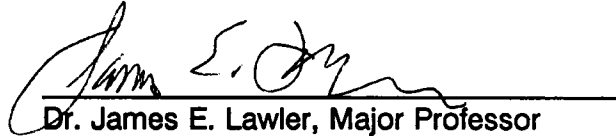
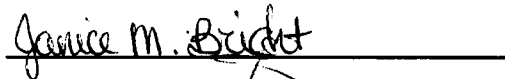


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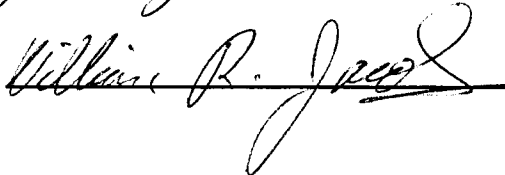

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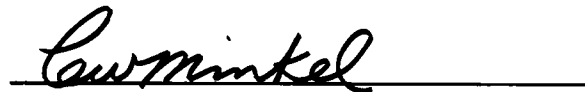

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SODIUM HOMEOSTASIS, pH REGULATION, AND HIGH ENERGY PHOSPHATE
METABOLISM IN HYPERTROPHIED HEARTS DURING ISCHEMIA

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Arrie Lynelle Golden

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ABSTRACT

Hearts with hypertrophy associated with pressure overload have an increased susceptibility to ischemic dysfunction and damage. There is evidence that hypertrophied hearts have altered Na^+ and Ca^{+2} homeostasis, and a greater dependence on anaerobic glycolysis that may promote increased ischemic damage and dysfunction via mechanisms involving disruption of cation gradients. The primary objectives of the studies described in the following chapters were to evaluate the role of an increase in intracellular Na^+ in promoting ischemic dysfunction and damage in hypertrophied hearts, and to relate the increase in intracellular Na^+ to changes in high energy phosphates and pH.

The gradual occurrence of hypertension in the Borderline Hypertensive Rat (BHR) fed a high salt diet suggested that this model might be useful in understanding early myocardial changes in hypertension. The objectives of the preliminary study described in Chapter 2 were to characterize the development of myocardial hypertrophy, and associated changes in creatine kinase (CK) isozyme expression in the BHR heart. The results indicated that BHR fed a high salt diet develop significant, but minimal, hypertrophy that does not increase in severity with age. In addition, the BHR did not develop changes in CK expression that are characteristic of models of more severe pressure overload hypertrophy such as the Spontaneously Hypertensive Rat (SHR).

The objective of the studies described in Chapter 3 was to develop an ischemic protocol to relate diastolic dysfunction with increased intracellular Na^+ . Interleaved ^{23}Na and ^{31}P NMR spectra were acquired in perfused hearts to assess changes in diastolic function, intracellular Na^+ , pH and high energy phosphates in BHR hearts during demand ischemia. The results indicated that induction of diastolic dysfunction during demand ischemia required a perfusate free $[\text{Ca}^{+2}]$ above 1mM, but a higher $[\text{Ca}^{+2}]$ was not

compatible with the ^{23}Na shift reagent TmDOTP⁻⁵. In addition, the increase in intracellular Na^+ was not greater in the hypertrophied BHR compared with control BHR. Thus, more severe ischemia, and SHR with greater hypertrophy were used in later experiments.

The objectives of the studies described in Chapter 4 were to determine if the increase in intracellular Na^+ was greater in hypertrophied SHR hearts compared with Wistar-Kyoto (WKY) controls during low flow ischemia, and to determine whether the accumulation of Na^+ in these hearts was associated with ischemic dysfunction and damage. In addition, the increase in Na^+ was related to decreases in high energy phosphates and pH. Interleaved ^{31}P and ^{23}Na (with the NMR shift reagent TmDOTP⁻⁵) spectra were acquired in perfused hearts from 8-10 month old SHR during low flow ischemia and reperfusion. Left ventricular pressures were monitored continuously.

The ischemic protocol distinguished two groups of hypertrophied SHR. The SHR group that exhibited contractile failure during ischemia will be referred to as the SHF, and the second group of SHR that sustained contractile function during ischemia will be referred to as the SHS. The SHS represented the majority of the SHR studied, and did not exhibit either a greater increase in intracellular Na^+ during ischemia, or greater ischemic dysfunction and damage compared with the WKY. In contrast, the SHF did exhibit a greater increase in intracellular Na^+ during ischemia compared with the WKY. Furthermore, the greater accumulation of Na^+ in the SHF hearts was associated with greater systolic and diastolic dysfunction during ischemia, and poorer recovery following reperfusion. Therefore, impaired Na^+ handling may indeed contribute to the greater sensitivity of hypertrophied hearts to ischemic dysfunction and damage. However, since not all hypertrophied hearts were affected, the impaired Na^+ handling was not associated with hypertrophy per se. We propose that impaired Na^+ handling may be indicative of

a subtle transition towards a less compensated stage of hypertrophy that may precede the development of congestive heart failure, and be manifest as an increased sensitivity to ischemia.

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CHAPTER I

INTRODUCTION

A. LITERATURE REVIEW

Pressure Overload Hypertrophy

Myocardial hypertrophy occurs as a compensatory response to chronically increased demand, such as pressure overload, and serves to normalize wall stress and maintain normal cardiac output (1-2). Hypertrophy is a complex process that involves reorganization of cellular and molecular processes as well as geometric restructuring of the heart (3). Increased myocardial mass in response to chronically increased pressure load is accomplished in adults by increasing the size of individual myocytes rather than by hyperplasia (4). Myocyte growth is characterized by increases in the number of myofibrils and organelles (5,6), increased protein synthesis (7-8) and altered gene expression (3,9). The hypertrophic process may also be accompanied by increased synthesis of connective tissue surrounding the myocytes (10-11) which may increase myocardial stiffness (12-13). Some capillary growth also occurs with myocardial hypertrophy, but the capillary proliferation is often inadequate to maintain normal capillary density (14-15). Similarly, the increase in mitochondria may be inadequate to maintain a normal myofibrillar to mitochondrial volume ratio (6). The balance of the molecular, cellular and structural changes that occur during the hypertrophic process determines the degree of functional compensation that is achieved by the heart (3,9).

