked changes in systemic and renal microcirculatory dynamics in congestive heart failure patients (260) may reflect, in part, significant increases in plasma renin and aldosterone levels. Plasma atrial natriuretic peptide levels in experimental animals 4 weeks after induction of infarction were shown to be elevated when compared to

control (261), as in humans with congestive heart failure (262), however plasma renin and aldosterone levels were unchanged. Ventricular levels of norepinephrine were decreased in experimental animals when compared to control (261). Thus, while the neurohormonal status of these experimental animals is altered to some extent 4 weeks after myocardial infarction, not all of the criterion for congestive heart failure in humans are met at this stage of failure. However, plasma atrial natriuretic peptide concentration was found to vary directly with size of myocardial infarction in rats (263), and atrial content of atrial natriuretic peptide mRNA was increased in animals with large infarct versus those control animals (264). Although the significance of increased plasma atrial natriuretic factor is not clear, injection of monoclonal antibodies specific for atrial natriuretic factor into rats with large myocardial infarction and high baseline plasma atrial natriuretic factor, was associated with a specific increase in the systemic vascular resistance (265). Venous capacitance, inferred by effective vascular compliance, was decreased in rats three weeks after the induction of large myocardial infarction (266); it has been suggested that elevated plasma atrial natriuretic factor levels affect central hemodynamics by reducing venous pressure and possibly by arterial dilatation. Since chronic treatment with captopril, an angiotensin-converting enzyme inhibitor, was shown to maintain normal left ventricular filling pressure and cardiac output, reduce ventricular hypertrophy and prolong survival in rats 3 - 12 months after induction of myocardial infarction, it seems logical that there is a pathophysiologic role for elevated levels of angiotensin in this model (53,267-271). In this regard, reduced glomerular plasma flow rate and single nephron glomerular filtration rate, as well as increased single nephron filtration fraction, efferent arteriolar resistance, and fractional proximal

tubule fluid reabsorption in rats with large myocardial infarction were normalized by treatment of these animals with teprotide, an angiotensin-converting enzyme inhibitor (272). As Na^+ excretion was shown to be impaired in rats with either large or small infarcts at 3 - 4 weeks, defective renal function was present in these animals (272,273).

Relatively little is known of biochemical parameters such as myofibrillar ATPase activity, myosin isozyme composition, myocardial high-energy phosphate content, sarcoplasmic reticular function, mitochondrial function, and sarcolemmal function in the viable myocardium of rat heart with myocardial infarction, especially in later stages of congestive heart failure. Myofibrillar ATPase activity was found to be 20 % lower in both sedentary and exercised infarct groups 11 week post-myocardial infarction when compared to sham-operated control animals (274). A shift in the myosin heavy chain isozyme content from V_1 toward V_3 is known to occur in infarcted hearts (275). Furthermore, although actomyosin ATPase activity and the percent V_1 myosin heavy-chain isozyme were shown to be decreased in all regions of the infarcted hearts by 3 weeks, regional variation was noted by further depression of V_1 myosin in the left ventricle and papillary muscle when compared to the right ventricular free wall and septum V_1 content from the same hearts (276). Two days following infarction, the ATP content of viable myocardium was found to be significantly lower than control; intravenous ribose administration attenuated this decrease and was associated with an improvement of cardiac pump function (277). These investigators also found that ATP content of the nonischemic portion of the myocardium was not different from control 4 days after occlusion of the left coronary. While other investigators confirmed that ATP and total adenine nucleotide contents were normal in viable tissue of hearts 1 or 3 weeks after the induction of

large myocardial infarction, they observed that tissue concentrations of creatine phosphate and free creatine were decreased (278). Sarcoplasmic reticular Ca^{2+} uptake and Ca^{2+} ATPase activity in purified vesicle preparations was found to be depressed in the viable left ventricular myocardium in experimental animals 16 weeks after induction of myocardial infarction (119). Very few studies to directly or indirectly assess sarcolemmal function have been attempted with this model of myocardial infarction, and none have assessed sarcolemmal function in decompensated stages of heart failure. One week after coronary occlusion, sensitivity of the surviving myocardium to verapamil was increased when compared to control (278). Accordingly, it was demonstrated that sensitivity to extracellular Ca^{2+} was decreased in viable tissue one or three weeks after myocardial infarction, and these authors suggested that inward Ca^{2+} channel activity was depressed in the infarcted hearts (278). Myocardial noradrenaline content was shown to be reduced and a small increase in dihydroalprenolol binding site density was noted in rats three weeks after coronary occlusion; these authors also concluded that the responsiveness of the nonischemic myocardium to isoprenaline was not changed in these animals (180). Nonetheless, these studies indicate that virtually nothing is known about sarcolemmal changes during the development of congestive heart failure secondary to myocardial infarction. This study was therefore undertaken to identify defects in the sarcolemmal membrane at different times of inducing myocardial infarction in rats.

III. METHODS

1. Experimental model. Myocardial infarction was produced in male Sprague-Dawley rats (200-250 g) by occlusion of the left coronary artery as described by Johns and Olson (244) and modified by Selye (279). The animals were anesthetized with ether, the skin incised along the left sternal border, the fourth rib cut proximal to the sternum and retractors were inserted. The pericardial sac was perforated and the heart was exteriorized through the intercostal space. The left coronary artery was ligated about 2 mm from its origin with a suture of 6-0 silk, and the heart was repositioned in the chest. Left coronary artery occlusion was ascertained by the paling to the suture. Throughout the course of the operation, rats were maintained on a positive-pressure ventilation delivering a mixture of 95% O_2 and 5% CO_2 mixed with ether. Closure of the wound was accomplished by a purse-string suture. The mortality of all animals operated upon in this fashion was about 35% within 48 hr. Sham-operated animals were treated similarly except the suture around the coronary artery was not tied. Animals were allowed to recover and received food and water ad libitum and maintained for a period of 4, 8 and 16 weeks prior to hemodynamic and biochemical assessment. For determination of the degree of hypertrophy, the percentage of the left ventricular free wall which was infarcted was estimated 3 weeks following coronary ligation by planimetric techniques; this percentage was extrapolated to the respective experimental groups. The mean ± SEM of scar weight in all groups was 0.36 ± 0.06 g; this was 44 ± 5% of the total left ventricular weight in 3 weeks post-operative animals. Experimental hearts showing normalized infarcted tissue mass < 30% of the total left ventricular mass were not included in this study.

2. Hemodynamic studies. The animals were anesthetized with an injection of sodium pentobarbitol (50 mg/kg Nembutal, i.p.). To maintain adequate ventilation, the trachea was intubated, the right carotid artery was exposed, and a microtip pressure transducer (model SPR-249, Millar) was introduced through a proximal arteriotomy (282,283). The catheter was advanced carefully through the lumen of the carotid artery until the tip of the transducer entered the left ventricle. The catheter was secured with a silk ligature around the artery and readings were taken from a dynograph recorder (model R511A, Beckman; Fullerton, CA). Left ventricular pressures, heart rate, rate of contraction (+dP/dt) and rate of relaxation (-dP/dt).

3. Crude membrane preparation. Rats were sacrificed by decapitation and hearts were removed. The atria, connective tissue and right ventricle were excised, and remaining left ventricle was processed for the preparation of membranes according to the method described by Wagner et al (5). In some experiments, the right ventricle was processed for crude membranes in parallel to the left ventricular tissue. In all experimental animals, the uninfarcted left ventricular tissue was used after removing the scar. In some experiments, the gastrocnemius muscle, the brain cortex, liver and right ventricle were also processed for the preparation of crude membranes. Briefly, the tissue was washed, minced, and then homogenized in 50 mM Tris-HCl, pH 7.4 (15 ml/g tissue) with a PT-20 polytron (2 x 20 sec, setting 5). The resulting homogenate was centrifuged at 1,000 g for 10 min, and the pellet was discarded. The supernatant was centrifuged at 48,000 g for 25 min. The resulting pellet was resuspended and centrifuged again 2 times in the same buffer at the same speed; the final pellet was resuspended in 50 mM Tris-HCl, pH 7.4. This preparation has been commonly used by various investigators for studying receptor mechanisms in the cell. In order to rule out artefactual

results related to the membrane preparation (185), some experiments were also carried out using crude membranes prepared exactly as above except that the 1000 g centrifugation step was excluded.

4. Preparation of cardiac sarcolemmal membranes. Purified light sarcolemmal membrane fraction was isolated from left ventriclar tissue according to the method of Pitts (216). Scar tissue from the viable left ventricular walls of infarcted hearts was excised and discarded before the isolation procedure. The ventricles were washed, minced, and then homogenized in 0.6 M sucrose, 10 mM imidazole-HCl, pH 7.0 (3.5 ml/g tissue) with a polytron PT-20 (5 X 20 s, setting 5). The resulting homogenate was centrifuged at 12,000 g for 30 min, and the pellet was discarded. After diluting (5 ml/g tissue) with 140 mM KCl - 20 mM 3-(N-morpholino)-propanesulphonic acid (MOPS), pH 7.4 (at 37°C), the supernatant was centrifuged at 95,000 g for 60 min. The resulting pellet was suspended in 140 mM KCl, 20 mM MOPS, pH 7.4 buffer and layered over a 30 % sucrose solution containing 0.3 M KCl - 50 mM $Na_4P_4O_7$, and 0.1 M Tris (hydroxymethyl) aminomethane; (Tris)-HCl, pH 8.3. After centrifugation at 95,000 g for 90 min (using a Beckman swinging bucket rotor) the band at the sucrose-buffer interface was taken and diluted with 3 vol of 140 mM KCl, 20 mM MOPS, pH 7.4 (at 37°C). A final centrifugation at 95,000 g for 30 min resulted in a pellet rich in sarcolemma. All isolation steps were carried out at 0 - 4°C. The heavy sarcolemmal membranes were isolated by the hypotonic shock/LiBr method (285). In some experiments, the final pellet was suspended in appropriate medium and used immediately; in others, the final pellet was suspended in 0.25 M sucrose - 10 mM histidine, pH 7.2 (3.5 mg/ml) and then quickly frozen and stored in liquid N_2. Freezing of membranes in such a manner was found to have no effect on the binding values. Protein concentration of all membranes was determined by the method of Lowry et al

(286). The marker enzyme activities as measured by the methods described elsewhere (22,284,287,288) revealed that the sarcolemmal membrane was 16.5 ± 0.4 fold purified in terms of digitoxigenin-sensitive Na^+-K^+ ATPase activity in the heart homogenate. K^+-p-nitrophenyl phosphatase (K^+-pNPPase) activity (sarcolemmal marker) was carried out according to methods previously described (287,292). The sarcoplasmic reticular and mitochondrial contaminations were negligible when assessed in terms of rotenone insensitive NADH cytochrome c reductase (0.30 ± 0.04 fold purification) and cytochrome c oxidase (0.24 ± 0.03 fold purification), respectively. Furthermore, the sarcolemmal yield from both control and experimental hearts was 1.12 ± 0.03 mg protein/g heart.

5. [^{3}H]-Nitrendipine binding assay. Binding of [^{3}H]-nitrendipine to membrane fractions was monitored according to a method reported earlier (5,8). Membrane preparations (0.08 - 0.1 mg protein/tube) were incubated with 0.035 to 5 nM[^{3}H]-nitrendipine, unless otherwise indicated in the text, in the absence or presence of 2.5 uM unlabelled nifedipine, a concentration sufficient to inhibit > 95 % of the specific [^{3}H]-nitrendipine binding. Assays were terminated after 1 hr at room temperature (22 - 23 °C) by filtration (Whatman GF/C filters). Filters were washed twice with 5 ml cold Tris-HCl buffer. The radioactivity of the filters was counted in a Beckman LS7500 scintillation counter at an efficiency of 39 - 41 %. The nonspecific [^{3}H]-nitrendipine binding (in the presence of nifedipine) was subtracted from the total binding (in the absence of nifedipine) to obtain the specific binding of [^{3}H]-nitrendipine. Binding of another dihydropyridine compound, [^{3}H]-PN200-110 to crude membrane fraction was also carried out to determine if the observed changes with nitrendipine were not due to any artifact of the radioligand employed here. Bindings of these radioligands were also measured

by using heart homogenates. Assay standards of [^{3}H]-nitrendipine and [^{3}H]-PN200-110 were prepared by dilution with 0.25 % ethanol. Control experiments indicated that 0.25 % ethanol did not significantly alter specific binding of either dihydropyridine. [^{3}H]-Nitrendipine and [^{3}H]-PN200-110 were obtained from New England Nuclear Medicine (Boston, USA). [^{3}H]-nitrendipine was 5-methyl-[^{3}H]-nitrendipine, with a specific activity of 80.9 Ci/mmol whereas [^{3}H]-PN200-110 was (+)-5-methyl- [^{3}H]-PN200-110, with a specific activity of 70.2 Ci/mmol.

6. [^{3}H]-dihydroalprenolol and [^{3}H]-prazosin binding assays. The binding of [^{3}H]-dihydroalprenolol (ß-adrenoceptor antagonist) and [^{3}H]-prazosin (alpha-adrenoceptor antagonist) to membrane fractions was monitored according to a method reported earlier (11). Membrane preparations (0.05 mg protein/tube of purified sarcolemmal vesicles or 0.1 mg protein/tube of crude membrane) were incubated with seven concentrations of [^{3}H]-dihydroalprenolol (0.035 - 4 nM) or with seven concentrations of [^{3}H]-prazosin (.01 - 1.25 nM) unless otherwise indicated in the text, in the abscence as well as presence of 10 uM l-propranolol or 5 uM phentolamine hydrochloride, respectively. The presence of unlabelled agonists in these concentrations was seen to inhibit > 95% of the specific binding of the respective labelled antagonist compounds. The total volume of each assay tube was 0.5 ml; each tube was incubated at 30 °C for a period of 30 min prior to filtration. Assays were terminated by filtration (Whatman GF/B filters) and the filters were washed thrice with 4.5 ml cold Tris-HCl buffer. Prior to filtration, the filters were soaked in a cold solution of Tris-HCl buffer containing 0.2 % - 0.3 % polyethylenimine. The radioactivity of the filters was counted in a Beckman LS7500 scintillation counter at an efficiency of 39 - 41%. The nonspecific [^{3}H]-drug binding (in the presence of excess unlabelled agonist) was subtracted from the total

binding (in the abscence of unlabelled agonist) to obtain the specific binding of [^{3}H]-drug. [^{3}H]-dihydroalprenolol and [^{3}H]-prazosin were obtained from New England Nuclear Medicine (Boston, USA). [^{3}H]-dihydroalprenolol was levo-[ring, propyl-^{3}H(N)]-dihydroalprenolol hydrochloride, with a specific activity of 92.2 Ci/mmol whereas [^{3}H]-prazosin was [7-methoxy-^{3}H]-prazosin, with a specific activity of 76.6 Ci/mmol.

7. Perfusion of hearts. Animals were killed by decapitation and the heart was quickly removed and washed in cold (4°C) saline. A polyethylene cannula was inserted into the aorta and the heart was perfused under non-recirculating conditions by Krebs-Henseleit solution containing (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.25, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 7.0, sodium pyruvate 2.0 and mannitol 1.1. Perfusion solution was saturated by a mixture of 95 % O_2 and 5 % CO_2 (pH 7.4) and its temperature was maintained at 37°C. Hearts were perfused under constant perfusion pressure corresponding to 100 cm H_2O. The hearts were electrically stimulated at a constant rate of 300 beats/min using silver electrodes attached to the base of the right ventricle. The voltage of stimulatory pulses was set at 50 % above the threshold value for each individual experiment. Coronary flow was measured by a timed collection of coronary effluent. The contractile function was measured by using an isometric force transducer (Grass FT.03) connected to the left ventricle. Contractile force (CF) and its first derivative (dF/dt) were registered on a Grass 7 recorded. After a sufficient stabilizing period (20 min) the resting force was gradually increased from 0 to approximately 6 g per gram of heart and maintained at this level. Under these conditions, the $+(dF/dt)_{max}$ was about 65 % of maximum reached at the optimum preload. Control experiments have shown that the contractile parameters in this

preparation were stable for 1.5 hrs.

The sensitivity of the isolated hearts to isoproterenol was measured in the presence of 10^{-3} M ascorbic acid to prevent the oxidation of isoprenaline; in these experiments 10^{-7} M phentolamine was used to eliminate the alpha-adrenergic activity. The sensitivity to phenylephrine was measured in the presence of 10^{-7} M propranolol to eliminate the residual ß-activity of phenylephrine. After the recording of control parameters the dose-response curves for both drugs were measured in a cumulative manner; the effect of each concentration was evaluated after reaching the steady state (about 4 min). At the end of each experiment, the resting force was gradually increased until the maximum level of $+(dF/dt)_{max}$ was reached (in the presence of the highest concentrations of drug used).

8. Measurement of ATPase activities. Estimation of Na^+-K^+ ATPase activity was carried out by a previously described method (286,287) with some modifications (288). Briefly, sarcolemmal vesicles (10 ug) were preincubated at 37°C, 1.0 mM EGTA (Tris), pH 7.4 at 37°C, 5 mM NaN_3, 6 mM $MgCl_2$, 100 mM NaCl and 10 mM KCl (except where indicated in the text), 2.5 mM phosphoenolpyruvate (PEP), and 10 I.U./ml pyruvate kinase. The reaction was started by the addition of 0.025 ml 80 mM Na_2 ATP, pH 7.4, and terminated after 10 min with 0.5 ml ice-cold 12% tricholoracetic acid. The liberated phosphate was measured by the method of Taussky and Shorr (289). In some experiments, 1 mM ouabain, 2.5 uM monensin or 10 uM digitoxigenin were added to the reaction medium before the addition of the sarcolemmal preparation. In parallel experiments either Na^+ plus K^+ or Mg^{2+} was omitted from the incubation medium. Na^+-K^+ ATPase activity was calculated as the difference between activities with and without Na^+ plus K^+. Mg^{2+} ATPase activity was estimated as the difference between the activities registered

with and without Mg^{2+} in the absence of Na^+ and K^+ in the medium. In a parallel set of experiments, Na^+-K^+ ATPase activity was measured by a different procedure previously used in our laboratory (22); the assay conditions of the experiments were similar except that Na^+-K^+ ATPase activity of the latter was calculated as the difference of activities in the presence and absence of 10 mM KCl. No significant difference in Na^+-K^+ ATPase activity was found among the two assay protocols. All measurements were carried out in duplicate. The concentrations of MgATP were calculated using the "SPECS" FORTRAN program developed by Fabiato (290). Alamethicin was directly included in the assay medium (no pretreatment) in some experiments at a ratio of 0.5 mg/1 mg sarcolemmal protein. Alamethicin was dissolved in ethanol; the final concentration of ethanol in the reaction tube did not exceed 0.5% (v/v); appropriate control experiment in the presence of ethanol was also carried out. For SDS pretreatment, sarcolemmal vesicles (0.5 mg/ml) were incubated for 25 min at 22 - 24°C in a buffer containing 0.25 M sucrose, 10 mM histidine, pH 7.4 and 0.03 mg/ml SDS. Then 10 ug sarcolemmal vesicles were transferred to the reaction medium.

9. [^{3}H]-ouabain binding. For determination of high and low-affinity ^{3}H-ouabain binding, experiments were carried out according to a method previously described (291). Sarcolemmal vesicles resuspended in 35 ug/tube 10 mM Tris-HCl, pH 7.5 were transferred to the reaction mixture which contained 1.5 mM $MgCl_2$, 1.0 mM phosphate, 10 mM Tris-HCl, pH 7.5 at 37°C, and 1 to 5000 nM [^{3}H] ouabain (specific activity of 4.42 Ci/mmol) in the absence or presence of 2.0 mM ouabain, a concentration sufficient to inhibit more than 95% of the specific [^{3}H]-ouabain binding. In order to make the sarcolemmal vesicles freely permeable to ouabain, SDS (9 ug/ml) was directly added to the incubation medium. Assays were terminated after 1 hour at 37°C by filtration

(Millipore, Bedford, Massachusetts; pore size = 0.45 uM). Filters were washed thrice with 2.5 ml ice-cold buffered washing solution (50 mM Tris-HCl, pH 7.5, 0.1 mM ouabain, 15.0 mM KCl). The radioactivity of the filters was counted in a scintillation counter (model LS1701, Beckman Industries, Fullerton, California) at an efficiency of 39 - 41 %. The nonspecific [^{3}H] ouabain binding (in the presence of excess unlabelled ouabain) was subtracted from the total binding (in the absence of unlabelled ouabain) to obtain the specific binding of [^{3}H] ouabain. Cold ouabain was dissolved in dimethyl sulphoxide (DMSO); the final concentration of the DMSO in the reaction tube did not exceed 0.25 % (V/V). Control experiments indicated that 0.25 % ethanol did not significantly alter specific binding of ouabain. [^{3}H] ouabain was obtained from New England Nuclear Medicine, Boston, Massachusetts. [^{3}H] ouabain was [^{3}H(G)] ouabain, with an undiluted specific activity of 15.4 Ci/mmol.

10. Sarcolemmal Ca^{2+}-transport measurements. The purity of the membrane preparations was examined by measuring the activities of marker enzymes such as digitoxigenin-sensitive Na^+-K^+ ATPase (sarcolemmal marker).

Na^+-dependent Ca^{2+} uptake measurement were carried out by a method described in detail elsewhere (30). In short, 5 ul of sarcolemmal vesicles (1.5 mg/ml; 7.5 ug protein/tube) preloaded with NaCl/MOPS buffer at 37°C for 30 min were rapidly diluted 50 times with Ca^{2+} uptake medium containing 140 mM KCl, 20 mM MOPS, 0.4 uM valinomycin, 0.3 uCi $^{45}Ca^{2+}$ and various Ca^{2+} concentrations (5 - 80 uM), pH 7.4 (at 37°C). After the appropriate time span, the reaction was stopped by the addition of ice-cold 0.03 ml stopping solution containing 140 mM KCl, 1 mM $LaCl_3$, 20 mM MOPS, pH 7.4 (at 37°C). Samples (0.25 ml from 0.28 ml of the total reaction mixture) were filtered through Millipore filters (pore size = 0.45 uM) and washed twice with 2.5 ml

of ice-cold 0.03 ml stopping solution containing 140 mM KCl, 0.1 mM $LaCl_3$, 20 mM MOPS, pH 7.4, at 37°C. Radioactivity of filters were measured using a Beckman LS 1701 counter. In parallel to these samples, nonspecific Ca^{2+} uptake was measured by placing the Na^+-loaded sarcolemmal vesicles in Ca^{2+} uptake medium which contained 140 mM NaCl instead of KCl. Na^+-dependent Ca^{2+} uptake activity was corrected by subtraction of the nonspecific Ca^{2+} uptake values.

For the determination of Mg^{2+} ATPase and Ca^{2+}-stimulated ATPase activities, experimental conditions were the same as reported elsewhere (18,31) with some modifications. Sarcolemmal vesicles (resuspended in 140 mM KCl, 20 mM MOPS, pH 7.4 at 37°C; 25 ug protein/tube) were preincubated at 37°C for 5 min in 0.5 ml of medium containing 140 mM KCl, 20 mM MOPS, pH 7.4 at 37°C, 2 mM $MgCl_2$, 5 mM sodium azide, 0.1 mM ethylene glycol-bis (ß-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 2.5 mM phosphoenolpyruvate (PEP), and 10 I.U./ml pyruvate kinase. The reaction for Mg^{2+} ATPase was started by the addition of 4 mM Tris-ATP, pH 7.4 at 37°C, and terminated 5 min later with 0.5 ml of ice-cold 12% trichloracetic acid; the liberated phosphate was measured by the method of Taussky and Shorr (289). Estimation of total (Ca^{2+} + Mg^{2+})-ATPase was made in the above mentioned medium containing 5 X 10^{-6} M free Ca^{2+} instead of EGTA. MgATP and free Ca^{2+} concentration in the incubation medium was calculated using the "SPECS" FORTRAN program of Fabiato (290). The Ca^{2+}-stimulated ATPase activity was the difference between the total ATPase and the Mg^{2+}-ATPase activities.

In order to measure ATP-dependent Ca^{2+} accumulation (293), sarcolemmal vesicles (22.5 ug protein/tube) were preincubated at 37°C for various times in 0.20 ml of medium containing 140 mM KCl, 10 mM MOPS, pH 7.4, at 37°C, 2 mM $MgCl_2$, and $^{45}CaCl_2$-EGTA which contained 5 X 10^{-6} M free Ca^{2+}, Ca^{2+}

accumulation was initiated by the addition of 4 mM Tris-ATP, pH 7.4 (at 37°C), 180 ul aliquots were immediately filtered through Millipore filters (pore size = 0.45 uM), washed twice with 2.5 ml ice cold 140 mM KCl, 20 mM MOPS, and 0.1 mM $LaCl_3$, pH 7.4 (at 37°C), dried, and radioactivity determined for calculating the total Ca^{2+} accumulation. Nonspecific Ca^{2+} binding was measured in the absence of ATP for each set of experiments and the ATP-dependent Ca^{2+} accumulation (uptake) was calculated by subtracting nonspecific Ca^{2+} binding from the total Ca^{2+} accumulation.

For the determination of passive Ca^{2+} accumulation, sarcolemmal vesicles (resuspended in 140 mM KCl, 20 mM MOPS, 22.5 ug/tube) were suspended in a medium (160 ul) containing 140 mM KCl and 20 mM MOPS, pH 7.4, and were preincubated for 3 min 37°C. Passive calcium accumulation was initiated by the addition of $^{45}CaCl_2$-EGTA bringing the total volume to 200 ul. After the appropriate times, 180 ul aliquots were immediately filtered through Millipore filters (pore size = 0.45 uM), washed twice with 2.5 ml ice-cold 140 mM KCl, 20 mM MOPS, pH 7.4 (at 37°C), dried and radioactivity determined for calculating the passive Ca^{2+} accumulation.

11. Statistical analysis. Results are presented as a mean ± SEM. The statistical differences between mean values for the two groups were evaluated by Student's t test. When appropriate, Duncan's multiple-range test was used to determine differences between the means. A value of $P < 0.05$ was considered as a significant difference between groups. Estimates of equilibrium binding parameters (K_d - dissociation constant, and B_{max} - maximal density) were obtained from the Scatchard plot analysis with the computer program "Ligand" of Munson and Robard (294).

IV. RESULTS

1. General characteristics and hemodynamic parameters in animals subsequent to myocardial infarction. A study of left ventricle, right ventricle, scar weight, left ventricle/body weight ratio, appearance of abdominal ascites, and lung weight revealed significant differences between experimental (16 weeks following coronary occlusion) and sham-operated animals (Table 2). Specifically, evidence of cardiac hypertrophy in experimental animals was noted by the increased mass of the remaining viable left ventricle as well as right ventricular myocardium. Furthermore, left ventricle/body weight ratio was increased and the accumulation of ascites in the abdominal cavity was evident in the experimental animals. Congestion of lungs in experimental animals was noted by increased wet lung weight and wet/dry lung weight ratio. Although no difference in liver weight or wet/dry liver weight ratio between sham-operated and experimental animals was seen, the livers of experimental animals had rounded edges and yellowish coloration. Signs of clinical congestive heart failure were also evident in experimental rats 8 weeks following myocardial infarction but the hearts of these animals were hypertrophied to a lesser extent than those of the 16 week experimental group. No difference in the scar weights of the left ventricular free wall was seen among the 4 week, 8 week or 16 week experimental groups. At a period of 4 weeks after surgery, the experimental animals were not significantly different from the sham-operated animals in any of the parameters indicated above except the accumulation of abdominal ascites (Table 2).

Assessment of hemodynamic performance of the 16 week experimental group revealed decreases in mean arterial pressure, left ventricular systolic pressure, rate of contraction, rate of relaxation, heart rate, and the total

Table 2. General characteristics of experimental rats 4, 8 and 16 weeks after induction of myocardial infarction.

Parameters	4-week		8-week		16-week	
	sham	experimental	sham	experimental	sham	experimental
Left ventricle (LV) wt. (g)	0.825 ± 0.091	0.782 ± 0.102	0.91 ± 0.09	1.25 ± 0.16	1.03 ± 0.10	1.45* ± 0.13
Right ventricle (RV) wt. (g)	0.230 ± 0.025	0.307 ± 0.039	0.239 ± 0.023	0.384* ± 0.042	0.258 ± 0.034	0.409* ± 0.045
LV wt./body wt. ($\times 10^{-3}$)	1.86 ± 0.21	1.95 ± 0.25	1.92 ± 0.17	2.71* ± 0.28	1.84 ± 0.24	2.79* ± 0.29
Scar wt. (g)	N.D.	0.357 ± 0.061	N.D.	0.369 ± 0.086	N.D.	0.396 ± 0.044
Ascites (ml)	N.D.	3.2* ± 1.1	N.D.	4.3* ± 1.8	0.5 ± 0.8	11.3* ± 2.1
Lung wet (wt. g)	1.67 ± 0.16	1.72 ± 0.21	1.81 ± 0.14	2.57* ± 0.16	1.94 ± 0.12	3.15* ± 0.17
Lung dry (wt.)	0.47 ± 0.03	0.45 ± 0.07	0.50 ± 0.08	0.53 ± 0.08	0.51 ± 0.03	0.58 ± 0.04
Lung wet wt./dry wt.	3.55 ± 0.36	3.82 ± 0.46	3.62 ± 0.28	4.85* ± 0.30	3.80 ± 0.23	5.43* ± 0.24

Data are expressed as means ± SEM of eight experiments. Left ventricular wt. indicated for experimental animals does not include scar tissue. N.D. - not detectable. * $P < 0.05$

mechanical energy output of these hearts (Table 3) as compared to sham-operated animals. The left ventricular end-diastolic pressure in the 4-, 8-, and 16-week experimental animals was significantly elevated when compared to control values. Physical examination of the chest cavity of experimental animals immediately after sacrifice revealed greatly enlarged hearts when compared to those of sham-operated animals. A marked reduction in heart rate and left ventricular systolic pressure was present in the 16 week experimental group; these animals were considered to be in a severe stage of failure. Although the 8 week experimental group did show significant reductions in many hemodynamic parameters as observed in 16 week experimental group, these changes were lesser in magnitude and this indicated that these animals were in a moderate stage of failure. Mean arterial pressure, heart rate and left ventricular systolic pressures were unchanged in the 4 week experimental group; however, left ventricular diastolic pressure, rate of contraction, rate of relaxation, and total mechanical energy output were reduced and thus it appears that these animals were at an early stage of heart failure. It is understood that the division of experimental animals into early, moderate, and severe stages of congestive heart failure with respect to time after occlusion of the coronary artery is arbitrary and is useful in the comparison of hemodynamic and clinical changes with biochemical parameters.

2. [^{3}H]-nitrendipine binding in congestive heart failure. Specific binding of [^{3}H]-nitrendipine was significantly reduced in the unscarred left ventricular myocardium in experimental animals 16 weeks following infarction of the left ventricular free wall (Figure 1 and Table 4). Figure 1 shows the saturation curves and Scatchard plots of [^{3}H]-nitrendipine binding with crude cardiac membranes from control and experimental animals. While two populations of [^{3}H]-nitrendipine binding sites were detected in left

Table 3. Hemodynamic characteristics of experimental rats 4, 8, and 16 weeks after myocardial infarction.

GROUP	MAP	LVSP	LVEDP	+dp/dt	-dp/dt	HR	Total Mechanical Energy
4-week							
sham	120 ± 2	144 ± 4	3.68 ± 0.8	5568 ± 107	4781 ± 249	420 ± 7	60615 ± 1781
experimental	109 ± 12	122 ± 10	11.3* ± 1.2	4500* ± 477	3318* ± 404	385 ± 28	47380* ± 4854
8-week							
sham	117 ± 8	135 ± 7	3.49 ± 0.73	5413 ± 332	5312 ± 180	391 ± 15	54180 ± 2574
experimental	90* ± 6	119 ± 9	12.6* ± 1.4	4457* ± 138	3985* ± 210	357 ± 9	45036* ± 2310
16-week							
sham	115 ± 6	132 ± 7	3.44 ± 0.92	5762 ± 528	5400 ± 672	380 ± 16	50705 ± 4483
experimental	65* ± 4	90* ± 6	14.2* ± 0.8	3769* ± 213	3043* ± 303	242* ± 5	21987* ± 1330

Data are expressed as means ± SEM of eight experiments. All measurements were made on Beckman dynograph by using a Millar microcatheter; the catheter was inserted into the left ventricle via cannulation of the right carotid artery. MAP - mean aterial pressure (mmHg); LVSP - left ventricular systolic pressure (mmHg); LVEDP - left ventricular end diastolic pressure (mmHg); HR - heart rate; + dP/dt, rate of contraction (mm/sec); - dP/dt, rate of relaxation (mm/sec); Total mechanical energy = HR x LVSP. *$P < 0.05$

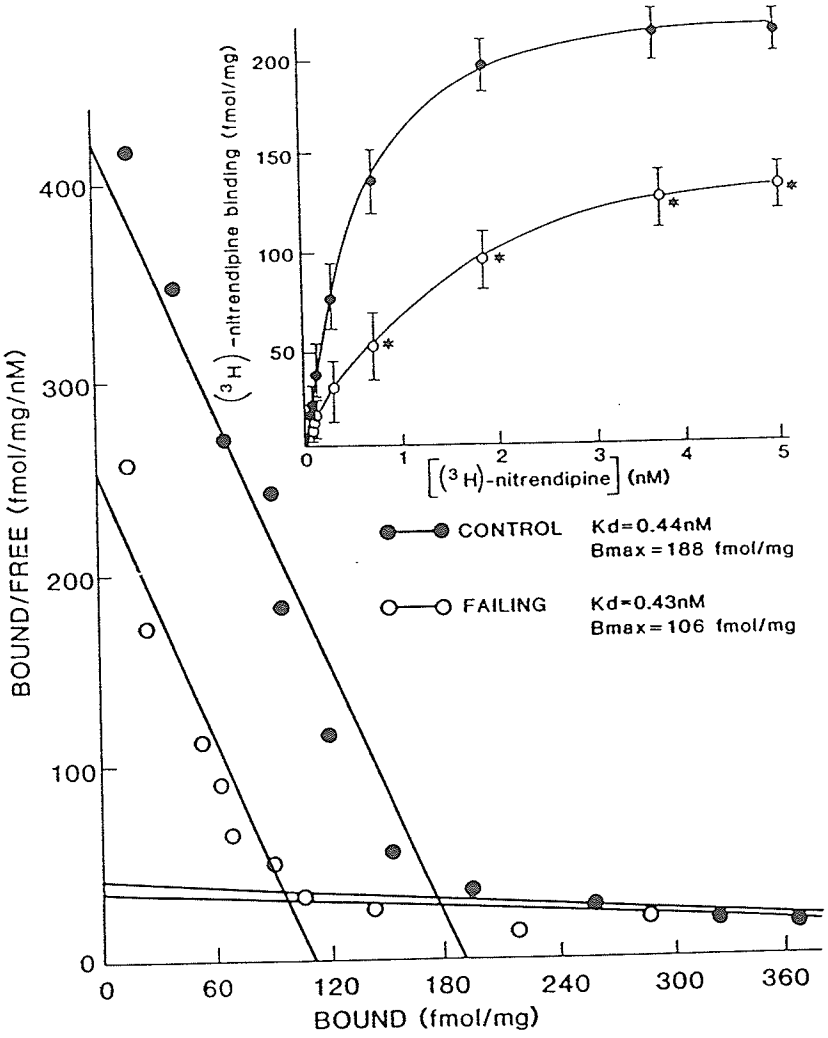

Figure 1. Scatchard plot analysis of specific [^{3}H]-nitrendipine binding to a crude membrane preparation of left ventricular tissue from control and failing (16 weeks) rats. Inset: Specific binding of [^{3}H]-nitrendipine to crude membrane preparations of left ventricle in control and failing animals at different concentrations of [^{3}H]-nitrendipine. Values represent mean $\pm$ SEM of eight experiments. Uninfarcted tissue of the left ventricle was used for failing preparations. K_d, dissociation constant; B_{max}, maximal density. * $P < 0.05$.

Table 4. [^{3}H]-nitrendipine binding (specific) with heart homogenate and crude membranes from control and 16-week failing animals.

	Control	Experimental
Yield of Crude Membranes (mg/g)	10.6 ± 0.6	10.9 ± 0.8
Heart Homogenate		
K_d (nM)	0.43 ± 0.06	0.49 ± 0.04
B_{max} (fmol/mg)	12.4 ± 1.02	6.8 ± 0.42*
Crude Membranes		
K_d (nM)	0.41 ± 0.06	0.40 ± 0.09
B_{max} (fmol/mg)	183 ± 12	103 ± 9*

The data expressed are means ± SEM of six experiments. Uninfarcted portion of the left ventricle of failing hearts was used. K_d, dissociation constant; B_{max}, maximal density. * $P < 0.05$

ventricular preparations (high and low affinity), no changes in [^{3}H]-nitrendipine binding characteristics (K_d and Bmax) of the low-affinity population were observed between control and experimental preparations. The data revealed that the maximal number of high affinity binding sites (B_{max}) was decreased in animals suffering from congestive heart failure without any change in the dissociation constant (K_d) (Table 4). It can also be seen from Table 4 that the [^{3}H]-nitrendipine binding in failing heart was also decreased when homogenate was employed for the assay. Table 5 illustrates the binding data of left ventricular preparations from control and experimental animals at different times following the induction of myocardial infarction. Both 8 and 16 week experimental groups showed significantly decreased [^{3}H]-nitrendipine receptor binding density with no change in K_d when compared to control values. No differences in binding characteristics of control and the 4 week experimental group was observed. The observed changes in ^{3}H-nitrendipine binding characteristics of the failing myocardium were also compared with normalized left ventricular hypertrophy in different experimental groups. It can be seen from Table 5 that a significant increase in cardiac hypertrophy 4 weeks after coronary ligation was not associated with any change in the ^{3}H-nitrendipine binding properties. Although Bmax values for ^{3}H-nitrendipine binding were depressed in 8 and 16 weeks following coronary ligation, no relationship between the extent of hypertrophy and changes in ^{3}H-nitrendipine binding was evident (Table 5). Figure 2 shows that the nonspecific binding of [^{3}H]-nitrendipine to membrane preparations of left ventricle from control and 16 week experimental animals was similar when bound [^{3}H]-nitrendipine was maximally displaced with high concentrations of unlabelled nifedipine; no further decrease in the total [^{3}H]-nitrendipine binding was seen when 5 to 10 uM unlabelled nifedipine was

Table 5. Binding (specific) characteristics of [^{3}H]-nitrendipine to crude membrane fraction prepared from uninfarcted portion of left ventricle in rat heart 4, 8, and 16 weeks after myocardial infarction.