Although myocardial hypertrophy in response to chronic pressure overload initially results in maintenance of normal cardiac output, severe pressure overload sustained over

a long period usually produces functional decompensation and congestive heart failure (3). The factors that promote the transition from the compensated state to congestive heart failure are incompletely understood (3,9). The rate at which the progression to failure occurs is dependent, in part, on the severity of the stress and the rate at which it develops or is applied (3). Thus, different methods of producing pressure overload will induce hypertrophy of varying severity and hypertrophied hearts will subsequently begin to fail at different rates (3).

Increased Susceptibility of Hypertrophied Hearts to Ischemia

Changes in the heart associated with even the compensated stage of hypertrophy may predispose hypertrophied hearts to ischemic dysfunction and injury. Myocardial ischemia is characterized by depletion of high energy phosphates (16-18), a decline in pH (18), alterations in transsarcolemmal ion gradients (18), and the accumulation of metabolites such as lactate (16-19). Hypoxia also promotes a substantial decrease in high energy phosphates and changes in ion gradients (18). The fundamental difference, however, between hypoxia and ischemia is that continuous washout of the extracellular space occurs only during hypoxia (17-18). The consequences of this washout are that metabolites do not accumulate in the myocardium, the pH decreases less, and anaerobic glycolysis may continue at a faster rate than during ischemia (18,20).

The mechanisms of ischemic dysfunction and damage in normal hearts are incompletely understood, and the causes of the increased sensitivity of hypertrophied hearts to ischemia are unknown. There is evidence of abnormal vascularization (21-25), altered metabolism (26-32), and changes in cation homeostasis (33) in hypertrophied hearts that may contribute to the increased sensitivity of these hearts to ischemic

dysfunction and damage.

The observation that hypertrophied hearts were more susceptible to ischemic damage was made initially in human patients undergoing cardiac by-pass surgery. The incidence of subendocardial necrosis (34) and myocardial contracture ("stone heart") (35-36) was much higher in hypertrophied hearts than in nonhypertrophied hearts. These observations have been confirmed by experimental studies in several animal models of pressure overload hypertrophy. Hypertrophied hearts have a decreased time to contracture (26,37-40), greater impairment of diastolic function during ischemia and hypoxia (27,33,41-43), and depressed recovery of both systolic and diastolic function following reperfusion or reoxygenation (26,27,44-48) compared with nonhypertrophied hearts.

Myocardial contracture during ischemia or hypoxia is characterized by cessation of systolic function and a progressive rise in diastolic tension (36,49,50). The development of contracture may be induced by a decline in the concentration of adenosine triphosphate (ATP) to very low levels, and may be potentiated by calcium overload (49-50). Inadequate ATP concentrations may result in the formation of 'rigor bonds' between myofilaments (36,49) and incomplete uptake of Ca^{2+} by the sarcoplasmic reticulum (51), and thereby produce a sustained increase in resting tension (49).

Some studies have shown that the development of contracture occurs when the ATP concentration falls to a critically low level (49). Other investigators, however, have found that the onset of contracture does not correlate with tissue averaged ATP content (50,52). It has been proposed that only the ATP in a compartment around the myofilaments (50), or ATP derived from glycolytic sources may be critical for preventing contracture (52-53). It is unlikely that changes in ATP in these compartments would be

detectable in tissue averaged measurements.

Earlier development of contracture has been observed in several models of pressure overload hypertrophy. Attarian and others (37) found that contracture occurred earlier in canine hearts in which hypertrophy was induced by subcoronary valvular stenosis. However, these hearts also had lower endocardial blood flow and ATP concentrations prior to ischemia (37). Similarly, an earlier onset of contracture was observed in hypertrophied hearts from spontaneously hypertensive rats (SHR) (38) and rats with aortic constriction (39) during zero flow ischemia. The control ATP concentration was lower in the aortic constricted hearts compared with nonhypertrophied controls suggesting that the sensitivity to ischemia may have been related to the depressed preischemic ATP concentrations (39). This concept is supported by a subsequent study, by the same investigators, in which preischemic cardiac arrest with KCl and perfusion with buffer containing glucose increased the basal ATP concentration and delayed the onset of contracture in hypertrophied hearts (40). The delayed onset of contracture was, however, not sufficient to completely eliminate the differences between groups, and the hypertrophied hearts continued to develop contracture earlier than controls.

Similarly, Anderson and others (26) used cardiac arrest to reduce energy demands and thereby increase preischemic high energy phosphate concentrations in aortic banded rats. Despite normalization of ATP concentrations, contracture still developed earlier in hypertrophied hearts than in nonhypertrophied hearts. Furthermore, the ATP concentrations did not differ between hypertrophied and control hearts at the onset of contracture (26). These observations indicate that factors other than lower preischemic ATP may contribute to the earlier development of contracture in hypertrophied hearts.

Hypertrophied hearts have also been shown to be more susceptible to diastolic dysfunction during relatively mild periods of hypoxia and ischemia that allowed the hearts to continue to beat for the entire protocol (27,33,41-43). Diastolic dysfunction may result from either decreased passive compliance, or incomplete relaxation of myofibrils (41). Impaired or incomplete relaxation may be caused by decreased uptake of calcium by the sarcoplasmic reticulum (51), and/or increased influx of calcium via mechanisms such as Na^+ - Ca^{2+} exchange (54).

Lorell and others (41) observed a significantly greater increase in left ventricular end diastolic pressure (LVEDP) in hypertrophied hearts from uninephrectomized deoxycorticosterone salt (DOCA-salt) hypertensive rats compared with controls during a brief 3 minute period of hypoxia. The rate and extent of isovolumic relaxation were also impaired in the hypertrophied hearts. The cause of the impaired diastolic function was not investigated in this study. However, the observation that the rate of relaxation was impaired as well as the extent of pressure decline suggests that the diastolic dysfunction was related to a decreased rate of removal of calcium and not due exclusively to abnormal compliance. Control and hypertrophied hearts were perfused at similar coronary flows which eliminated the possibility that inadequate perfusion was the cause of the greater diastolic dysfunction in the hypertrophied hearts. Vogel and others (42) also reported increased diastolic dysfunction during hypoxia in the hypertrophied DOCA-salt rat heart. In addition, they found that differences in diastolic dysfunction between hypertrophied hearts and controls were eliminated by treatment with the combined alpha- and beta-adrenergic antagonist, labetalol, which promoted regression of hypertrophy.