	Left Ventricle		Normalized left ventricular hypertrophy
	K_d (nM)	Bmax (fmol/mg)	(% mass of control left ventricle)
Control	0.41 ± 0.05	186 ± 11	100
4 week	0.42 ± 0.04	165 ± 9	135 ± 7
8 week	0.40 ± 0.08	149* ± 5	197 ± 11
16 week	0.45 ± 0.07	112* ± 10	201 ± 9

Data are expressed as means ± SEM of eight experiments for each of 4, 8 and 16 week failing hearts and 12 experiments for control hearts. Because the values for 4, 8 and 16 week sham controls were overlapping, the results were grouped together as "control". Uninfarcted tissue of the failing left ventricle was used for experimental preparations. For the determination of degree of hypertrophy, the percent of the infarcted left ventricle was estimated 3 weeks after coronary ligation by planimetric techniques; this percentage was extrapolated to the respective experimental groups. K_d, dissociation constant; B_{max}, maximal density. *$P < 0.05$

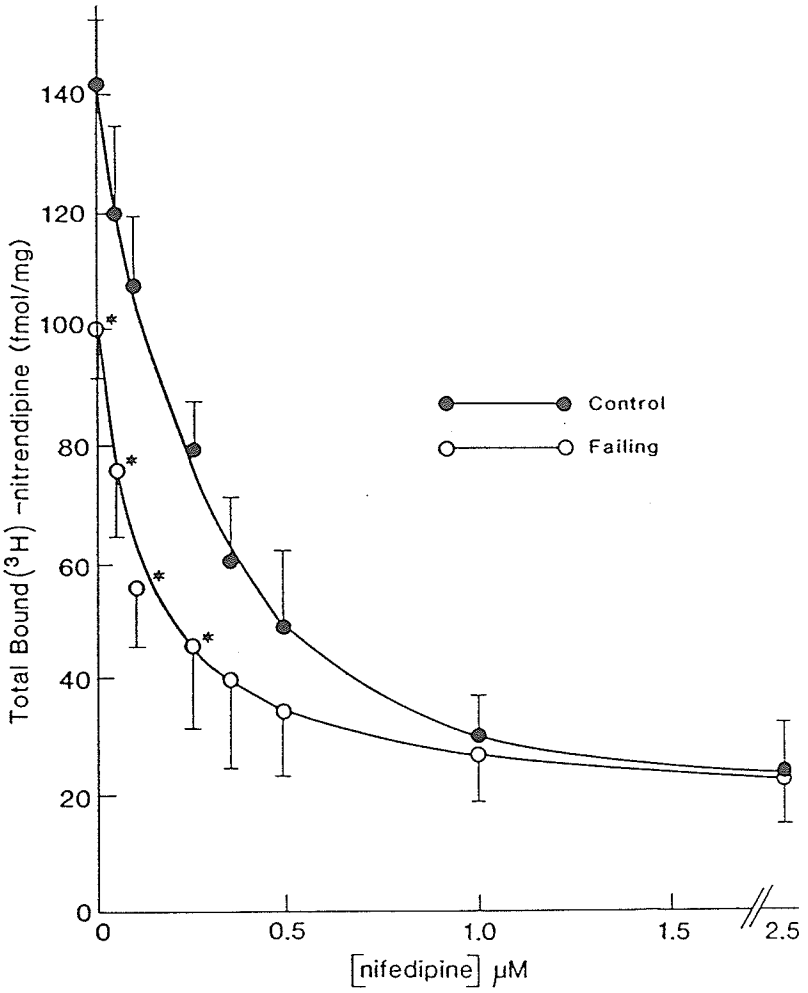

Figure 2. Inhibition by unlabelled nifedipine of [^{3}H]-nitrendipine binding to crude membrane fraction prepared from left ventricle of rats 16 weeks following myocardial infarction and age-matched control rats. Values represent mean + SEM of eight experiments. Uninfarcted tissue of the left ventricle from failing hearts was used. * $P < 0.05$.

used in the incubation medium.

To determine if changes in [^{3}H]-nitrendipine binding with failing hearts were of a specific nature, we prepared the medial gastrocnemius (skeletal) muscle, brain cortex, cardiac right ventricle, and liver membranes from controls and 16 week experimental groups. In brain and skeletal muscle preparations, the density of [^{3}H]-nitrendipine receptor sites was significantly decreased in the experimental animals when compared to control, with no change in K_d (Table 6). Right ventricle and liver preparations of control and experimental groups were not changed with respect to receptor density or dissociation constant (Table 6).

[^{3}H]-nitrendipine binding characteristics of two types of purified sarcolemmal membranes (216,285) were also compared to rule out the possibility of any artefacts (Table 7). No difference in protein yield between experimental and control groups were seen in each of these membrane preparations. Similarly, the K_d was unchanged in both types of preparations from the experimental and control left ventricles, whereas the [^{3}H]-nitrendipine receptor density was significantly decreased in these membranes from 16 week experimental group. To test if the observed changes in failing hearts were limited to the [^{3}H]-nitrendipine binding, another radioligand ([^{3}H]-PN200-110) was used for studying the binding characteristics. Scatchard plot analysis of [^{3}H]-PN200-110 binding to homogenate and crude membrane fraction derived from the 16 week sham-operated and experimental groups is given in Table 8. A reduction in the receptor density without any changes in K_d was evident in failing heart homogenate as well as crude membrane preparations.

3. [^{3}H]-dihydroalprenolol and [^{3}H]-prazosin binding in congestive heart failure. Figure 3 shows the saturation curves and Scatchard plots of [^{3}H]-

Table 6. Binding (specific) characteristics of [^{3}H]-nitrendipine to crude membrane fractions of various tissues in rats 16 weeks after myocardial infarction.

	K_d (nM)		Bmax (fmol/mg)	
Tissue	Control	Experimental	Control	Experimental
Right Ventricle	0.40 ± 0.06	0.44 ± 0.07	92 ± 12	85 ± 8
Brain (Cortex)	0.27 ± 0.06	0.23 ± 0.09	117 ± 12	77* ± 9
Skeletal Muscle	5.9 ± 0.6	6.5 ± 0.9	1361 ± 216	750* ± 186
Liver	8.3 ± 0.6	9.7 ± 1.8	69.2 ± 8.1	79.8 ± 7.6

Data are expressed as means ± SEM of eight experiments. K_d, dissociation constant; B_{max}, maximal density. * $P < 0.05$

Table 7. Comparison of [^{3}H]-nitrendipine binding to (specific) purified sarcolemmal preparations isolated by two different methods from control and 16-week experimental hearts.

		Sarcolemma	
		Sucrose gradient	Hypotonic shock
Yield (mg/g)	Control	1.3 ± 0 .4	3.7 ± 0.7
	Failing	1.2 ± 0.7	3.6 ± 0.5
Kd (nM)	Control	0.45 ± 0.08	0.39 ± 0.07
	Failing	0.44 ± 0.07	0.40 ± 0.08
Bmax (fmol/mg)	Control	162 ± 14	148 ± 13
	Failing	88* ± 9	77* ± 11

Data are expressed as means ± SEM of eight experiments. The light sarcolemmal preparation was obtained by the sucrose density gradient method, whereas the heavy sarcolemmal preparation was obtained by the hypotonic shock-LiBr treatment method. Uninfarcted tissue from the left ventricle of failing hearts was used 16 weeks after induction of myocardial infarction was used. K_d, dissociation constant; B_{max}, maximal density. *$P < 0.05$

Table 8. Comparison of binding (specific) characteristics of the dihydropyridine compound [^{3}H]-PN200-110 to homogenate and crude membrane preparations from control and 16-week experimental hearts.

	Homogenate		Crude Membrane	
	Control	Experimental	Control	Experimental
Bmax (fmol/mg protein)	23.3 ± 1.6	18.0 ± 0.8*	276 ± 12.9	206 ± 8.6*
Kd (nM)	0.29 ± 0.01	0.28 ± 0.01	0.25 ± 0.01	0.26 ± 0.02

Values are means ± SEM of six experiments. Uninfarcted left ventricle from failing rats 16 weeks after inducing infarction was used. K_d, dissociation constant; B_{max}, maximal density. * $P < 0.05$

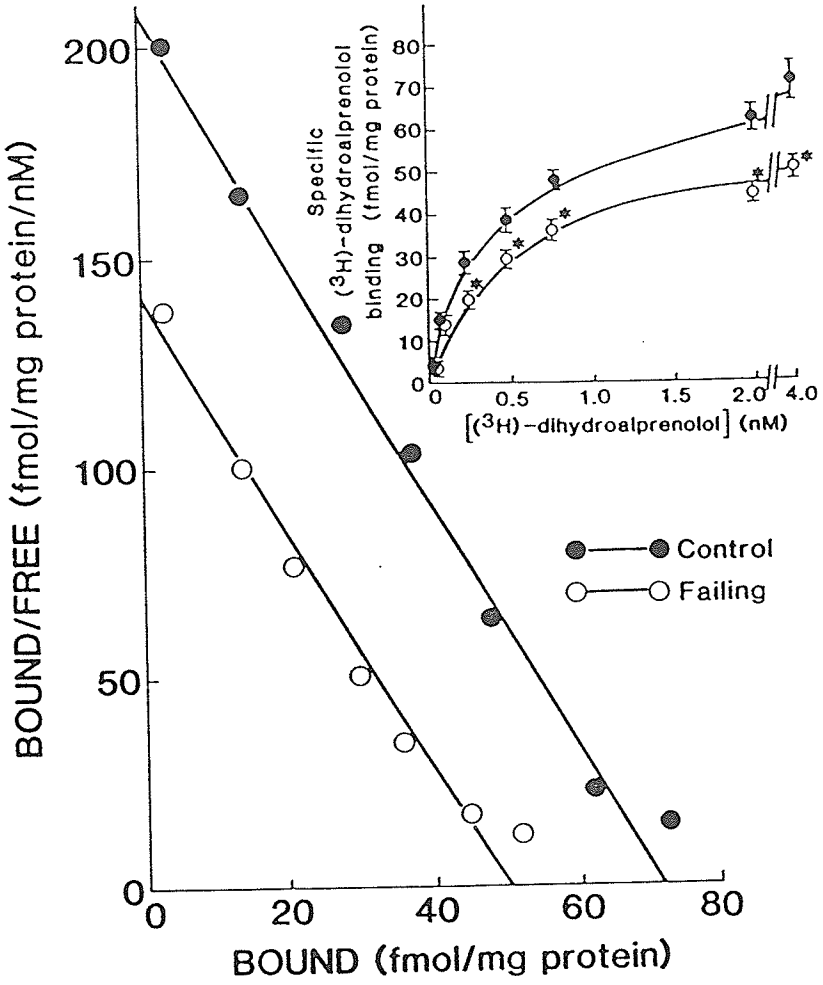

Figure 3. Scatchard plot analysis of specific [^{3}H]-dihydroalprenolol binding to a crude membrane preparation of left ventricular tissue from control and early stage failing (4 week) rats. Inset: Specific binding of [^{3}H]-dihydroalprenolol to crude membrane preparations of left ventricle in control and experimental animals at different concentrations of [^{3}H]-dihydroalprenolol. Values represent mean ± SEM of eight experiments. Uninfarcted tissue of the left ventricle was used for experimental preparations. * $P < 0.05$.

dihydroalprenolol specific binding with crude cardiac membranes from control and 4 weeks experimental animals. The data revealed that the maximal number (B_{max}) of a single population of high affinity binding sites was decreased in the experimental animals with early stage of heart failure without any change in the dissociation constant (K_d). A significant reduction of the Bmax value for specific binding of [^{3}H]-dihydroalprenolol was seen in preparations of animals suffering early, moderate, and severe congestive heart failure (Table 9). Figure 4 (bottom) shows that the nonspecific binding of [^{3}H]-dihydroalprenolol to membrane preparations of left ventricle from control and 8 week experimental animals was similar when bound [^{3}H]-dihydroalprenolol was maximally displaced with high concentrations of unlabelled propranolol; these concentrations were similar to that used for the determination of nonspecific [^{3}H]-dihydroalprenolol binding.

Figure 5 shows the saturation curves and Scatchard plots of [^{3}H]-prazosin binding with crude cardiac membranes from control and 4 week experimental animals; no change in either the B_{max} or K_d values were seen in the 4 week experimental group when compared to the control group. Scatchard analysis of the specific binding of [^{3}H]-prazosin revealed a significant increase in the B_{max} value in preparations of the 8 and 16 week experimental group without any change in K_d values (Table 9). Therefore an increase in the maximal number of [^{3}H]-prazosin binding sites was only apparant in animals suffering moderate or severe congestive heart failure. Figure 4 (top) shows that the nonspecific binding of [^{3}H]-prazosin to membrane preparations from control and 8 week experimental animals was similar when bound [^{3}H]-prazosin was maximally displaced with high concentrations of unlabelled phentolamine.

The observed changes in the [^{3}H]-drug binding characteristics of the

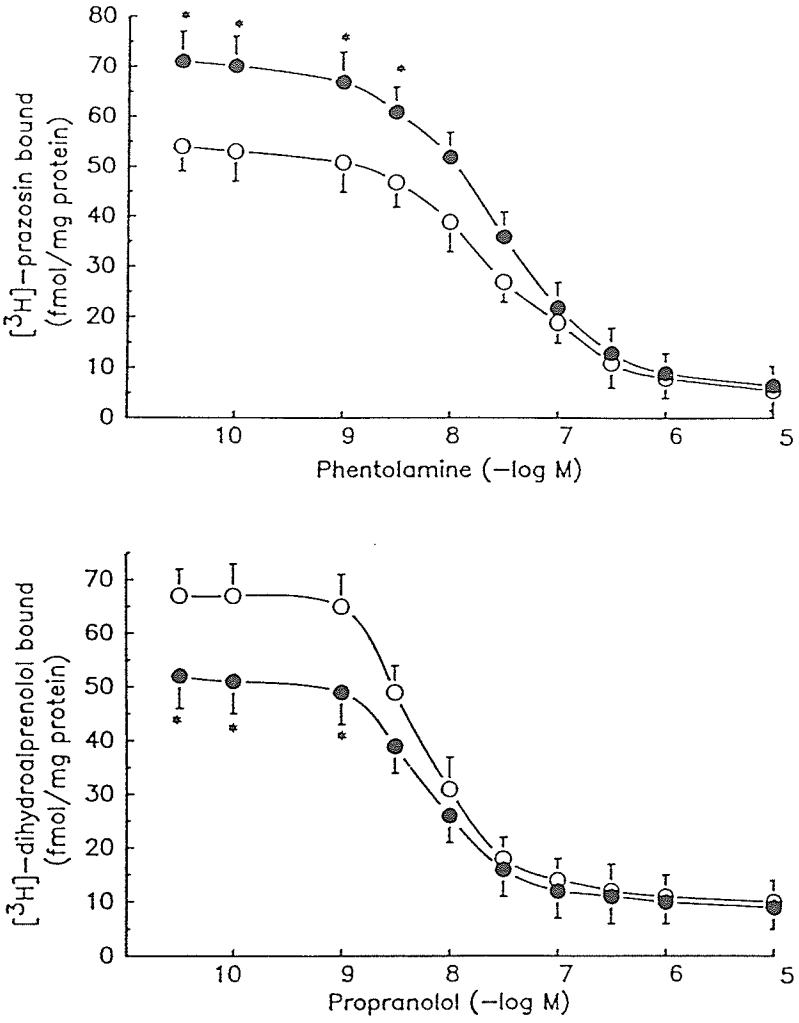

Figure 4. Inhibition by unlabelled phentolamine of [^{3}H]-prazosin binding (TOP) and by unlabelled propranolol of [^{3}H]-dihydroalprenolol binding (BOTTOM) to crude membrane fraction prepared from left ventricle of rats 8 week following myocardial infarction and age-matched control rats. In all experiments, values represent mean ± SEM of eight experiments. (0---0) and (●---●) denotes values of [^{3}H]-drug bound for control and experimental preparations, respectively. Uninfarcted tissue of the left ventricle from failing hearts was used. * $P < 0.05$.

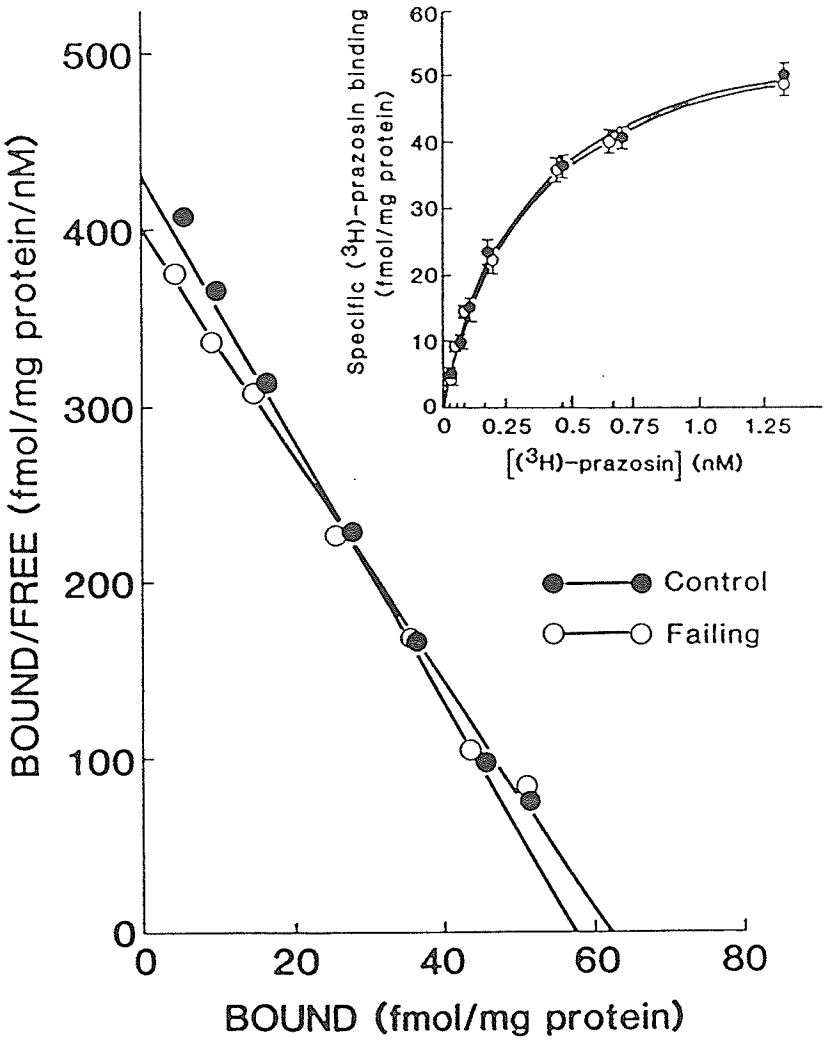

Figure 5. Scatchard plot analysis of specific [^{3}H]-prazosin binding to a crude membrane preparation of left ventricular tissue from control and early stage failure (4 week) rats. Inset: specific binding of [^{3}H]-prazosin to crude membrane preparations of left ventricle in control and experimental animals at different concentrations of [^{3}H]-prazosin. Values represent mean ± SEM of eight experiments. Uninfarcted tissue of the left ventricle was used for failing preparations. * $P < 0.05$.