The relationship between diastolic dysfunction and high energy phosphates was investigated in another study using the DOCA-salt model of hypertrophy (33). Although

the phosphocreatine (PCr) concentrations were lower in the hypertrophied hearts during control perfusion, the greater increase in LVEDP in the hypertrophied hearts was not related to the rate of decline in ATP and PCr during 15 minutes of hypoxia (33). These investigators concluded that the sensitivity of hypertrophied hearts to hypoxic dysfunction was not related to a differential change in the energy status of these hearts during ischemia. As an alternative, they proposed that the exaggerated diastolic dysfunction in these hearts was due to some primary defect in calcium homeostasis (33).

A greater increase in diastolic tension was also observed during demand ischemia in blood perfused hypertensive rabbit hearts during the early stages of hypertrophy (43). Demand ischemia was initiated by simultaneously increasing the heart rate and decreasing the coronary flow. Hypertrophied hearts were perfused at coronary flows which were not different from controls. Since the rabbit hearts had only minimal hypertrophy, it is unlikely that decreased compliance due to fibrosis contributed to the diastolic dysfunction in this study. In addition, the effects of increased vascular turgor which may contribute to the observed diastolic dysfunction during hypoxia are not present during demand ischemia (43). These investigators again proposed that the increased diastolic dysfunction of hypertrophied hearts might be due to impaired Ca^{2+} handling by these hearts (43).

There are a number of mechanisms which have been proposed to account for irreversible myocardial damage following ischemia or hypoxia in normal hearts. The loss of adenine nucleotides and an inability to resynthesize ATP during reperfusion is considered important by many investigators (55-57). Additionally, many other factors have been investigated including inhomogeneity of reperfusion (no-reflow) (58-59), the accumulation of metabolites such as lactate (16,19,60), and cellular Ca^{2+} overload

secondary to disruption of transsarcolemmal gradients for Na^+ and Ca^{2+} (18).

Poorer recovery of both systolic and diastolic function has been observed in hypertrophied hearts following reperfusion or reoxygenation. Hearse and others (44) found that isolated working rat hearts with hypertrophy produced by aortic banding recovered cardiac output more slowly than control hearts following 70 minutes of hypothermic zero flow ischemia. In addition, functional recovery was significantly less complete following reperfusion in hearts from aortic banded rats subjected to zero flow ischemia (26). These hearts had decreased recovery of developed pressure and increased diastolic stiffness compared with controls. Menasche and others (45) also observed that hearts from aortic constricted rats exhibited a greater sustained increase in diastolic pressure compared with controls following 30 minutes of normothermic zero flow ischemia.

Snoeckx and others (46) observed that isolated working hearts from 16 month old SHR exhibited poor recovery of cardiac output and a greater loss of enzymes following 30 minutes of zero flow ischemia compared with WKY. Similar results were reported for 18 month old SHR (47). Although PCr and ATP were lower in the SHR hearts at control, there were no differences in high energy phosphates between SHR and WKY after reperfusion that would account for differences in functional recovery (46). Recovery of developed pressure was also less complete in rat hearts hypertrophied by suprarenal aortic constriction compared with controls (48). The hypertrophied hearts in the latter study had greater loss of purine nucleotides during reperfusion and subsequently poorer recovery of inorganic phosphate, ATP, and the phosphorylation potential.

Hypertrophied hearts have vascular abnormalities which may contribute to the increased susceptibility of these hearts to ischemic damage and dysfunction. Hypertrophy

is associated with decreased capillary density (14,21), and increased coronary resistance or decreased coronary reserve (22-25,61) compared with nonhypertrophied hearts. The decreased ATP concentrations observed in hypertrophied hearts exhibiting an earlier development of contracture may, therefore, reflect the effect of decreased coronary flows due to increased coronary resistance in these hearts. In fact, two of the studies which found lower ATP concentrations in hypertrophied hearts also reported lower coronary flows in these hearts (26,37). However, decreased high energy phosphate concentrations have been observed in hypertrophied hearts perfused at coronary flows equivalent to those in control hearts (33). This observation indicates that reduced high energy phosphate concentrations in hypertrophied hearts may not be accounted for solely by deficiencies in coronary flow.

Vascular abnormalities may also contribute to ischemic damage following reperfusion in hypertrophied hearts. Hypertrophied hearts have been shown to have larger areas of myocardium which are not reperfused following ischemic contracture (no-reflow) compared with controls (38,47,62). It has been hypothesized that abnormalities in the smaller vessels in hypertrophied hearts may predispose these vessels to injury during ischemia and reflow, and result in poor reperfusion of the subendocardium (47). However, poorer functional recovery has also been observed in hypertrophied hearts compared with controls following hypoxia (27) which is not characterized by inhomogeneities in reperfusion (26). Therefore, although incomplete reperfusion probably contributes to ischemic damage in hypertrophied hearts under some circumstances, vascular abnormalities are not the sole cause of the increased sensitivity of these hearts to ischemic dysfunction and damage.

Evidence of Altered Metabolism in Hypertrophied Hearts

Lower concentrations of high energy phosphates in hypertrophied hearts in the absence of differences in flow indirectly suggest that there may be fundamental differences in energy production or utilization in these hearts (33). It has been proposed that decreased capillary density and coronary reserve in hypertrophied hearts may predispose these hearts to intermittent ischemia (63). Although the vasculature may be adequate to support normal perfusion under basal conditions, stress due to rapid pacing has been shown to produce ischemia, particularly in the subendocardium, in hypertrophied hearts (64-65).

It has been hypothesized that intermittent ischemia may be responsible for promoting changes in the genetic expression of various enzymes such as creatine kinase (CK) and lactate dehydrogenase which may alter metabolism in hypertrophied hearts (32,63). There is evidence of either an increased (26,30,32) or decreased (27) capacity of hypertrophied hearts to recruit anaerobic glycolysis compared with control hearts. The ability of hypertrophied hearts to recruit anaerobic pathways may be important in determining the response of these hearts to ischemia and hypoxia (26-27).

Altered expression of creatine kinase (CK) has been observed in hearts hypertrophied by pressure overload. Creatine kinase catalyzes the reversible transfer of high energy phosphate from ATP to creatine (66-67). The enzyme is important for normal synthesis, transport, and utilization of high energy phosphates (66-67). Creatine kinase exists as four different isozymes that consist of two polypeptide chains (M and B) that are synthesized by different genes (67). The mitochondrial isozyme is located on the inner mitochondrial membrane (66). The MM isozyme is associated primarily with the myofibrils and sarcolemmal ion transport sites (68,69), and the BB and MB isozymes are believed

to be distributed in the cytosol (67). The mitochondrial and MM isozymes of CK normally predominate in adult myocardium, whereas the BB and MB are normally more prominent in neonatal myocardium (70).

Ingwall and others (63) measured total CK activity and isozyme distributions in biopsies from human patients with left ventricular hypertrophy secondary to aortic stenosis. The total creatine kinase activity was 32 to 39% lower in hypertrophied myocardium, and the MB isozyme activity was 12 to 20 times higher than in normal hearts.

Changes in the total activities and isozyme distributions for creatine kinase have also been observed in several experimental models of pressure overload hypertrophy. Hypertrophied hearts from rats with two kidney, one clip hypertension exhibited a significant increase in the sum of MB and BB isozyme activity and a corresponding decrease in the MM and mitochondrial isozyme activities (71). Significant increases in the relative proportion of CK-MB was also observed in canine hearts hypertrophied by aortic or pulmonic stenosis (67). Changes in isozymes in the latter study were specific for the hypertrophied ventricle and did not occur in the unaffected contralateral chamber (67). Significant increases in MB and BB isozyme activities have also been observed in non-failing hypertrophied hearts from six and 12 month old SHR (67), and from aortic banded rats (72). The myocardium from aortic banded rats, like hypertrophied human myocardium (63), exhibited lower total CK activity (72).

In hypertensive canine myocardium the increase in MB and BB isozyme activity was greatest in the subendocardium (71). Given that the subendocardium is also susceptible to intermittent ischemia (64-65), greater isozyme changes in the subendocardium indirectly support the hypothesis that intermittent ischemia may promote

changes in CK expression. Additional circumstantial evidence that isozyme changes are linked to periodic ischemia was provided by the observation that human patients with coronary artery disease also exhibited increased expression of MB and BB isozymes of creatine kinase (63).

Significant changes in the activities of lactate dehydrogenase (LDH), and the expression of different isoforms of the enzyme have also been observed in hypertrophied hearts. Lactate dehydrogenase is an enzyme found in cardiac and skeletal muscle that catalyzes the interconversion of lactate and pyruvate (73). The enzyme contains two subunits, the heart type (H), and the muscle type (M) which combine as tetramers to produce 5 distinct isozymes with different ratios of H and M subunits (74). Isozymes of LDH in the normal heart and in muscle that is dependent primarily on aerobic metabolism contain a predominance of H subunits (74). Conversely, the LDH in glycolytic skeletal muscle is rich in M subunits (74). The M subunit is also prominent in embryonic myocardium, but decreases with age as the H subunit increases (75).

An increase in the relative proportion of M subunits of LDH was found in hypertrophied human myocardium at autopsy (76). Fox and Reed observed a significant increase in M subunits and decrease in H subunits of LDH in hypertrophied right ventricles from dogs with experimental pulmonic stenosis (73). This observation was repeated using a similar canine model of pulmonic stenosis in a different lab (32). There were no significant changes in total LDH activity reported in any of these studies.

Smith and others observed a significant increase in the total LDH activity in hypertrophied left ventricles from dogs with two kidney, one clip hypertension, and the increase was greatest in the subendocardium. (71). In contrast, an increase in the proportion LDH-M in aortic banded rats was associated with a decrease in total LDH

activity (72). These hearts, however, also showed evidence of left ventricular failure, which was not true of the hypertensive dog hearts with increased LDH activity (71). Therefore, hypertrophied hearts have consistently exhibited increased expression of LDH-M, the fetal isozyme of LDH, while changes in total LDH activity have been variable.

The functional significance of the increased expression of the fetal isozymes of CK and LDH has not been established. The velocity of the creatine kinase reaction in vivo is dependent on the total CK activity and on the concentrations of creatine and MgADP, but is independent of the CK isozyme composition under basal conditions (77). Therefore, changes solely in the isozyme composition apparently do not affect the rate of the creatine kinase reaction under basal conditions. The MB and BB isozymes, however, have a higher affinity for creatine phosphate than the MM isozyme (78), and it has been proposed that the increase in the MB and BB isozymes may allow more efficient transfer of high energy phosphate from creatine phosphate to ADP in hypertrophied hearts (63). This increased efficiency may have functional significance in hypertrophied hearts in which abnormally low creatine concentrations have also been observed (63).

Increased expression of LDH-M was promoted by hypoxia in cardiocytes in culture (79) and has also been observed in ischemic human myocardium (76). In addition, the M subunit of LDH is less sensitive to inhibition by pyruvate, and therefore favors the conversion of pyruvate to lactate (74). These observations, and the predominance of M-LDH in embryonic tissues and in muscle dependent on anaerobic glycolysis, are the basis for the proposal that increased expression of M-LDH may increase the efficiency of anaerobic glycolysis in myocardium (32,72). The BB and MB isozymes of CK are also characteristic of neonatal myocardium, and myocardium

exposed to chronic hypoxia (63,70). Consequently, it has been argued that increased expression of these isozymes of CK, like increased expression of LDH-M, may be indicative of an increased dependence on, or capacity to use, anaerobic glycolysis (26).