Table 9. Binding characteristics of ^{3}H-dihydroalprenolol and ^{3}H-prazosin to crude membrane fraction prepared from uninfarcted portion of left ventricle in rat heart 4, 8, and 16 weeks after myocardial infarction.

	^{3}H-dihydroalprenolol		^{3}H-prazosin	
	Kd (nM)	Bmax (fmol/mg protein)	Kd (nM)	Bmax (fmol/mg protein)
Control	0.43 ± 0.07	79 ± 3	0.15 ± 0.03	58 ± 3
4 weeks	0.41 ± 0.04	50 ± 3*	0.13 ± 0.03	65 ± 3
8 weeks	0.39 ± 0.06	55 ± 4*	0.13 ± 0.02	78 ± 3*
16 weeks	0.44 ± 0.06	59 ± 4*	0.11 ± 0.02	76 ± 4*

Data are expressed as mean ± SEM of eight experiments for each of 4, 8, and 16 weeks failing hearts and 12 experiments for control hearts. Uninfarcted tissue of the failing left ventricle was used for experimental preparations. K_d, dissociation constant; B_{max}, maximal density. * $P < 0.05$

failing myocardium were compared with normalized left ventricular hypertrophy in different experimental groups. It can be seen from Table 5 and Table 9 that a significant increase in cardiac hypertrophy 4 weeks after coronary ligation was not associated with any change in the [^{3}H]-prazosin binding properties. Although the B_{max} values for [^{3}H]-dihydroalprenolol binding were depressed in animals 4, 8, and 16 weeks following coronary ligation, no relationship between the extent of hypertrophy and changes in the [^{3}H]-drug binding was evident whereas the increase of B_{max} values for [^{3}H]-prazosin binding was apparent in the 8 and 16 week groups.

[^{3}H]-dihydroalprenolol and [^{3}H]-prazosin binding characteristics of purified sarcolemmal vesicles preparation were compared to rule out the possibility of artifacts. Table 10 shows the binding characteristics of [^{3}H]-dihydroaprenolol and [^{3}H]-prazosin to purified sarcolemmal vesicles prepared from uninfarcted portion of the left ventricle in 4 week experimental animal ([^{3}H]-dihydroalprenolol) and in 16 week experimental animals ([^{3}H]-prazosin) and in respective control groups. The K_d value for [^{3}H]-dihydroalprenolol binding was similar in the 4 week experimental group compared to control value; however the B_{max} value was significantly decreased. The K_d value of [^{3}H]-prazosin binding in the 16 week experimental group was unchanged and the B_{max} value was increased when compared to control. No difference in the protein yield between experimental and control groups were seen in the purified sarcolemmal preparation (mean ± SEM: 1.1 ± 0.1 vs. 1.0 ± 0.1 mg protein/g wet tissue). The binding characteristics of [^{3}H]-dihydroalprenolol and [^{3}H]-prazosin to the modified crude membrane preparation in which the initial 1000 g spin was excluded were not significantly different from binding characteristics to the crude membrane preparations (data not shown).

Table 10. Binding characteristics of ^{3}H-dihydroalprenolol and ^{3}H-prazosin to purified sarcolemmal vesicles prepared from uninfarcted portion of the left ventricle in rat heart following myocardial infarction.

	^{3}H-dihydroalprenolol		^{3}H-prazosin	
	Control	4 weeks	Control	16 weeks
Kd (nM)	0.49 ± 0.06	0.42 ± 0.04	0.14 ± 0.02	0.11 ± 0.03
Bmax (fmol/mg)	251 ± 19	166 ± 12*	475 ± 21	571 ± 18*

Data are expressed as mean + SEM of four experiments for each group. K_d, dissociation constant; B_{max}, maximal density. * $P < 0.05$

4. Myocardial responsiveness to isoproterenol and phenylephrine in early and moderate stages of congestive heart failure. Figure 6 shows the response (expressed as % of the control maximal rate of contraction) of isolated heart preparations induced by infusion of various concentrations of isoproterenol and phenylephrine in early stage failure animals (4 weeks following induction of myocardial infarction) and moderate stage failure animals (8 weeks following induction of infarction). In heart preparations of the 4 week experimental group of animals (upper panel), a significant depression of myocardial responsiveness at several concentrations of isoproterenol was noted when compared to the control values. On the other hand, the effect of phenylephrine on hearts of this group not different from that of the control group. Isolated hearts of the 8 week experimental group (lower panel) showed blunted responses to several concentrations of infused isoproterenol, whereas the responsiveness of these hearts to phenylephrine was slightly increased but these differences were not found to be significant.

5. Na^+-K^+ ATPase activity in moderate congestive heart failure (8 weeks). The results in Table 11 indicate that sarcolemmal Na^+-K^+ ATPase and ouabain-sensitive ATPase activities in the 8 weeks experimental hearts were markedly decreased in comparison to the control values. It should be mentioned that control membranes used in this study have been characterized as being predominantly of inside-out orientation (22,288). Since ouabain is known to bind on the outer or extracellular face of the sarcolemmal membrane (19) whereas ATP hydrolysis occurs at the inner side or cytoplasmic face, (18,19) any inhibition of activity by ouabain must occur in right-side out and leaky vesicles. The data from this study indicate that about 20% of the total Na^+-K^+ ATPase activity in the untreated control and experimental preparations was ouabain-sensitive (Table 11). On the other hand,

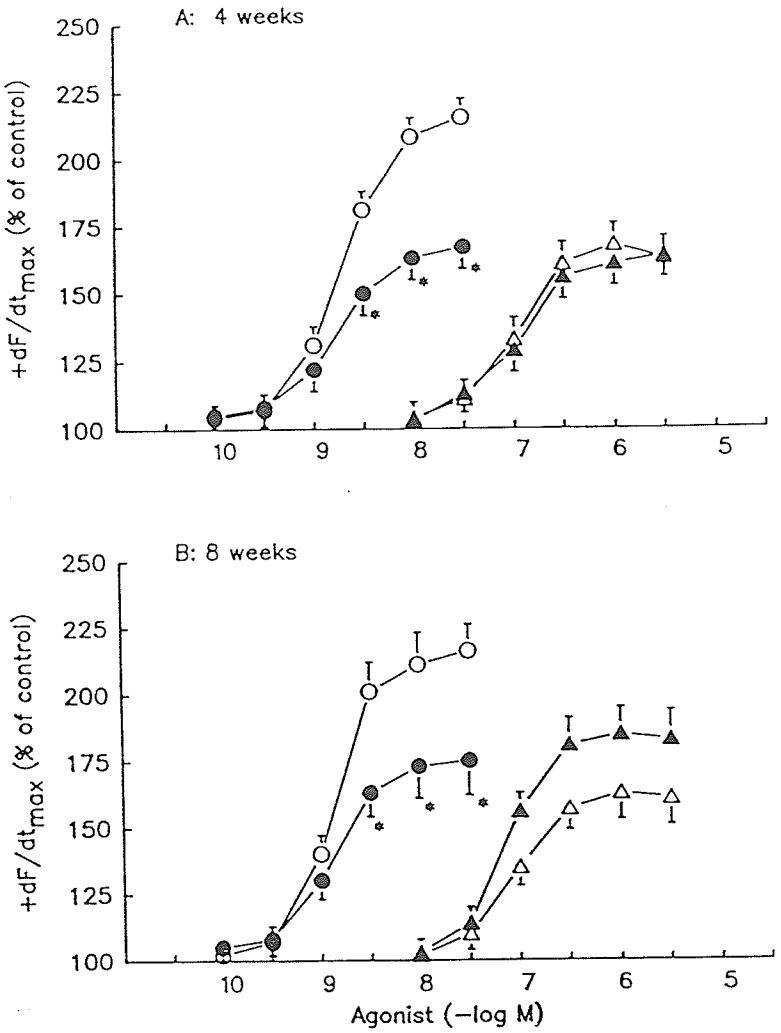

Figure 6. Maximal rate of contraction in isolated perfused hearts from control and experimental animals (Panel A: 4 week, Panel B: 8 week). (O---O) indicates response of control hearts to isoproterenol (●---●) indicates the response of failing hearts to isoproterenol. (△---△) indicates the response of control hearts to phenylephrine; (▲---▲) indicates the response of failing hearts to phenylephrine. All values represent mean ± SEM of eight experiments. * $P < 0.05$.

Table 11. Effects of alamethicin and sodium dodecyl sulphate (SDS) on the rat heart sarcolemmal Na^+-K^+ ATPase activities from control and experimental (8 weeks after induction of myocardial infarction) animals.

Group	Mg^{2+}-ATPase (umol Pi/mg protein/hr)	Na^+-K^+ ATPase (umol Pi/mg protein/hr)	Ouabain-sensitive Na^+-K^+ ATPase (umol Pi/mg protein/hr)
Control	80.2 ± 4.2	19.9 ± 1.1	4.7 ± 0.3
Experimental	57.1 ± 3.2*	12.4 ± 0.9*	2.9 ± 0.2*
Control (Alamethicin)	59.2 ± 1.8†	15.0 ± 1.7†	12.8 ± 1.1†
Experimental (Alamethicin)	45.9 ± 2.1*†	9.7 ± 0.5*†	8.1 ± 0.6*†
Control (SDS)	60.7 ± 3.2†	14.1 ± 1.1†	14.0 ± 1.6†
Experimental (SDS)	45.4 ± 2.9*†	9.6 ± 0.6*†	9.0 ± 0.6*†

Each value is a mean ± SEM of 4-8 experiments. Experimental animals were sacrificed 8 weeks following surgical occlusion of the left coronary artery. According to the type of experiment, the assay medium (final) contained i) alamethicin/sarcolemmal protein ratio of 0.5:1 ii) SDS/sarcolemmal protein ratio of 0.03:1. Purified sarcolemmal vesicles were prepared from left ventricular tissue; in the experimental groups, only viable left ventricular tissue was used for sarcolemmal membrane isolation. * $P < 0.05$ compared to control values. † $P < 0.05$ compared to control untreated and experimental untreated values, respectively.

digitoxigenin, which unlike ouabain is freely permeable across the membrane vesicles, was capable of the Na^+-K^+ ATPase activity completely; digitoxigenin-sensitive Na^+-K^+ ATPase activity in control and 8 weeks experimental sarcolemmal preparations were 21.8 ± 1.9 and 10.4 ± 1.2 umol Pi/mg protein/hr, respectively. The results in Table 11 also indicate that Mg^{2+} ATPase activity in the experimental sarcolemmal preparations was depressed in comparison to the control membranes. Both Na^+-K^+ ATPase and Mg^{2+} ATPase activities in control and experimental preparations were only slightly altered in the presence of monensin, a Na^+- and K^+-ionophore. In this regard, Na^+-K^+ ATPase activities in control and 8 weeks experimental preparations in the presence of 2.5 uM monensin were 22.4 ± 2.4 and 11.6 ± 0.8 umol Pi/mg protein/hr whereas Mg^{2+}-ATPase activities were 86.7 ± 5.2 and 57.3 ± 1.9 umol Pi/mg protein/hr, respectively. As maximal activation of the Na^+-K^+ ATPase activity in both control and experimental preparations was attained in the abscence of monensin, it is unlikely that the observed depression in the Na^+-K^+ ATPase in experimental preparations was due to any differences in the accessibility of cations to the enzymes in the vesicular preparations.

The Na^+-K^+ ATPase activities in the control and experimental preparations were also measured upon treating the sarcolemmal vesicles with sodium dodecyl sulfate (SDS), a detergent commonly used to increase membrane permeability, and alamethicin, which is known to produce holes in the membrane. The data in Table 11 show that both Mg^{2+} ATPase and Na^+-K^+ ATPase activities in the experimental preparations remained depressed in comparison to the control membranes upon treatment with SDS or alamethicin. It may be noted that in contrast to the untreated membranes, the Na^+-K^+ ATPase activities in both control and experimental preparations was almost

fully inhibited by ouabain upon treating the membranes with SDS or alamethicin. Furthermore, treatment of sarcolemmal preparations with alamethicin and SDS was seen to inhibit both Mg^{2+} ATPase and Na^+-K^+ ATPase activities in control and experimental preparations when compared to activities of untreated samples (Table 11). This finding is in accord with previous observations with similar control preparations (288). As reported in earlier studies (22,25) the Mg^{2+}-ATPase activity was higher than that for Na^+-K^+ ATPase in rat sarcolemma.

To determine the pH optima of Na^+-K^+ ATPase activity in control and experimental sarcolemmal preparations, the enzyme activity was measured in the incubation medium at different pH. It is evident from Figure 7 that the response of Na^+-K^+ ATPase to various pH levels was almost parallel between control and experimental samples; the pH optima of activity from both preparations was 7.2 - 7.4. In another series of experiments, the concentrations of Na^+ and K^+ in the incubation medium were varied while the osmolarity of the medium was kept constant (Figure 8). The results reveal that the optimal activating concentrations of Na^+ and K^+ were about 95 and 25 mM respectively for both the control and experimental sarcolemmal preparations. It is pointed out that a marked depression of Na^+-K^+ ATPase activity was seen in 8 weeks experimental left ventricle samples at every point in the pH response study when compared to control values; a similar trend was apparent on varying Na^+ and K^+ concentrations.

6. Kinetic characteristics of Na^+-K^+ ATPase in congestive heart failure. Na^+-K^+ ATPase activity of the sarcolemma from control and 8 weeks experimental rat hearts was studied by varying the concentration of MgATP in the incubation medium (Figure 9). Lineweaver-Burk analysis of the data revealed a depression of V_{max} (indicating maximal velocity of the

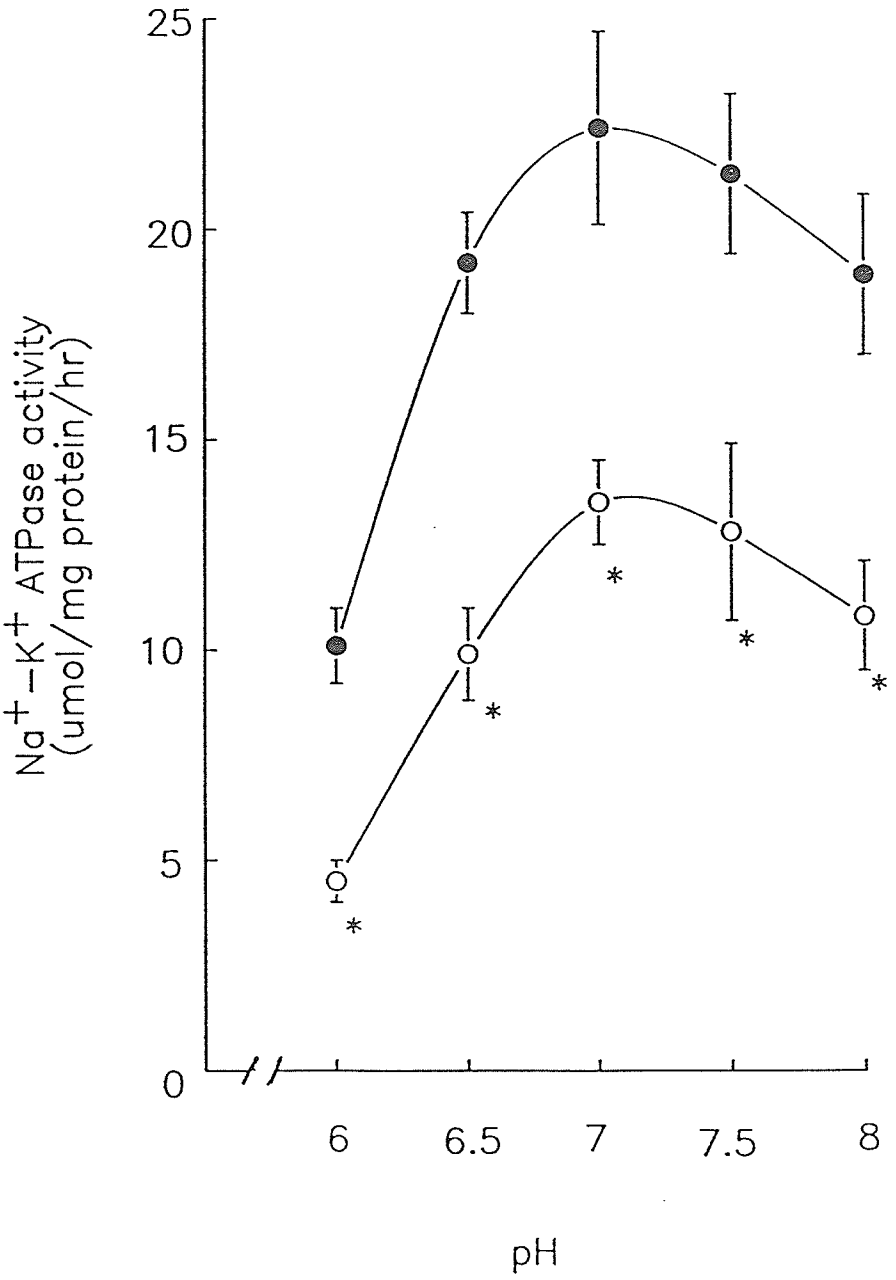

Figure 7. Relation of pH and Na^+-K^+ ATPase activity in sarcolemmal preparations from control and experimental (8 weeks after induction of myocardial infarction) left ventricle. Control - ●--●; failing - O--O; * $P < 0.05$ when compared to the control values. Each value is a mean ± SEM of six experiments.

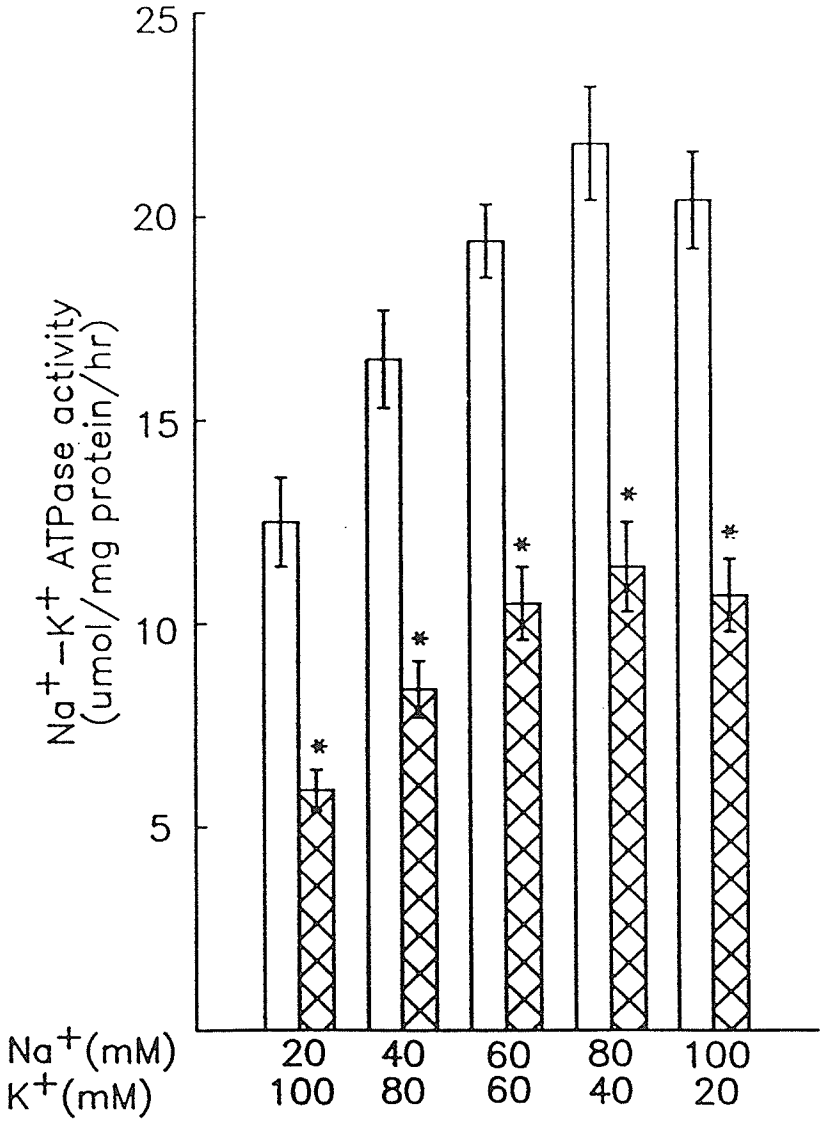

Figure 8. Na^+-K^+ ATPase activity in sarcolemmal preparations from control ([ ]) and experimental ([XXX]) left ventricle (8 weeks after induction of myocardial infarction) measured in the presence of different concentrations of Na^+ and K^+ ions. The indicated concentrations of Na^+ and K^+ ions in the incubation medium represented actual additions. * $P < 0.05$ when compared to the control values. Each value is a mean ± SEM of six experiments.