The hypothesis that hypertrophied hearts may have a greater dependence on glycolytic metabolism is supported by one study in which dual tracer autoradiography was used to measure regional fatty acid and glucose uptake in hypertensive DAHL rats (30). The hypertensive hearts had decreased uptake of fatty acids, and increased uptake of glucose compared with controls under basal conditions. The increase in glucose uptake was greatest in left ventricular free wall and subendocardium (30). These data indicate that hypertrophied myocardium, particularly the subendocardium, may preferentially recruit glycolytic pathways even when global oxygen supplies are not limited. This hypothesis is supported by the observations that the basal ratio of lactate to pyruvate was higher in myocardium taken from hypertrophied SHR hearts compared with WKY (80), and that homogenates from hypertrophied hypertensive dog hearts produced more lactate than controls (32).

More direct studies of the capacity of hypertrophied hearts to utilize glycolysis, and the possible connection between anaerobic glycolysis and the response of these hearts to ischemia and hypoxia have been done using isolated hearts (26,27). Anderson and others (26) observed increased rates of lactate production during hypoxia and ischemia in rat hearts hypertrophied by aortic constriction compared with controls (26). They concluded that hypertrophied hearts do indeed have a greater potential to utilize anaerobic glycolysis. During zero flow ischemia increased lactate production in aortic banded hearts resulted in greater lactate accumulation, earlier development of contracture, and poorer functional recovery following reperfusion. In contrast, greater

lactate production in hypertrophied hearts during 15 minutes of hypoxic perfusion without substrate resulted in better functional recovery in these hearts compared with controls (26).

The differential effects of hypoxia and ischemia on functional recovery were explained in terms of the potential damaging effects of lactate and other by-products of anaerobic glycolysis that accumulated during ischemia, but were washed out during hypoxia (26). Increased anaerobic glycolysis would serve to preserve high energy phosphate concentrations during both ischemia and hypoxia. However, the damaging effects of accumulated lactate and H^+ ions have been shown to outweigh the beneficial effects of increased energy production during ischemia (16,17). Thus, these results indicate that hypertrophied hearts may have an enhanced capacity to use anaerobic glycolysis that may increase the susceptibility of these hearts to damage and dysfunction during ischemia.

In contrast, on the basis of isolated heart studies using the DOCA-salt rat, Cunningham and others (27) concluded that hypertrophied hearts may actually have a decreased capacity to recruit anaerobic glycolysis. Lactate production was lower in hypertrophied hearts from DOCA-salt rats during 15 minutes of hypoxic perfusion with buffer containing 5.5mM glucose and 1mM lactate. Lower lactate production in the hypertrophied hearts was associated with a greater increase in LVEDP, and a greater decrease in systolic pressure during hypoxia. Furthermore, recovery of both diastolic and systolic function following reoxygenation was less complete in the hypertrophied hearts with attenuated lactate production (27).

Increasing the glucose concentration to 27.5 mM and providing insulin improved function during hypoxia, and recovery following reoxygenation in both DOCA-salt and

nonhypertrophied hearts (27). Furthermore, the functional improvement with higher glucose was greater in the hypertrophied hearts so that differences in hypoxic dysfunction and damage between these hearts and controls were eliminated. Decreased dysfunction during ischemia and improved functional recovery in hypertrophied hearts were correlated directly with the normalization of lactate production relative to controls (27). These results support the hypothesis that differences in anaerobic metabolism may contribute to the increased sensitivity of hypertrophied hearts to hypoxia, but suggest that the DOCA-salt hearts may be at greater risk due to a decreased, rather than increased, capacity to utilize anaerobic glycolysis.

Improved resistance to hypoxia with higher glucose concentrations was also reported by Brooks and others (31). These investigators observed that differences in functional impairment between SHR papillary muscles and controls during hypoxia were eliminated by increasing the superfusate glucose concentration from 5.5 mM to 22 mM. Lactate production, however, was not measured in this study (31).

The reasons for the differences in lactate production in hearts hypertrophied by aortic banding and DOCA-salt hypertension are unclear. There were, however, a number of important differences in the hypoxic conditions in these two studies. Specifically, Anderson and others perfused hearts with hypoxic buffer without energy substrates (26), whereas Cunningham and others perfused the DOCA-salt hearts with buffer containing relatively low concentrations of glucose in combination with lactate (27). Subsequently, both the control and banded hearts stopped beating after 3 to 4 minutes of hypoxia (26), whereas the DOCA-salt hearts and their controls continued to beat throughout the 15 minutes of hypoxia (27). Thus, there were differences in both energy supply and demand in these experiments which may have contributed to the observed differences in lactate

production in the two models of pressure overload hypertrophy.

Another possible explanation for the observed differences in anaerobic metabolism between DOCA-salt and aortic banded rats is that these hearts were at different stages of the hypertrophic process. That is, the DOCA-salt hearts may have been less compensated (closer to failure) than the aortic banded hearts. This concept is supported by a recent study in aortic banded canine hearts that evaluated the response of hypertrophied hearts with and without evidence of failure to 60 minutes of zero flow ischemia (81). Hypertrophied hearts with failure exhibited less lactate production during early reperfusion compared with both control hearts and hypertrophied hearts without failure. The decreased lactate production was associated with greater impairment of diastolic function following reperfusion in the failing hearts. These results suggest that the transition to failure in hypertrophied hearts may be associated with a decreased capacity to recruit anaerobic glycolysis (81).

Therefore, there is significant indirect evidence that suggests that hypertrophied hearts may have a greater dependence on, or capacity to recruit, anaerobic glycolysis compared with nonhypertrophied hearts. However, studies designed to test this hypothesis directly, and to relate metabolic changes with the increased sensitivity of hypertrophied hearts to ischemia have, to date, yielded disparate results. Additional research will be required to determine the reasons for this disparity, and to evaluate the significance of altered enzyme expression and changes in metabolism associated with the compensated and failing hypertrophied heart. This information may be important to a complete understanding of the susceptibility of these hearts to ischemic dysfunction and damage.

Evidence of Abnormal Na^+ and Ca^{2+} Regulation in Hypertrophied Hearts

Another possible mechanism for the exaggerated sensitivity of hypertrophied hearts to ischemia involves impaired handling of Na^+ . The transsarcolemmal gradient for Na^+ is controlled, in part, by the Na^+ - K^+ ATPase pump that transports three Na^+ ions out of the cell in exchange for two K^+ ions (82). The Na^+ - K^+ ATPase is also important for maintenance of the transmembrane gradient for K^+ and the electrical potential across the sarcolemma (82). The Na^+ - K^+ ATPase activity is decreased in at least some types of pressure overload hypertrophy (83-87). Additionally, the isoform expression and functional characteristics of the Na^+ - K^+ ATPase may be altered in hearts with pressure overload hypertrophy (88-89).

Several studies have shown a decrease in the Na^+ - K^+ ATPase activity in hypertrophied hearts. Lee and others (83) found a 35-41% reduction in the Na^+ - K^+ ATPase activity in myocardial membranes from 16-19 week old SHR compared with WKY controls using both sodium dependent phosphorylation, and ^3H -ouabain binding assays. The number of phosphorylation sites and ^3H -ouabain binding sites were decreased in the SHR hearts, but there were no significant differences between the SHR and WKY hearts in the K_D for ouabain binding, or the I_{50} for inhibition of the enzyme by ouabain. Thus, the decrease in activity of the Na^+ - K^+ ATPase in the SHR hearts was attributed to a decrease in the number of active pumps (83).

Sharma and others (84) reported a 36% decrease in the ouabain sensitive Na^+ - K^+ ATPase activity in myocardial homogenates, and a 43% decrease in the activity in sarcolemmal microsomes from 16 week old SHR compared with WKY. Similar decreases in ouabain sensitive Na^+ - K^+ ATPase activity were observed by other investigators in 16 week old (85) and 22 week old SHR (86). A reduction in Na^+ - K^+ ATPase activity of a

similar magnitude as that found in SHR was observed in rats with one-kidney, one-clip hypertension (87). The observation of decreased $\text{Na}^+\text{-K}^+$ ATPase activity in the renal model of pressure overload hypertrophy indicated that the defect was not a genetic characteristic of the SHR. However, no reduction in the $\text{Na}^+\text{-K}^+$ ATPase activity was found in a third model of pressure overload in which hypertrophy was produced rapidly by severe constriction of the abdominal aorta (86).

Structural changes in the $\text{Na}^+\text{-K}^+$ ATPase which alter the binding of the enzyme to ouabain were observed in hearts in which hypertrophy is produced by aortic constriction (88,89). Myocardial membranes have two $\text{Na}^+\text{-K}^+$ ATPase binding sites for ouabain with different affinities for the drug (90,91). The affinity of the binding site for the drug is believed to be conferred by the presence of different isoforms of the $\text{Na}^+\text{-K}^+$ ATPase (90,91). Chevalier et al. (88) found that the ratio of low affinity ouabain binding sites to high affinity sites was reduced in hypertrophied hearts from aortic banded rats. This change in the proportion of low affinity sites was explained as a probable shift in the predominant isoform of the $\text{Na}^+\text{-K}^+$ ATPase (88).

Additional evidence that pressure overload hypertrophy may alter the expression of different forms of myocardial $\text{Na}^+\text{-K}^+$ ATPase is provided by a study by Charlemagne and others (89). Hearts in which hypertrophy was induced by constriction of the abdominal aorta had increased expression of a neonatal form of $\text{Na}^+\text{-K}^+$ ATPase. This neonatal $\text{Na}^+\text{-K}^+$ ATPase exhibited decreased rates of ouabain binding and dissociation compared with the enzyme expressed in control hearts (89).

Therefore, alterations in both the number of active $\text{Na}^+\text{-K}^+$ pumps and the structure of the enzyme have been observed in different models of pressure overload hypertrophy. Although the functional significance of these changes has not been established, it has

been proposed that decreased activity of the $\text{Na}^+\text{-K}^+$ ATPase may serve to improve contractility in hypertrophied hearts by augmenting the uptake of Ca^{2+} via $\text{Na}^+\text{-Ca}^{2+}$ exchange (85,86).

Abnormalities in Ca^{2+} transport may also occur in hypertrophied hearts (84,86,92-98). Calcium transients in the heart are complex, and there are several mechanisms which may be important in the influx and removal of calcium from the cytoplasm. In general, the rise in cytoplasmic calcium which precedes contraction is due to release of Ca^{2+} from the sarcoplasmic reticulum, influx of Ca^{2+} through the sarcolemmal Ca^{2+} channels, and influx via $\text{Na}^+\text{-Ca}^{2+}$ exchange (99,100). The removal of Ca^{2+} from the cytoplasm that is required for relaxation is accomplished by reuptake of Ca^{2+} by the sarcoplasmic reticulum, and transport across the sarcolemma via $\text{Na}^+\text{-Ca}^{2+}$ exchange (99,100). The sarcolemmal Ca^{2+} ATPase also contributes to a lesser extent to the removal of Ca^{2+} from the cytoplasm (100). The relative contribution of these mechanisms to the uptake and removal of Ca^{2+} is apparently altered in hypertrophied hearts. Specifically, uptake and removal of Ca^{2+} by the sarcoplasmic reticulum in hypertrophied hearts may be depressed while mechanisms of influx and removal of Ca^{2+} across the sarcolemma may be enhanced (84,86,92-98).

Using aequorin to measure the Ca^{2+} transient, Gwathmey and Morgan (92) demonstrated that the duration of the calcium transient was increased in hypertrophied right ventricular papillary muscles. Right ventricular hypertrophy was induced in this study by banding the pulmonary artery. Prolongation of the Ca^{2+} transient was associated with a subsequent prolongation of the isometric contraction in these muscles. These observations were thought to be indicative of depressed uptake of Ca^{2+} by the sarcoplasmic reticulum in the hypertrophied myocardium (92).