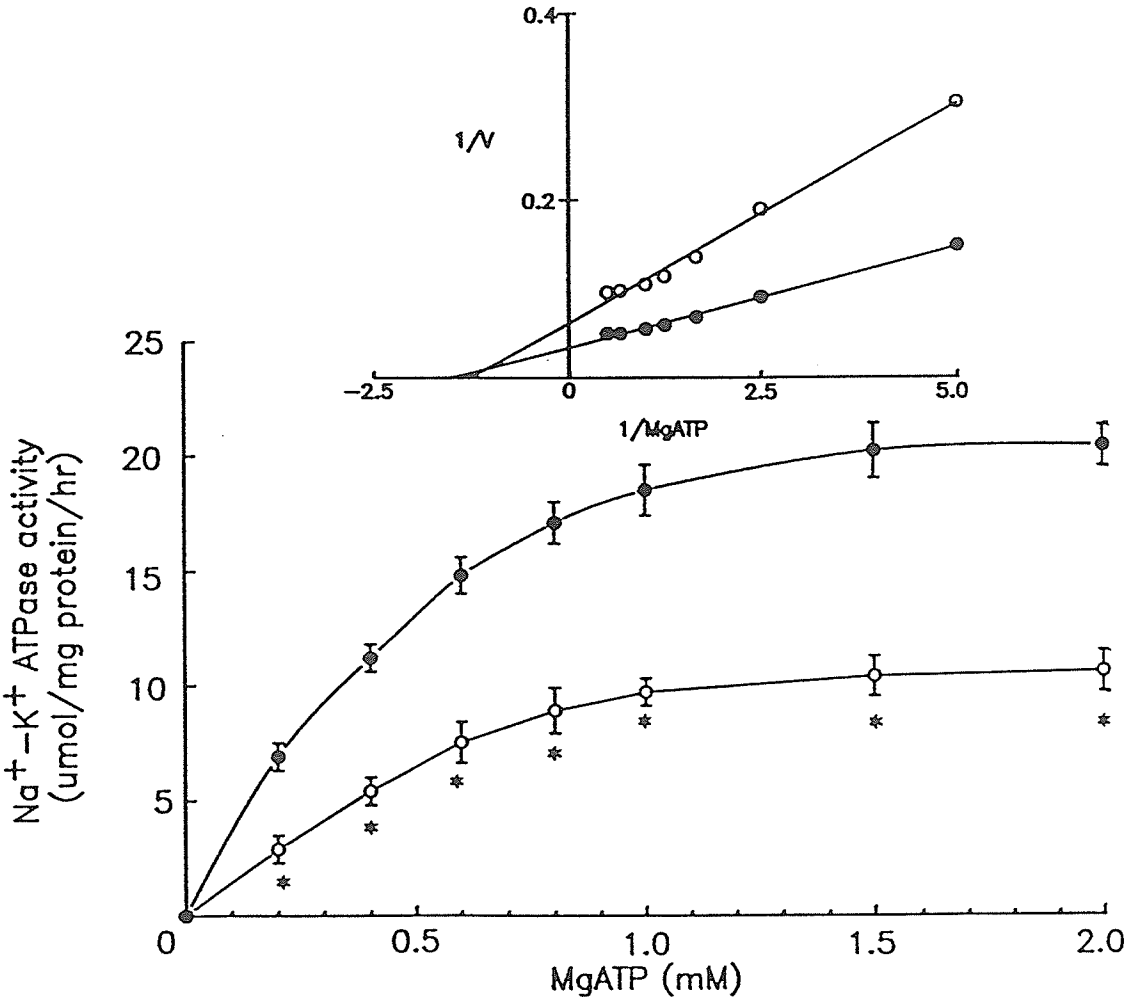

Figure 9. Saturation kinetics of Na^+-K^+ ATPase activity with MgATP in sarcolemmal preparations from control and experimental (8 weeks following induction of myocardial infarction) left ventricle. INSET: Lineweaver-Burk plot of the data. Control - ●--●; failing - O--O; * $P < 0.05$ when compared to the control values. Each value is a mean ± SEM of six experiments.

reaction) in the experimental preparations without any changes in the Km value for MgATP (Table 12). The kinetic properties of Na^+-K^+ ATPase activity from the control and experimental hearts were studied by changing the Na^+ concentration while maintaining the K^+ concentration (10 mM) or by changing K^+ concentration while maintaining Na^+ concentration (100 mM). Under these conditions, the reaction velocity curves were parabolic in shape and the Na^+-K^+ ATPase activity of the experimental preparations was depressed at most points along the Na^+-response (Figure 10) or K^+-response (Figure 11) curves, when compared to control values. Lineweaver-Burk plot analysis of these data revealed that V_{max} for Na^+-K^+ ATPase in experimental membranes was decreased in response to alteration of either Na^+ or K^+ (Figure 10 and Figure 11, respectively), but no change in the Ka for Na^+ or K^+ was evident (Table 12).

7. Na^+-K^+ ATPase in early (4 weeks) and severe (16 weeks) stages of congestive heart failure. In a seperate set of experiments, the Na^+-K^+ ATPase activity in 4 weeks experimental animals was studied and the data are shown in Table 13. The Na^+-K^+ ATPase, Mg^{2+}-ATPase, and ouabain-sensitive Na^+-K^+ ATPase activities in the experimental preparations showed a slight depression when compared to control values; however, none of these changes were significant. Pretreatment of sarcolemmal preparations with SDS (0.03 mg SDS/mg protein) was associated with inhibition of all activities, but no differences were found among the two groups in any assay. The Na^+-K^+ ATPase, ouabain-sensitive Na^+-K^+ ATPase and Mg^{2+} ATPase activities in the control and 16 weeks experimental preparations were also measured by employing 4 animals in each group. All these activities in 16 weeks failing heart preparations were significantly decreased in comparison to the control values (data not shown) but the magnitudes of these depressions were not

Table 12. Kinetic parameters for Na^+-K^+ ATPase in the presence of various concentrations of MgATP, K^+, and Na^+ in cardiac sarcolemma isolated from viable left ventricular myocardium of control and experimental (8 weeks after induction of myocardial infarction) rats.

	Control	Experimental
V_{max} for Na^+-K^+ ATPase (umol Pi/mg protein/hr)	25.6 ± 1.6	14.7 ± 0.8*
K_m for MgATP (mM)	0.70 ± 0.09	0.81 ± 0.07
K_a for K^+ (mM)	2.6 ± 0.3	3.1 ± 0.4
K_a for Na^+ (mM)	10.5 ± 0.1	9.9 ± 0.1

Data are expressed as mean ± SEM of 4 experiments. Experimental conditions were the same as those indicated in Figs. 9, 10 and 11. * $P < 0.05$

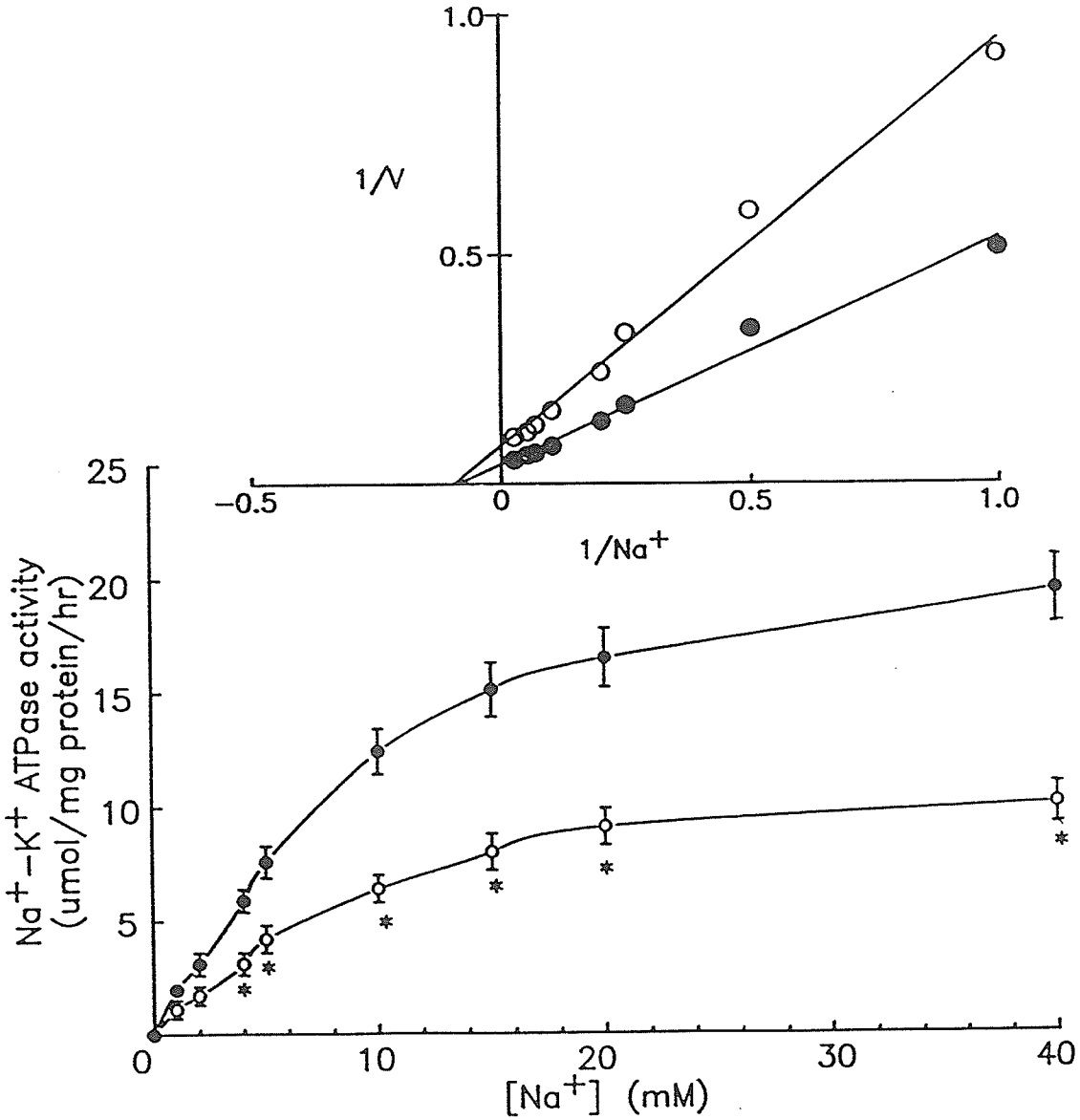

Figure 10. Response of Na^+-K^+ ATPase activity to Na^+ ion concentration in sarcolemmal preparations from control and experimental (8 weeks after induction of myocardial infarction) left ventricle. Control - ●--●; failing - O--O; * $P < 0.05$ when compared to the control values. Each value is a mean ± SEM of six experiments.

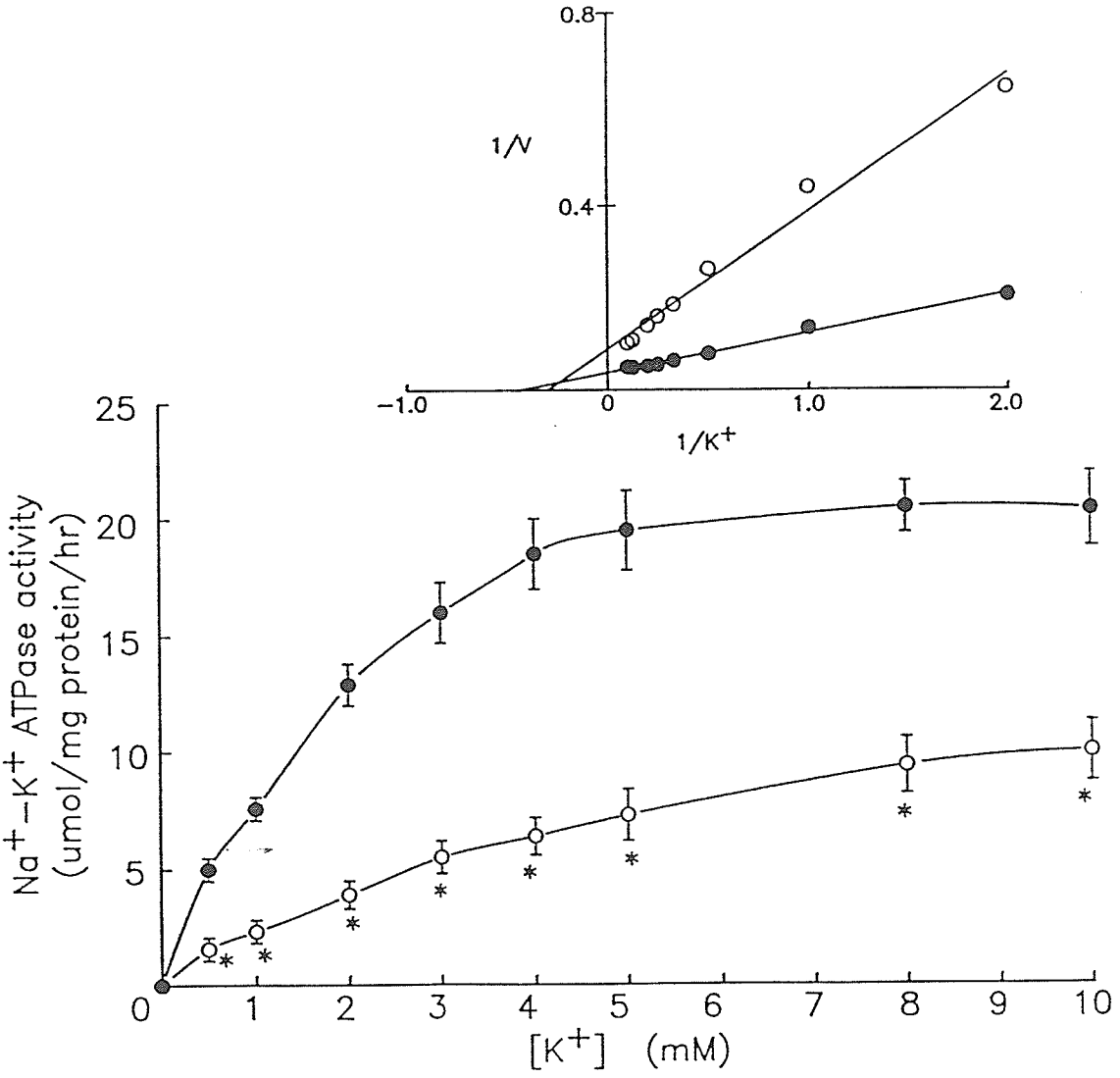

Figure 11. Response of Na^+-K^+ ATPase activity to K^+ ion concentration in sarcolemmal preparations from control and experimental (8 weeks after induction of myocardial infarction) left ventricle. Control - ●--●; failing - O--O; * $P < 0.05$ when compared to the control values. Each value is a mean ± SEM of six experiments.

Table 13. Na^+-K^+ ATPase activities in sarcolemma vesicles isolated from the viable left ventricular tissue from control and experimental (early failure stage - 4 weeks after induction of myocardial infarction) rats.

	Mg^{2+} ATPase	Na^+-K^+ ATPase	Ouabain-sensitive Na^+-K^+ ATPase
		(umol Pi/mg protein/hr)	
A. Untreated preparations			
Control	85.6 ± 3.2	20.8 ± 1.3	5.2 ± 0.4
Experimental	72.5 ± 3.1*	17.1 ± 1.4	4.8 ± 0.6
B. SDS - treated preparations			
Control	68.4 ± 2.9	15.3 ± 1.8	15.0 ± 1.2
Experimental	57.1 ± 2.2*	13.9 ± 0.9	13.5 ± 0.6

Data are expressed as mean ± SEM of 6 experiments. Treatment with 0.03 mg SDS/mg protein was carried out for 25 min at 20° C. Ouabain - sensitive ATPase was that portion which was inhibited by 1 mM ouabain. * $P < 0.05$

different from those observed for 8 weeks experimental samples.

8. [^{3}H]-ouabain binding activity in experimental left ventricle. The Na^+-K^+ ATPase protein is considered to be a receptor for the cardiac glycosides; two classes of ouabain binding sites have been implicated by ouabain dose-response contractile force experiments on cardiac muscle preparations (237) and by biochemical ligand-binding techniques (295). Recent data from experiments with rat cardiac tissue indicates that high- and low-affinity binding sites may correspond to two different isozymes of the Na^+-K^+ ATPase catalytic subunit, designated alpha + and alpha, respectively (296); however, the relative contribution of each Na^+-K^+ ATPase isozyme to the glycoside-mediated cardiac inotropic effect in vivo is not fully understood (237,238). Figure 12 shows the Scatchard plots of [^{3}H]-ouabain binding with purified cardiac sarcolemmal membranes from control and 8 weeks experimental animals. These results confirm the presence of two classes of ouabain binding sites and show that the maximal densities (B_{max}) for both high and low affinity sites were similar in the control and 8 weeks experimental preparations (Figure 12 and Table 14). On the other hand, a significant increase in the K_d (dissociation constant) was noted in both high-affinity and low-affinity binding sites in experimental preparations (Figure 12 and Table 14).