Schouten and others (93) also found evidence of depressed uptake of Ca^{2+} by the sarcoplasmic reticulum in hypertrophied rat trabeculae and papillary muscles. They observed prolongation of the action potential and a greater degree of postextrasystolic potentiation in muscles from hearts in which hypertrophy was induced by constriction of either the aorta or the pulmonary artery. These observations were considered to be electrical and mechanical correlates of an increase in the contribution of extracellular Ca^{2+} to the calcium transient (93). In addition, they observed delayed relaxation of isometric force, and a faster decay of postextrasystolic potentiation that may have reflected a reduction in the sequestration of Ca^{2+} by the sarcoplasmic reticulum. Therefore, the study provided evidence for both decreased uptake and release of Ca^{2+} by the sarcoplasmic reticulum in hypertrophied hearts (93).

Kimura and others (94) found that Ca^{2+} accumulation by sarcoplasmic reticulum in chemically skinned fibers from hypertrophied hearts was significantly slower compared with controls. Hypertrophy in these hearts was induced by aortic constriction. The uptake of Ca^{2+} by the sarcoplasmic reticulum was estimated in this study as the area under the force curve for a caffeine induced contracture. Prior to the contracture the sarcoplasmic reticulum were preloaded with Ca^{2+} by superfusing the muscle with solutions with graded concentrations of Ca^{2+} for different loading periods (94).

Decreased Ca^{2+} uptake has been directly observed in isolated sarcoplasmic reticular microsomes from SHR hearts (95). Decreased Ca^{2+} uptake by the sarcoplasmic reticulum was associated with decreased phosphorylation of the protein phospholamban. The decreased phosphorylation may have been due to a deficiency in the intrinsic cyclic AMP dependent protein kinase in the SHR hearts (95). In a rabbit model of aortic constriction, Lamers and Stinis (97) measured both a reduction in Ca^{2+} uptake in

microsomes of sarcoplasmic reticulum, and decreased Ca^{2+} ATPase activity. Additionally, the reduced Ca^{2+} uptake by the sarcoplasmic reticulum in the aortic constricted hearts could not be reversed with excess exogenous cyclic AMP and protein kinase. Thus, the defect in this model was apparently due to a reduction of the number of Ca^{2+} ATPase transport sites rather than a deficiency in endogenous protein kinase (97).

Depressed function of the sarcoplasmic reticulum in SHR hearts increased progressively with age from 4 to 22 weeks which indicated that the defect may have been related to the severity of hypertrophy in this model (95). Furthermore, in a subsequent study these investigators were able to show that the activity of the sarcoplasmic reticulum was improved by treatment with α -methyldopa which promoted regression of hypertrophy as well as a reduction in blood pressure. Reduction of blood pressure alone, with hydralazine, did not improve function of the sarcoplasmic reticulum. Thus, impaired uptake of Ca^{2+} by the sarcoplasmic reticulum in hypertrophied hearts appeared to be related specifically to hypertrophy rather than to elevated arterial pressure in this model (96).

In contrast with decreased Ca^{2+} uptake by the sarcoplasmic reticulum, Ca^{2+} transport by Na^+ - Ca^{2+} exchange may be enhanced in the hypertrophied heart. The direction of Ca^{2+} movement across the sarcolemma by Na^+ - Ca^{2+} exchange is determined by the prevailing electrochemical gradients for Na^+ and Ca^{2+} (100). These gradients favor an influx of Ca^{2+} during membrane depolarization and removal of Ca^{2+} following repolarization (100).

Sarcolemmal Na^+ dependent Ca^{2+} transport was shown to be significantly increased in 22 week old SHR using isolated microsomes in which the Na^+ and Ca^{2+} gradients were controlled experimentally.(86). Similar increases in Na^+ - Ca^{2+} exchange

were observed in the same study for hearts in which hypertrophy was induced by aortic banding for 7 and 28 days. Since there was no change in Na^+ - Ca^{2+} exchange in younger SHR or banded animals without evidence of hypertrophy, the increase in Na^+ - Ca^{2+} exchange was apparently associated with the development of hypertrophy (86).

The importance of Ca^{2+} removal by the sarcolemmal Ca^{2+} ATPase for myocardial relaxation is incompletely understood, but the enzyme does contribute to Ca^{2+} homeostasis by actively pumping Ca^{2+} out of the cardiocyte (100). Increased activity of the sarcolemmal Ca^{2+} ATPase was detected in sarcolemmal vesicles from three week old SHR (98) and seven and 22 week old SHR (86). Similarly, the sarcolemma isolated from hearts hypertrophied by aortic constriction also had increased Ca^{2+} ATPase activity (86).

The Ca^{2+} channels contribute to calcium influx during the myocardial action potential (100). The number of Ca^{2+} channels measured using a ^3H -nitrendipine binding assay in sarcolemmal microsomes from 7 week old (86), 16 week old (84) and 22 week old SHR was increased compared with age matched WKY controls (86). However, the number of Ca^{2+} channels measured using the same technique in rat hearts in which hypertrophy was induced by aortic banding was actually decreased (86). The discrepancy between studies in whether the number of channels was increased or decreased suggests that changes in Ca^{2+} channel density may vary in different models of pressure overload hypertrophy.

Although there is some variability between different models of pressure overload hypertrophy with respect to changes in specific transport processes and the mechanisms involved in these changes, there is general agreement that Ca^{2+} uptake and release by the sarcoplasmic reticulum is depressed in hypertrophied hearts, and that mechanisms of Ca^{2+} influx and efflux across the sarcolemma are enhanced. There is also evidence

from several experimental models of hypertrophy that the $\text{Na}^+\text{-K}^+$ ATPase activity is reduced.

It has been argued that the enhancement of sarcolemmal mechanisms for Ca^{2+} transport in hypertrophied hearts may compensate for the depression of Ca^{2+} uptake and removal by the sarcoplasmic reticulum and thereby produce a Ca^{2+} transient of normal amplitude (92), and maintain normal systolic function (92,93). As noted earlier, it has also been argued that the decreased $\text{Na}^+\text{-K}^+$ ATPase activity in hypertrophied hearts may also be compensatory and serve to augment contractile function.