9. Sarcolemmal Na^+-Ca^{2+} exchange in congestive heart failure. Figure 13 shows Na^+-dependent Ca^{2+} uptake monitored at different times of incubation in control and experimental samples. It can be seen that vesicles from left ventricle of experimental animals showed a significant depression in both the initial rate and the maximal Na^+-dependent Ca^{2+} uptake in comparison to the control preparations. It should be noted that the initial rates of Na^+-dependent Ca^{2+} uptake were linear during the 5s period in both control and

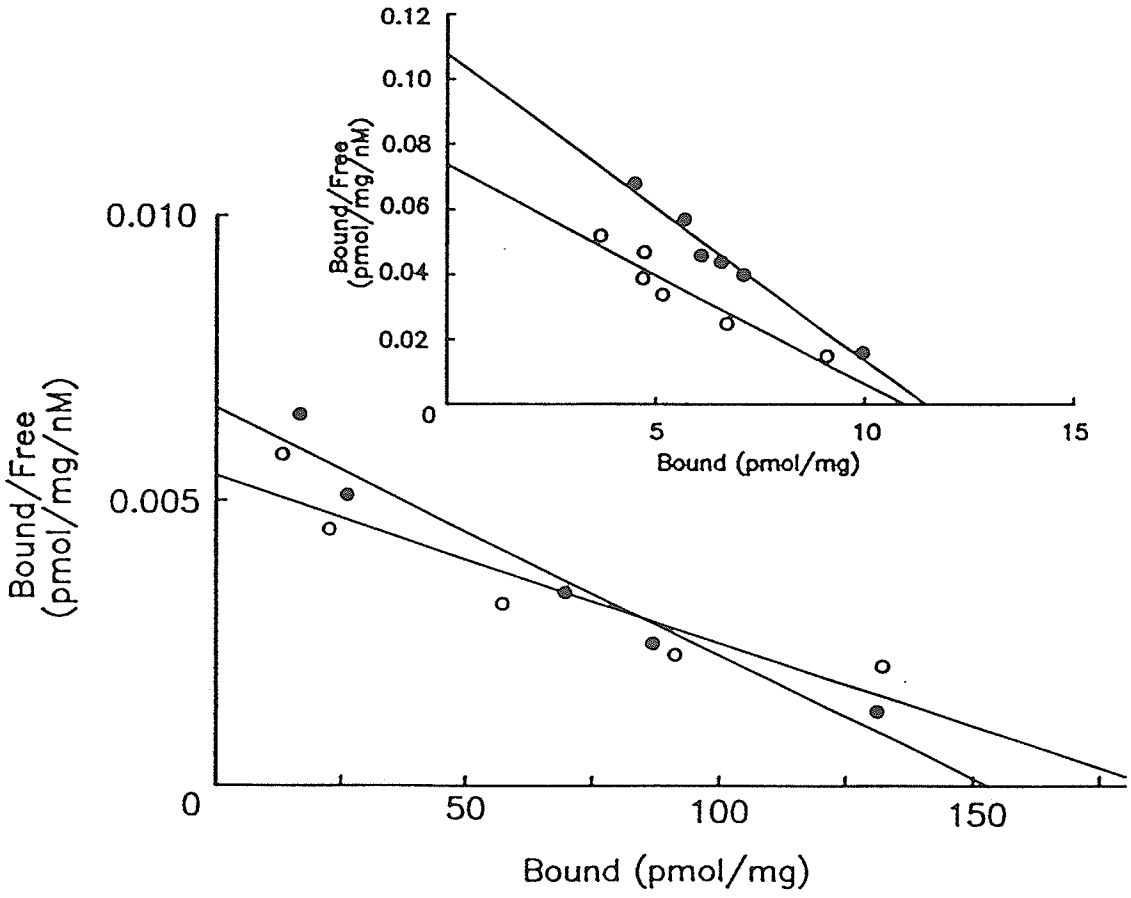

Figure 12. Scatchard plot analysis of specific [^{3}H]-ouabain binding to sarcolemmal preparations of left ventricular from control and failing (8 weeks after induction of myocardial infarction) hearts. Control - ●--●; failing - O--O; * $P < 0.05$ when compared to the control values. Each value is a mean ± SEM of six experiments.

Table 14. Summary of Scatchard plot analysis of specific [^{3}H]-ouabain binding to purified cardiac sarcolemmal vesicles isolated from viable left ventricular myocardium from control and experimental (8-week) rats.

		Control	Experimental
K_d	high affinity (nM)	119 ± 26.1	239 ± 34.7*
	low affinity (uM)	12.0 ± 3.6	28.9 ± 1.9*
B_{max}	high affinity (pmol/mg protein)	11.0 ± 1.2	12.7 ± 1.1
	low affinity (pmol/mg protein)	153 ± 12	174 ± 18

The values represent the mean ± SEM of 3 experiments. Experimental conditions are the same as described in Fig. 6. B_{max}, maximal density; K_d, dissociation constant. * $P < 0.05$

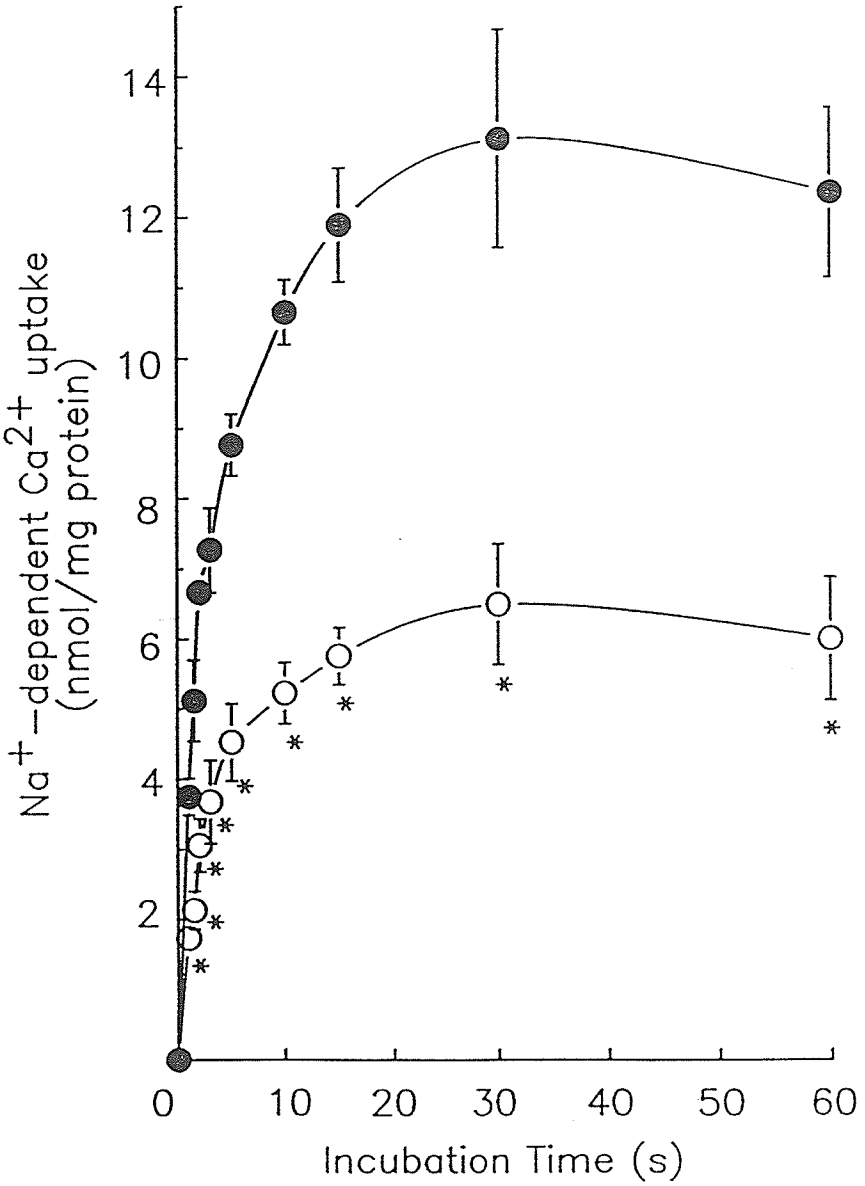

Figure 13. Time-course of Na^+-dependent Ca^{2+} uptake in control and experimental (8 weeks after inducing myocardial infarction) rat heart sarcolemma. The concentration of Ca^{2+} used in these experiments was 30 uM. Control - ●--●; failing - O--O; * $P < 0.05$ in comparison to the control values. Each value is a mean ± SEM of 6 experiments.

experimental samples. Sarcolemmal Na^+-dependent Ca^{2+} uptake activities were also examined in control and experimental preparations upon varying the concentration of Ca^{2+} in the incubation medium (Figure 14). A significant depression of Na^+-Ca^{2+} exchange activity at different concentrations of Ca^{2+} was observed in vesicles of experimental samples as compared with control values. Representation of these data on a double-reciprocal plot illustrates the noncompetitive nature of the depression of Na^+-Ca^{2+} exchange activity in the experimental preparations. The value of Ka for Na^+-Ca^{2+} exchange in cardiac sarcolemma (24.6 ± 1.6 and 21.5 ± 1.2 uM Ca^{2+} for control and experimental samples, respectively) was not altered in experimental samples whereas the Vmax of Na^+-dependent Ca^{2+} uptake (10.0 ± 0.8 and 5.3 ± 0.6 nmol Ca^{2+}/mg protein/2 s for control and experimental, respectively) is decreased by 47% in the experimental samples. No alteration of nonspecific Ca^{2+} accumulation were found between control and experimental samples when measured at different concentrations of Ca^{2+} (Table 15). In order to further show that the apparent defect in Na^+-dependent Ca^{2+} uptake was not due to altered membrane permeability of the vesicle preparations, passive Ca^{2+} accumulation of control and experimental preparations was examined at different times of incubation. The data in Table 16 indicate no difference in the passive Ca^{2+} accumulation between control and experimental vesicles.

10. ATP-dependent Ca^{2+} pump in experimental hearts. To examine the possibility of altered sarcolemmal Ca^{2+} pump activity, ATP-dependent Ca^{2+} accumulation was assayed in control and experimental preparations (Table 17). Vesicles from experimental (8 weeks after myocardial infarction) hearts showed no changes in the ATP-dependent Ca^{2+} accumulation from the control value at 1 min and 5 min incubation. Nonspecific Ca^{2+} accumulation under the Ca^{2+}

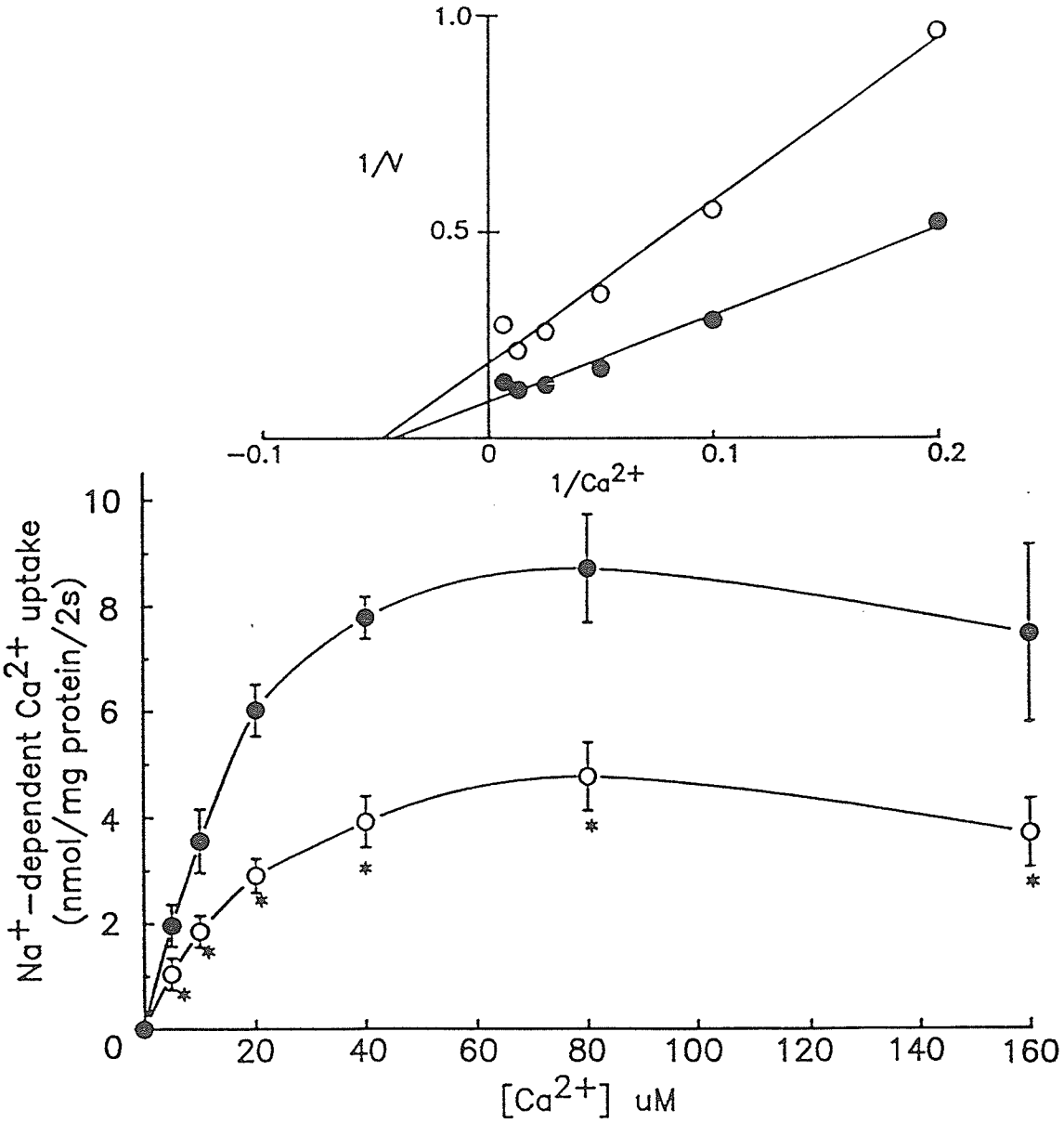

Figure 14. Na^+-dependent Ca^{2+} uptake activity in control and experimental (8 weeks after inducing myocardial infarction) rat heart sarcolemma at different concentrations of Ca^{2+}. The exchange activity in control (0--0) and failing (●--●) heart preparations was measured for 2s. Lineweaver plot of a typical experiment is also shown in this figure. * $P < 0.05$ in comparison to the control values. Each value is a mean ± SEM of 6 experiments.

Table 15. Nonspecific Ca^{2+} accumulation under conditions employed for Na^{+}-Ca^{2+} exchange in cardiac sarcolemma isolated from the viable left ventricular myocardium of control and failing (8 weeks after myocardial infarction) rats.

Concentration of Ca^{2+}	Control	Experimental
	nmol Ca^{2+}/2 sec	
5 uM	0.78 ± 0.08	0.69 ± 0.04
10 uM	1.42 ± 0.15	1.22 ± 0.11
20 uM	2.69 ± 0.31	2.45 ± 0.22
40 uM	4.87 ± 0.38	4.23 ± 0.27
80 uM	6.21 ± 0.52	6.54 ± 0.47
160 uM	6.89 ± 0.60	6.73 ± 0.56

Data are expressed as mean ± SEM of 4 experiments.

Table 16. Passive Ca^{2+} accumulation of sarcolemmal vesicles isolated from the viable left ventricular myocardium of control and failing (8 weeks after induction of myocardial infarction) rats.

Incubation time (min)	Passive Ca^{2+} accumulation (nmol/mg protein)	
	Control	Experimental
0.25	4.03 ± 0.28	3.77 ± 0.35
1	5.17 ± 0.42	4.73 ± 0.39
3	5.52 ± 0.49	4.88 ± 0.52
5	6.73 ± 0.59	6.04 ± 0.34
10	7.87 ± 0.63	7.22 ± 0.45
30	8.81 ± 0.41	8.23 ± 0.64

Data are expressed as mean ± SEM of 4 experiments. The concentration of Ca^{2+} employed in these experiments was 30 uM.

Table 17. ATP-dependent Ca^{2+} accumulation in cardiac sarcolemmal vesicles isolated from the viable left ventricular myocardium in control and failing (8 weeks after myocardial infarction) rats.

	1 min incubation		5 min incubation	
	ATP-dependent	Nonspecific	ATP-dependent	Nonspecific
	Ca^{2+} uptake (nmol/mg protein)			
A. In the absence of oxalate				
Control	12.7 ± 0.9	2.0 ± 0.1	18.9 ± 1.2	3.9 ± 0.2
Experimental	12.5 ± 1.1	1.8 ± 0.2	19.4 ± 0.9	4.1 ± 0.3
B. In the presence of 2 mM oxalate				
Control	13.2 ± 1.1	1.4 ± 0.3	20.1 ± 0.8	4.6 ± 0.4
Experimental	13.1 ± 1.4	2.3 ± 0.1	21.7 ± 1.3	4.2 ± 0.2

Data are mean ± SEM of 4 experiments. The concentration of Ca^{2+} used in these experiments was 5 uM.

uptake conditions was also unchanged in control and experimental samples. It should be noted from Table 17 that the ATP-dependent Ca^{2+} uptake in the sarcolemmal vesicles of either group was not augmented by the presence of 2 mM oxalate, which is known to increase the ATP-dependent Ca^{2+}-accumulation in the sarcoplasmic reticulum.

In another series of experiments, the activities of Ca^{2+}-pumping ATPase (Ca^{2+}-stimulated ATPase) were determined in sarcolemmal preparations from control and experimental left ventricle. Although Mg^{2+}-ATPase activity was significantly depressed in experimental preparations when compared to control, Ca^{2+}-stimulated ATPase activity was unchanged (Table 18). To rule out that these ATPase activities were not a manifestation of mitochondrial contamination, we measured activities of these enzymes in the presence of 2 mM sodium azide (NaN_3). While NaN_3 could slightly inhibit Mg^{2+}-ATPase activity in control and experimental preparations (Table 18) it was clear that mitochondrial contamination was not a factor in the ATPase activity determination; this result corresponds to work done previously in our laboratory (22).

As the rat heart has a well-developed sarcoplasmic reticular network, the possibility that ATP-dependent Ca^{2+}-pumping activities of the control and experimental sarcolemmal preparations may be due to contamination by fragments of sarcoplasmic reticulum. To rule out such a possibility, experiments were conducted to examine whether Ca^{2+} accumulated via the ATP-dependent mechanism in the sarcolemmal vesicles, unlike the sarcoplasmic reticulum, is released by Na^+. We observed a rapid release of Ca^{2+} from the sarcolemmal vesicles upon exposure to medium containing 40 mM Na^+; at 10 s, 15.4 ± 3.3% and 17.2 ± 4.6% of intravesicular Ca^{2+} remained in the control and experimental preparations, respectively. At 20 s from the

Table 18. Ca^{2+}-stimulated ATPase activities of sarcolemmal vesicles isolated from the viable left ventricular myocardium in control and failing (8 weeks after myocardial infarction) rats.

	Mg^{2+}-ATPase	Ca^{2+}-stimulated ATPase
	(umol Pi/mg protein/hr)	
A. In the abscence of sodium azide		
Control	89.7 ± 6.1	21.6 ± 1.4
Experimental	66.5 ± 3.7*	20.2 ± 0.8
B. In the presence of 2 mM sodium azide		
Control	86.8 ± 7.5	22.0 ± 0.8
Experimental	59.8 ± 5.8*	19.8 ± 1.2

Data are expressed as mean ± SEM of 4 experiments. $P < 0.05$ when compared to sham control. The concentration of Ca^{2+} was 5 uM.

addition of Na^+, the intravesicular Ca^{2+} content was decreased to 11.6 ± 7.2% and 9.5 ± 5.3% in control and experimental samples, respectively. Furthermore, the sarcolemmal Ca^{2+}-stimulated ATPase activities in both control and experimental preparations were equally inhibited by 0.5 - 4.0 uM vanadate (Figure 15). Such low concentrations of vanadate are known not to affect the Ca^{2+}-stimulated ATPase activity in rat heart sarcoplasmic reticular preparations (22,297).

11. Marker enzyme studies. The yield of sarcolemmal membrane protein obtained by the sucrose density gradient technique did not differ significantly between the control and experimental groups (Table 19). To exclude the possibility that the observed alterations of Ca^{2+}-transport may be due to the differential purification of the sarcolemmal vesicles from control and experimental left ventricle, activities of marker enzymes of different subcellular organelles were examined (Table 19). Digitoxigenin, a lipid-soluble agent, was capable of inhibiting the Na^+-K^+ ATPase activities completely in the intact sarcolemmal preparations from control and experimental left ventricle. The digitoxigenin-sensitive Na^+-K^+ ATPase activity was significantly depressed in the experimental samples; however, this ATPase activity was purified to a similar extent in control and experimental preparations. Likewise, the K^+-p-nitrophenolphosphatase (K^+-pNPPase) activity was depressed in experimental samples when compared to control but the relative fold purification of this activity was similar in both preparations. Mitochondrial and sarcoplasmic reticular contamination in the sarcolemmal membrane fractions from control and experimental samples were negligible as indicated by relatively low activities of cytochrome c oxidase and rotenone-insensitive NADPH cytochrome c reductase, respectively (Table 19). The activities of cytochrome c oxidase and rotenone-insensitive NADPH

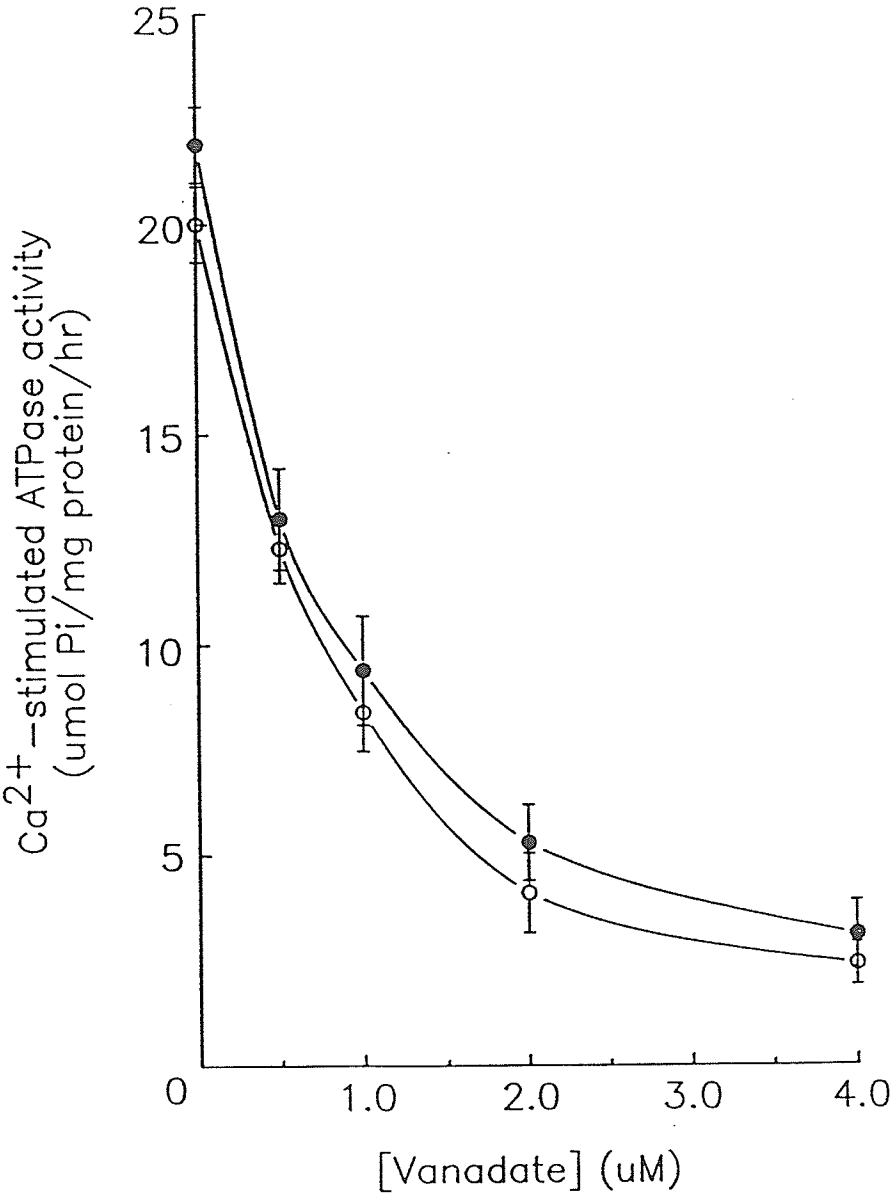

Figure 15. Effect of low concentrations of vanadate on the sarcolemmal Ca^{2+}-stimulated ATPase activities in control and experimental (8 weeks after inducing myocardial infarction) rat heart. Each value is a mean ± SEM of 4 control (●--●) and 4 failing hearts (O--O) preparations.

Table 19. Characteristics of purified cardiac sarcolemmal membrane vesicle preparations isolated from the viable left ventricular myocardium of control and experimental (8 week after induction of myocardial infarction) rat hearts.

	Activities in sarcolemma		Purity factor	
	Control	Experimental	Control	Experimental
Protein yield (mg protein/g heart)	1.0 ± 0.2	1.1 ± 0.2	--	--
Digitoxigenin-sensitive Na^+-K^+ ATPase (umol Pi/mg protein/hr)	20.9 ± 1.7	11.5 ± 0.9*	17.1	17.6
K^+ pNPPase (umol phenolate/mg protein/hr)	6.8 ± 0.35	3.9 ± 0.23*	16.1	15.7
Rotenone - insensitive NADPH cytochrome c reductase (nmol cytochrome c reduced/ mg protein/min)	2.3 ± 0.3	2.7 ± 0.2	0.32	0.34
Cytochrome c oxidase (nmol cytochrome c/mg protein/min)	55 ± 7	50 ± 4	0.25	0.31

Data are expressed as the mean ± SEM of 5 experiments. The purity factor was calculated as the ratio of the enzyme activity in sarcolemma and of that in the heart homogenate. * $P < 0.05$ when compared to sham control.

cytochrome c reductase were 0.33 and 0.25-fold of those in the heart homogenate; no difference in the purification of these enzymes existed in control and experimental samples. These data suggest that the relative contamination of sarcolemma membrane by mitochondria and sarcoplasmic reticulum in both groups was minimal but similar.

12. Sarcolemmal Ca^{2+} transport at early and late stages of congestive heart failure. To show that defects in sarcolemmal Ca^{2+}-transport were related to the degree of congestive heart failure in this experimental model, an investigation of sarcolemmal function was carried out in rats at 4 and 16 weeks following induction of myocardial infarction (Table 20). Sarcolemmal Na^{+}-dependent Ca^{2+} uptake was significantly reduced in the 4 weeks group; however, sarcolemmal Ca^{2+}-pump activity (indicated by ATP-dependent Ca^{2+} accumulation and Ca^{2+}-stimulated ATPase activity) was not different from control values. The Na^{+}-dependent Ca^{2+} uptake (6.1 ± 0.7 nmol Ca^{2+}/mg protein/30 s) and ATP-dependent Ca^{2+}-uptake (19.2 ± 0.7 nmol Ca^{2+}/mg protein/5 min) activities were significantly depressed and unaltered in 16 weeks experimental animals (n = 4) when compared to control values, respectively; these values were similar to those seen in 8 weeks experimental preparations.

Table 20. Some aspects of Ca^{2+}-transport in sarcolemma isolated from the viable left ventricular tissue of control and early failure stage (4 weeks after induction of myocardial infarction) in rats.

	Control	Experimental
Na^{+}-Ca^{2+} exchange activity (nmol/mg protein/30s)	13.4 ± 0.4	10.3 ± 0.6*
ATP-dependent Ca^{2+} accumulation (nmol/mg protein/5 min)	19.7 ± 1.6	20.3 ± 1.2
Ca^{2+}-stimulated ATPase activity (umol Pi/mg protein/hr)	21.7 ± 0.7	19.3 ± 1.4

Data are expressed as mean ± SEM of 6 experiments. * $P < 0.05$ when compared to sham control.

V. DISCUSSION

1. Experimental model of congestive heart failure. Congestive heart failure secondary to myocardial infarction of the left ventricular free wall has been reported to occur in rats following surgical ligation of the left coronary artery (53,243,278,298). Animals with large healed infarcts were reported to show characteristic rightward movement on the pressure-volume relationship (ventricular dilatation), elevated left and right filling pressures, and signs of pulmonary edema (53,243,298). In this study we have examined changes in cardiac function during the course of 16 weeks from the induction of myocardial infarction to investigate the possibility of graded heart failure. We are able to confirm the presence of an early stage of failure and moderate failure in the 4 week and 8 week experimental groups, respectively; severe congestive heart failure was present in the 16 week experimental group. This classification of varying degrees of heart failure subsequent to myocardial infarction in rats was based on our observations regarding general characteristics of the experimental animals and hemodynamic data. However, it is understood that such a categorization is arbitrary and is meant to examine the relationship between different degrees of heart failure and biochemical alterations.

No single mechanism for the explanation of reduced cardiac pump function in congestive heart failure has been elucidated. Although a reduction in the rate of cardiac contraction is associated with a shift in myocardial V_1 to V_3 myosin isozyme content in different experimental models of heart failure (69-71), this change does not occur in the failing ventricles of large mammals (72-77). Disruption of intracellular Ca^{2+}-metabolism in the failing myocardium is also suspected to cause defects in the delivery or removal of

Ca^{2+} from the contractile machinery thereby causing abnormal contractile function (1,2). Although the mitochondrial, sarcoplasmic reticular, and sarcolemmal membranes are all involved in the transport of Ca^{2+} to and from the myoplasm, only the latter two membranes are deemed crucial to the process of excitation-contraction coupling in normal myocardium. However, changes in uptake of Ca^{2+} by mitochondria or sarcoplasmic reticulum respectively can be seen to lead to disruption of intracellular Ca^{2+} homeostasis. Since myocardial Ca^{2+} metabolism may change as a result of an alteration in the sarcolemmal Ca^{2+} handling capacity, we have sought to define alterations in some specific aspects of sarcolemmal function either directly or indirectly associated with Ca^{2+} transport for explaning the depressed contractility at different stages of congestive heart failure secondary to myocardial infarction.

2. **Alterations of Ca^{2+}channels in failing hearts.** Although we have demonstrated that a progressive loss of cardiac function in congestive heart failure was paralleled by a significant decrease in Ca^{2+}-channel density at 8 and 16 weeks of inducing myocardial infarction, it should be noted that alterations in heart function seen at 4 weeks were not accompanied by any changes in the characteristics of Ca^{2+}-channels. Thus it is apparent that the observed changes in Ca^{2+}-channels in moderate to severe stages of congestive heart failure are a consequence of events occurring during the progression of disease. Depression in Ca^{2+}-channel density as monitored by [^{3}H]-nitrendipine binding in failing hearts was not only seen in crude membrane fraction, which is commonly used for the detection of these receptor sites, but it was also evident in heart homogenate as well as purified sarcolemmal preparations obtained by two different methods. Furthermore, such changes were also seen when another radioligand, [^{3}H]-PN200-110, was

employed. It should be pointed out that the observed decrease in Ca^{2+}-channel density in failing heart was not due to any alterations in the nonspecific binding of the radioligands because values for the nonspecific binding of ^{3}H-Ca^{2+} antagonists in control and failing heart preparations were not different from each other. Since hypertrophy of the right ventricle in experimental animals did not exhibit any changes in Ca^{2+}-channel density, myocardial hypertrophy of the left ventricles cannot be considered to account for the observed changes in [^{3}H]-nitrendipine binding. The decrease in Ca^{2+}-channel density in the failing left ventricle was not specific because a marked depression in [^{3}H]-nitrendipine binding with brain and skeletal muscle from the experimental animals was also seen. Since [^{3}H]-nitrendipine binding densities in right ventricular and liver preparations from animals with congestive heart failure were not decreased, it is unlikely that the observed decrease in Ca^{2+}-channels is due to a generalized down-regulation of these sites as a result of some circulating hormones and other factors in congestive heart failure. Thus the exact mechanisms for the observed decrease in Ca^{2+}-channel density are far from clear; however, it can be argued that such changes are due to functional ischemia commonly seen in congestive heart failure (187). This view is supported by the fact that both myocardial ischemia and hypoxia-reoxygenation were reported to result in a depression of Ca^{2+}-channel density (8,9).

Since Ca^{2+}-channels in the cell membrane are considered to play a critical role in the proper functioning of the excitation-contraction coupling in the myocardium (2,299), a loss of functional Ca^{2+}-channels at moderate and severe stages of heart failure can be seen to cause a derangement of cardiac function. On the other hand, it is pointed out that various vasodilator substances such as nitroglycerin, hydralazine, and captopril,

which may not have any direct effect on the myocardium, have been shown to exert a beneficial effect in congestive heart failure (53,300,301); this is considered to be due to a reduction in the afterload imposed on the heart. Furthermore, the use of Ca^{2+}-antagonists in congestive heart failure is controversial (131,300-304) in spite of their marked vasodilator effect which results in lowering the afterload on the failing hearts. Such a controversy may be due to the varying degrees of the negative inotropic effects of Ca^{2+}-antagonists in view of the differences in the Ca^{2+}-channel densities observed at different stages of heart failure. It should be pointed out that a decrease in the sensitivity of failing heart to extracellular Ca^{2+} has been demonstrated in this experimental model (278) and this can be seen to be due to the observed decrease in Ca^{2+}-channel density. However, it needs to be recognized that changes in the Ca^{2+}-channel density may be only one of several mechanisms responsible for disturbance of Ca^{2+} movements in the failing heart (1,2) and thus it is not our intention to rule out other molecular abnormalities for explaining cardiac dysfunction in congestive heart failure. Nonetheless, a depression in Ca^{2+}-channel density would result in a decrease in Ca^{2+}-influx in myocytes from moderate to severe degrees of failing hearts and under these conditions, therapeutic applications promoting the entry of Ca^{2+} through mechanisms other than Ca^{2+}-channels would be beneficial in congestive heart failure.

3. Alterations in adrenoceptors of failing hearts. We have demonstrated that a progressive loss of cardiac function was associated with a significant reduction of ß-adrenoceptor density 4, 8, and 16 weeks after induction of myocardial infarction, and a significant increase of alpha-adrenoceptor density at was seen at 8 and 16 weeks. It should be noted that alterations in heart function seen at 4 weeks were not accompanied by any changes in the

characteristics of alpha-adrenoceptors, and thus the observed changes in cardiac adrenoceptors in congestive heart failure are a consequence of events occurring during the progression of this disease. Since changes in both alpha-adrenoceptors and ß-adrenoceptors did not show any linear relationship with the occurrence of normalized cardiac hypertrophy in the experimental animals, it is evident that these changes are not a function of the occurrence of cardiac hypertrophy. The pattern of alterations in sarcolemmal Ca^{2+}-channel density was not closely linked to such changes in cardiac sarcolemmal adrenoceptor populations. In this regard, it is pointed out that coregulation of ß-adrenoceptor and dihydropyridine binding site densities, as well as Ca^{2+}-channel functional properties have recently been demonstrated in cultured chick ventricular myocyte preparations when exposed to 1 uM isoproterenol (305). However, since the density of sarcolemmal Ca^{2+}-channels was not decreased significantly in 4 week experimental animals (157) and the ß-adrenoceptor density was found to be significantly depressed when compared to control values, the possibility of specific co-regulation of these sarcolemma-bound proteins in early stage failure due to myocardial infarction appears unlikely.

Depressed ß-adrenoceptor density and increased alpha-adrenoceptor density in the left ventricle of 8 week experimental animals were seen not only in the crude membrane fraction which is commonly used for the detection of these receptor sites, but were also evident in the purified sarcolemmal preparations. It is pointed out that these changes were not due to any alterations in the nonspecific binding of the radioligands because values for the nonspecific binding of both ^{3}H-drug in control and experimental left ventricles were not different from each other. The functional implications of altered ß-adrenoceptor distribution were noted by a reduced positive inotropic

response of isolated hearts to various concentrations of isoproterenol in animals 4, 8, and 16 weeks following myocardial infarction. No significant alteration in the responsiveness of 4 and 8 week of experimental hearts to phenylephrine was seen, when compared to control values. This finding indicates that the mechanisms involved in regulation of cardiac contractility via the alpha-adrenoceptor pathway are complex. It is known that plasma norepinephrine levels are increased in heart failure (306,307), and it has been suggested that increased levels of norepinephrine may cause down-regulation of ß-adrenoceptor number which is associated with a blunted positive inotropic response to ß-adrenoceptor agonists, in failing hearts (11). Since the alpha-adrenoceptor density in left ventricular preparations in 8 and 16 weeks animals with congestive heart failure was increased, it is unlikely that the sole factor responsible for the observed changes in adrenoceptor densities is a generalized down-regulation of these sites as a result of some circulating hormones and other factors in congestive heart failure. Whether or not the observed differential changes in alpha-adrenoceptors and ß-adrenoceptors during the development of congestive heart failure occur at the genetic level remains to be explored by employing the molecular biology techniques. However, it can be argued that such changes are due to functional ischemia of the myocardium commonly seen in congestive heart failure (187). In this regard, it should be pointed out that in dilatated and hypertrophied failing hearts, increased wall stress can lead to a reduction of coronary perfusion due to arterial vessel compression whcich may give rise to ischemia. Furthermore, inadequate adaptation of the capillary vasculature when compared to the degree of hypertrophy of rat cardiac myocytes following induction of large myocardial infarction has been demonstrated (250). Thus the viable hypertrophied ventricle subsequent to myocardial infarction is

vulnerable to additional ischemic episodes. The view that functional ischemia in congestive heart failure may cause alterations of cardiac sarcolemmal adrenoceptor number is supported by the fact that [^{125}I]-iodocyanopindolol or [^{3}H]-CGP 12177 binding sites were decreased in rabbit myocardium subjected to ischemia and cultured chick myocytes challenged with hypoxic injury, respectively (12,13). Furthermore, in regions of cat heart subjected to ischemia and in adult canine myocytes exposed to hypoxia, [^{3}H]-prazosin binding sites were shown to be increased (209,210).

Since ß-adrenoceptors in the cardiac cell membrane are known to be an important cellular transduction mechanism which confer the adrenergic hormone signal to the cell via the activation of the adenylate cyclase enzyme anf formation of cAMP (2), a loss of functional ß-adrenoceptors at early, moderate, and severe stages of heart failure can be seen to cause a derangement of autonomic control of cardiac function. Although the exact role of the alpha-adrenoceptors in the regulation of cardiac function is not well understood, alpha-agonists have long been known to produce a positive inotropic effect associated with action potential prolongation not accompanied by cAMP elevation (212). The positive inotropic action of alpha-adrenoceptor agonists is believed to occur by liberation of inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) via the cleavage of phosphatidylinositol 4,5-bisphosphate by phospholipase C which is directly activated by the occupied alpha-adrenoceptors. IP_3 is believed to cause the release of Ca^{2+} from intracellular sites; DAG is known to activate protein kinase C (212) and thus may indirectly increase phosphorylation of the sarcolemmal Ca^{2+}-transporting proteins. Therefore, activation of cardiac alpha-adrenoceptors may directly and indirectly contribute to increased levels of cytoplasmic Ca^{2+} concentration. Furthermore, it has been recently demonstrated that

norepinephrine or phenylephrine mediated activation of cardiac alpha-adrenoceptors is accompanied by a strong inhibition of the transient outward K^+ current which substantially slowed the action potential repolarization (308). The observed increase in the number of alpha-adrenoceptors at moderate and severe stages of heart failure can be seen to maintain some balance in cardiac autonomic control when seen with a concomitant loss of ß-adrenoceptor number and thus can be considered to serve as an adaptive mechanism in this experimental model of congestive heart failure.

Other investigators have assessed cardiac adrenoceptor density and function in various experimental models of heart failure (13-16,180,181). In rats tested for ß-adrenoceptor density and positive inotropic response to isoprenaline 3 weeks following induction of myocardial infarction, no significant differences were noted when compared to control animals (180). Similarly, ß-adrenoceptor density was not changed in right ventricle of the same experimental model after either 2 or 7 days of coronary occlusion (181). On the other hand, a loss of sensitivity to adrenergic stimulation and a decrease in the number and affinity of ß-adrenoceptors has been reported in the uninvolved right ventricle of guinea pigs 3 days following left ventricular infarction (15,16). Similarly, a net loss of high-affinity ß-adrenoceptor sites has been found in a canine model of heart failure due to thoracic aortic banding (14). Contrary to other reports, an up-regulation of both ß- and alpha-adrenoceptors has been observed in hearts of cardiomyopathic hamsters with the onset of heart failure (13). Although some differences exist between our findings and earlier reports, these differences could be attributed to different species, model, or to the stage of heart failure. It should be recognized that changes in the alpha- or ß-adrenoceptor densities may be only one of several mechanisms responsible for disturbance of Ca^{2+}

movements in the failing heart, and we cannot rule out other molecular abnormalities for explaining cardiac dysfunction in congestive heart failure. Nevertheless, altered ß-adrenoceptor densities can be seen to result in the loss of autonomic control of cardiac inotropism in heart failure.

4. Alterations in sarcolemmal Na^+-K^+ ATPase in failing heart. Since the sarcolemmal Na^+-K^+ ATPase activity in 4 weeks experimental preparations was not altered, it appears that the observed depression of the enzyme activity at moderate and severe stages of heart failure is a consequence of events occuring during the development of congestive heart failure. However, the possibility must be considered that the observed alterations in Na^+-K^+ ATPase activity were not specific for the development of congestive heart failure but were related to hypertrophy of myocardium in experimental animals. In this regard, it should be noted that no alteration of ouabain-sensitive Na^+-K^+ ATPase activities were found in sarcolemmal preparations from hypertrophied rabbit hearts secondary to induction of pressure-overload (309). Furthermore, about 35% hypertrophy of the left ventricle in 4-week experimental animals was not associated with any change in sarcolemmal Na^+-K^+ ATPase activity. Thus myocardial hypertrophy of the left ventricle cannot explain the alteration of Na^+-K^+ activity in congestive heart failure. It can also be argued that the changes in Na^+-K^+ ATPase are due to functional hypoxia commonly seen in congestive heart failure (187). This view is supported by the fact that both ischemia and hypoxia have been shown to decrease the myocardial Na^+-K^+ activity (21,157).

The alterations in the sarcolemmal Na^+-K^+ ATPase activities were not confounded by excessive sarcoplasmic reticular or mitochondrial contamination since marker enzyme activities revealed that the cross contamination by these organelles was minimal in both control and experimental sarcolemmal

preparations. Furthermore, the depression in Na^+-K^+ ATPase activity cannot be explained on the basis of any difference in the availability of the enzyme sites in the sarcolemmal vesicles because ouabain inhibition of the Na^+-K^+ ATPase activity was 20 - 25 % of the values in untreated control and experimental samples. Treatment of membrane vesicles with alamethicin or SDS to unmask the latent ouabain-sensitive Na^+-K^+ ATPase activity also revealed depression in the enzyme acitivty of experimental preparations in comparison to the control membranes. Although Mg^{2+} ATPase activity in the 4 and 8 weeks experimental preparations was also decreased, no depression in the Na^+-K^+ ATPase activity was evident in 4 weeks failing heart preparations. Likewise, a decrease in sarcolemmal ß-adrenergic activity in 4 weeks experimental preparations was not associated with any changes in the Ca^{2+} channel activity (157,310). Thus it appears that the observed changes in Na^+-K^+ ATPase activity are not due to any nonspecific alterations in the sarcolemmal membrane in failing hearts. In fact such results suggest that defects in the failing myocardium may occur at multiple sites in the sarcolemmal membrane during the course of congestive heart failure.

The depression in Na^+-K^+ ATPase activity in experimental animals was associated with a decrease in V_{max} for the enzyme and such a defect was not due to any changes in the affinities of the enzyme for MgATP, Na^+, and K^+ or pH optimum. Since the [^{3}H]-ouabain binding affinities at both low and high affinity sites in Na^+-K^+ ATPase were significantly decreased in the 8 weeks experimental preparations when compared to control values, it is possible that some specific defect in the sarcolemmal Na^+-K^+ ATPase is occurring concomitant with loss of the enzyme activity. This defect could be seen to impart reduced sensitivity of the remaining viable myocardium to cardiac glycosides in the failing heart and thus may change the ratio of

therapeutic and toxic doses of these drugs. Since the densities for both high and low affinities sites of ouabain were not altered in failing heart preparations, it is likely that the observed depression in Na^+-K^+ activity is not due to any change in the number of enzyme molecules in the experimental heart preparations.

Because cardiac Na^+-K^+ATPase is an essential part of the Na^+-K^+ pump which is suggested to participate in the maintenance of cellular resting potential (311), a decline of Na^+-K^+ ATPase activity could cause disturbance in the electrical behavior of the failing myocardium. Inhibition of Na^+-K^+ATPase activity could also explain the attenuation of normal diastolic transmembrane potential in papillary muscle taken from failing feline hearts following cooling and rewarming of the muscle (239). Altered depolarized plateau levels in failing preparations during electrical stimulation and attenuated post-stimulation hyperpolarization in these hearts have been reported (239). It is also conceivable that depressed sarcolemmal Na^+-K^+ ATPase could lead to decreased maximum diastolic potential across the sarcolemma in remaining viable left ventricular myocardium of 8 weeks experimental animals. Subsequent partial inactivation of fast Na^+-channels in these hearts may occur and could reduce the magnitude of Na^+ current to the myocardium, known to be involved in release of intracellular Ca^{2+} from sarcoplasmic reticular (SR) stores (29). In this respect, depression of Na^+-K^+ ATPase may contribute to reduced cardiac pump function, the hallmark of congestive heart failure. However, such a change in the sarcolemmal Na^+-K^+ ATPase was not seen at early stages of congestive heart failure and thus cannot be considered to be the cause of contractile failure in this experimental model. On the other hand, inhibition of sarcolemmal Na^+-K^+ ATPase in failing heart can be considered to augment the

contractile force development in a manner similar to that proposed for cardiac glycosides (18,19). Such an augmentation of contractile force may tend to maintain the ability of cardiac muscle at moderate degree of congestive heart failure to pump blood and in this context the observed depression in Na^+-K^+ ATPase may be adaptive in nature.

5. Alterations in sarcolemmal Ca^{2+}-transport in failing hearts. The results of the present study demonstrate a significant depression in Na^+-Ca^{2+} exchange in sarcolemmal vesicles isolated from hearts of rats 8 weeks after induction of myocardial infarction. As the sarcolemmal membrane preparation was minimally but equally contaminated by other subcellular organelles in control and experimental samples, the difference observed in Na^+-Ca^{2+} exchange activity was unlikely to be due to any artifact related to isolation of these membranes. The decrease in Na^+-Ca^{2+} exchange activity in the experimental samples was characterized by a marked decrease in V_{max} values when compared to control whereas no change in the affinity of Na^+-Ca^{2+} exchange for Ca^{2+} was noted between the two groups. These changes in Na^+-Ca^{2+} exchange were specific since no alterations in the activities of sarcolemmal Ca^{2+}-pump (ATP-dependent Ca^{2+} accumulation and Ca^{2+}-stimulated ATPase) were evident in experimental animals 8 weeks after induction of myocardial infarction. Lack of quantitative differences of ATP-dependent Ca^{2+} uptake in the presence or absence of oxalate, or of Ca^{2+}-stimulated ATPase in the presence or absence of sodium azide indicate that measurement of these activities was not confounded by excessive sarcoplasmic reticular or mitochondrial contamination, respectively. The Ca^{2+}-stimulated ATPase activities in the sarcolemmal preparations, unlike the sarcoplasmic reticulum, were also inhibited by low concentrations of vanadate. In addition, no difference was observed between the experimental and control

preparations with respect to nonspecific Ca^{2+} binding as well as passive accumulation of Ca^{2+}.

The activity of Mg^{2+}-ATPase was demonstrated to be depressed in 8 week experimental animals when compared to control values. This ATPase, which is known to be activated by millimolar concentrations of either Ca^{2+} or Mg^{2+} and utilizes ATP as a substrate, is often referred to as Mg^{2+}-ATPase, Ca^{2+}-ATPase, basal, basic, or nonspecific ATPase (312). This enzyme was found to have the highest ATPase activity in our cell membrane preparations. Although the precise physiological role of this ATPase is unclear, it has been suggested that it may function as a gating system for sarcolemmal Ca^{2+}-channels in the cardiac cell (312) and it is conceivable that Ca^{2+} movement to the intracellular space may be depressed in myocardium of these animals by this mechanism. In fact Mg^{2+}-ATPase was found to be depressed in sarcolemmal preparations from 4 weeks experimental animals and it is possible that the defect may be one of the early lesions in the sarcolemmal membrane during the development of congestive failure in this experimental model. It should be pointed out that decreased Na^{+}-dependent Ca^{2+} uptake without any changes in ATP-dependent Ca^{2+}-pump activities was also evident in early stages of congestive heart failure (4 weeks following myocardial infarction). Although the magnitude of depression in Na^{+}-dependent Ca^{2+} uptake at early stages of failure was less than that seen in preparations from animals at moderate stages of congestive heart failure, the relationship between the magnitude of depression in Na^{+}-Ca^{2+} exchange and degree of congestive heart failure in this experimental model was not straightforward. This view is based on our observations that the magnitude of alterations in sarcolemmal Na^{+}-dependent Ca^{2+} uptake were not different betweeen moderate and severe stages of congestive heart failure. Thus it is possible that the defective

Na^+-Ca^{2+} exchange, which may play a role in cardiac pump dysfunction during initial stages of congestive heart failure, may not be involved in further deterioration of cardiac function at late stages of congestive heart failure.

The possibility must be considered that the alterations observed in Na^+-dependent Ca^{2+} uptake were not specific for events occuring during the development of congestive heart failure but were directly related to hypertrophy of the experimental hearts. Reports from this laboratory (309) and elsewhere (34) have assessed sarcolemmal Na^+-dependent Ca^{2+} uptake and Ca^{2+}-pump activities in hypertrophied rabbit or rat heart resulting from aortic banding and spontaneous hypertension. No difference in Na^+-dependent Ca^{2+} uptake was found in preparations of pressure overloaded (aortic banded) rabbit heart, whereas Ca^{2+}-pump activity was increased in these hearts (309). In hearts of both aortic banded and hypertensive rats, both Na^+-dependent Ca^{2+} uptake and Ca^{2+}-pump activities were significantly increased (34). Thus it seems unlikely that the mechanism for depression of Na^+-Ca^{2+} exchange activity in congestive heart failure can be explained by myocardial hypertrophy. It can be argued that functional hypoxia occuring in the hypertrophied failing heart may be responsible to inducing sarcolemmal changes in the congestive heart failure in this experimental model. However, this does not seem to be the case because, unlike the observed changes in congestive heart failure, both sarcolemmal Na^+-dependent Ca^{2+} uptake and ATP dependent Ca^{2+} uptake have been reported to be depressed in hypoxic or ischemic hearts (22,31).

Sarcolemmal Na^+-Ca^{2+} exchange and Ca^{2+}-pump are both suggested to participate in the efflux of Ca^{2+} ions from the cytosolic space, and therefore maintain the diastolic cytosolic Ca^{2+} concentration below 10^{-6} M

(1,213,224,297). Accordingly, depressed relaxation of the myocardium at 4 to 8 weeks of myocardial infarction may be partly explained on the basis of observed decrease in the Na^+-Ca^{2+} exchange activity. On the other hand, recent work has shown that in the absence of calcium entry through voltage-dependent calcium channels, depolarization of membranes elicited release of calcium from stores in the sarcoplasmic reticulum which was dependent upon extracellular calcium concentration (29); these authors have suggested that the Na^+-Ca^{2+} exchange mechanism may contribute to Ca^{2+} entry in the myocardium. Recent measurement of the intracellular Ca^{2+} transient was made using the fluorescent Ca^{2+} indicator dye fura-2 in rat ventricular cells (50) and it was concluded that most of the activator Ca^{2+} in rat ventricular cells was released from sarcoplasmic reticulum as a graded response to sarcolemmal Ca^{2+} influx. Therefore a reduction of sarcolemmal Ca^{2+} influx by depressed Na^+-Ca^{2+} exchange activity could lead to decreased contractility in the myocardium. Reduced sequestration and release of Ca^{2+} from sarcoplasmic reticular stores may occur in association with defective sarcolemmal Ca^{2+}-influx in the failing hearts, as sarcoplasmic reticular Ca^{2+} uptake and Ca^{2+}-stimulated ATPase activities were recently shown to be decreased in hearts of rats with congestive heart failure due to myocardial infarction (119). A decrease in the sensitivity of the failing heart to perfused extracellular Ca^{2+} has been demonstrated in this experimental model (278), and we have suggested that this may be due to decreased voltage-sensitive Ca^{2+}-channel density (157). The present results can also be interpretted to indicate that decreased Ca^{2+} sensitivity of failing hearts may also be due to decreased Ca^{2+}-entry through abnormal handling of Ca^{2+} by sarcolemmal Na^+-Ca^{2+} exchange system. As there are other mechanisms responsible for disturbance of Ca^{2+} movements in the failing heart (1), the

exact sequence of subcellular events which lead to loss of Ca^{2+}-homeostasis and depressed cardiac pump function in congestive heart failure is as yet unclear.

6. Significance of sarcolemmal changes in congestive heart failure. From a summary of results obtained in this study (Table 21), it is apparent that we have observed alterations in the sarcolemmal Na^{+}-Ca^{2+} exchange and ß-adrenoceptor density in hearts with early stage of failure. Reduction of both the rates of contraction (+ dP/dt) and relaxation (-dP/dt) is evident at this stage. This suggests that these defects in sarcolemma may be responsible for, rather than a consequence of, impaired contractility seen in 4-week experimental rats. For example, a decline of cAMP-dependent phosphorylation of sarcolemmal Ca^{2+}-channels as a result of depressed ß-adrenergic receptor density could cause a reduction of the signal Ca^{2+} for intracellar Ca^{2+} release and subsequent decrease in contractile activity at early stages of congestive heart failure. Thus even though Ca^{2+}-channel number is normal in hearts with an early stage of failure, the regulation of Ca^{2+}-channel function may be deranged. Similarly the reduction of sarcolemmal Ca^{2+} influx by Na^{+}-Ca^{2+} exchange upon excitation of failing myocardium may be a primary defect responsible for reduced myocardial contractile force development. Alternatively, a depression of sarcolemmal Na^{+}-Ca^{2+} exchange in the failing myocardium could contribute to the reduction of rate of relaxation by reduced removal of cytoplasmic Ca^{2+} by this mechanism. It is pointed out that a decline in the ability of hearts to maintain arterial pressure at moderate stages of congestive heart failure coincides with the significant depression of sarcolemmal Ca^{2+} channel density in membrane preparations but such a change may be a consequence of events occuring in heart failure.

Although the clinical signs of congestive heart failure becomes prominent in the 8- and 16-week experimental groups, the depression of rates of contraction and relaxation is comparable to that observed in the early stage of failure. This finding suggests that some mechanism(s) may be operating to ameliorate the sarcolemmal defects seen in the failing hearts. In this regard, alpha-adrenoceptor density is increased in 8- and 16-week experimental groups and because activation of these adrenoceptors is associated with an increase in intracellular Ca^{2+} levels in the myocardium by acting through the inositol trisphosphate pathway, such a alteration may increase intracellular Ca^{2+} concentration in these hearts and thus may serve as an adaptive mechanism. Furthermore, the observed increased in the alpha-adrenoceptor density suggests that any defect in cAMP-dependent phosphorylation of Ca^{2+}-channnels may be compensated for by the activation of protein kinase C via augmented release of diacylglycerol. Since the sarcolemmal Na^{+}-K^{+} ATPase activity is depressed in 8- and 16-week animals, but not in the 4-week group, this alteration may also serve to increase the intracellular concentration of Ca^{2+} to compensate for earlier defects in the sarcolemmal function. Normal sarcolemmal Ca^{2+}-pump activities (ATP-dependent Ca^{2+} accumulation and Ca^{2+}-stimulated ATPase) indicate that removal of cytoplasmic Ca^{2+} was intact by this mechanism and in light of depressed of mechanisms for Ca^{2+}-influx, this system may contribute towards decreasing the availability of Ca^{2+} for contractile machinery in the failing myocardium. Thus it appears that sarcolemmal defects in Na^{+}-Ca^{2+} exchange and ß-adrenergic receptors may be associated with depressed contractile force development whereas changes in sarcolemmal alpha-adrenoceptors and Na^{+}-K^{+} ATPase may be adaptive in nature during the development of congestive heart failure.

Table 21. Summary of results on sarcolemmal changes at different stages of congestive heart failure in rats subsequent to coronary occlusion.

	4 weeks (early)	8 weeks (moderate)	16 weeks (severe)
Ca^{2+} channels	No change	Decreased	Decreased
ß-adrenoceptors	Decreased	Decreased	Decreased
alpha-adrenoceptors	No change	Increased	Increased
Na^{+}-K^{+} ATPase	No change	Decreased	Decreased
Na^{+}-Ca^{2+} exchange	Decreased	Decreased	Decreased
Ca^{2+}-pump	No change	No change	No change

VI. CONCLUSIONS

1. On the basis of changes in general characteristics and hemodynamic performance, early, moderate and severe stages of congestive heart failure were observed to occur in rats at 4, 8 and 16 weeks following the induction of large infarcts in the left ventricle, respectively.
2. The depression of sarcolemmal Na^+-Ca^{2+} exchange activity and ß-adrenoceptor density in early stages of congestive heart failure may be primary defects causing reduction of sarcolemmal Ca^{2+}-influx and therefore reduced myocardial contractility.
3. Depression of cardiac sarcolemmal Ca^{2+}-channel density was associated with the incidence of moderate and severe congestive heart failure and this may also contribute towards an insufficient supply of Ca^{2+} for the contractile machinery.
4. Changes in $alpha_1$-adrenoceptor density and Na^+-K^+ATPase activity appear to be compensatory in nature and may serve as adaptive mechanisms for the maintenance of cardiac contractile force development at moderate and severe stages of congestive heart failure.
5. As Ca^{2+}-pump function was normal, removal of Ca^{2+} from the cytoplasm by this mechanism may assist in reducing the availability of Ca^{2+} for contractile apparatus during the development of congestive heart failure.

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