Although enhanced sarcolemmal transport of Ca^{2+} may be compensatory under normal conditions, these changes may be detrimental during ischemia and hypoxia. An increased dependence on $\text{Na}^+\text{-Ca}^{2+}$ exchange for the removal of Ca^{2+} would be especially detrimental for myocardial relaxation during ischemia when intracellular Na^+ is increased. Elevated intracellular Na^+ would inhibit removal of Ca^{2+} by $\text{Na}^+\text{-Ca}^{2+}$ exchange, and perhaps actually promote cellular uptake of Ca^{2+} in exchange for intracellular Na^+ . In combination with depressed function of the sarcoplasmic reticulum, increased cellular Ca^{2+} uptake would produce elevated cytosolic free Ca^{2+} and, subsequently, an increase in active stiffness during diastole. As outlined earlier, diastolic dysfunction during even mild ischemic or hypoxic conditions is a common finding in hypertrophied hearts (41,42,43). Increased cellular uptake of Ca^{2+} during ischemia or hypoxia could also potentiate the development of contracture (49,50), and perhaps result in poorer functional recovery following reperfusion.

The relationship between altered Na^+ and Ca^{2+} homeostasis and ischemic dysfunction and damage has not been investigated in hypertrophied hearts. There is, however, a substantial amount of evidence that imbalances in cation homeostasis

contribute to ischemic dysfunction and injury in normal hearts.

The Role of Sodium in Ischemic Dysfunction and Damage

In addition to a depression of high energy phosphate concentrations and pH, and the accumulation of metabolites, myocardial ischemia is characterized by imbalances in the distribution of cations across the sarcolemma. An increase in intracellular Na^+ has been observed during myocardial ischemia and early reperfusion (101-108). This increase in Na^+ may occur as a result of increased uptake of Na^+ via Na^+ - H^+ exchange in response to increased lactate and H^+ production (101,102,106-109), and/or decreased removal of Na^+ by the Na^+ - K^+ ATPase due to decreased energy availability (102,110). The increase in Na^+ during ischemia is important because increased Na^+ during ischemia may promote an increase in Ca^{2+} during ischemia and reperfusion via Na^+ - Ca^{2+} exchange (101,102,108,109). Elevated Ca^{2+} is associated with ischemic injury and dysfunction (101,102,108,111). Therefore, the accumulation of intracellular Na^+ may be a primary step in the production of mechanical dysfunction during ischemia and irreversible damage following reperfusion.

Increased intracellular Na^+ during ischemia or hypoxia has been observed using several techniques in several animal species. Tani and Neely observed a 50% increase in Na^+ after 15 minutes of zero flow ischemia in perfused rat hearts (101). Increasing the duration of ischemia to 30 or 40 minutes produced a four to five-fold increase in Na^+ in this study. The Na^+ rose approximately linearly during ischemia, and continued to increase during the initial two minutes of reperfusion (101). Elevation of intracellular Na^+ was also observed in canine myocardium following occlusion of the left anterior descending coronary artery (LAD) (103). Partial occlusion of the LAD produced a two-fold

increase and complete occlusion produced a 4 to 5-fold increase in Na^+ . Forty minutes of hypoxia, in the absence of glucose, in perfused rat hearts produced an increase in intracellular Na^+ similar to that observed during zero flow ischemia (104). The intracellular Na^+ was measured in all of these studies in homogenized tissue using ion selective electrodes (101-102) or flame photometry (103-104) in combination with ^{14}C -D-sorbitol (101-102), ^{51}Cr (103) or ^{14}C -inulin (104) as extracellular markers.

Similar results were found in studies in which NMR was used to measure intracellular Na^+ . Pike and others (105) observed a five-fold increase in Na^+ after 30 minutes of zero flow ischemia in ferret hearts using ^{23}Na NMR and the shift reagent dysprosium (III) triethylenetetraminehexaacetate (DyTTHA). An 80% increase in Na^+ was measured following 21 minutes of zero flow ischemia in perfused rat hearts using NMR and the Na^+ shift reagent thulium (III) 1,4,7,10-tetraazacyclodecane- $\text{N},\text{N}',\text{N}'',\text{N}'''$ -tetra(methylenephosphonate) ($\text{Tm}(\text{DOTP})^{5-}$) (106). Additionally, thirty minutes of low flow ischemia at a coronary flow equal to 10% of the control flow produced a significant, but smaller, increase in intracellular Na^+ of ~50% (106). Other investigators have measured a 54% increase in intracellular Na^+ following 10 minutes of zero flow ischemia (107), and a three-fold increase following 20 minutes of ischemia in perfused rat hearts (108) using NMR and the Na^+ shift reagent $\text{Tm}(\text{DOTP})^{5-}$.

The observation of increased intracellular Na^+ during hypoxia and ischemia is not universal. Kleber et al were unable to detect an increase in intracellular Na^+ using sodium selective microelectrodes in guinea pig hearts after 15 minutes of zero flow ischemia (112). This inconsistency may reflect exceptional Na^+ handling in the guinea pig, or may be an artifact of the technique used to measure Na^+ . Microelectrodes measure Na^+ in only a few cells on the epicardial surface where ischemia is least severe. Therefore, it is

possible that changes in Na^+ may have occurred in the inner layers of the ventricle in these hearts that were not detected using microelectrodes. In support of this argument Regan and others (104) found that the magnitude of the increase in cell Na^+ in ischemic dog hearts was greater in the inner layer of the left ventricular wall than in the outer layer. All of the studies which reported substantial increases in Na^+ during ischemia used methods which measured the average Na^+ either for the whole heart (101-103,105-108) or for large sections of the ventricle (104). In summary, a large number of studies have indicated that intracellular Na^+ increases during ischemia and reperfusion, and that the magnitude of the increase is dependent on the severity and duration of ischemia.

Decreased activity of the Na^+/K^+ ATPase which could promote an increase in intracellular Na^+ has been observed in myocardium during ischemia (113,114). This decrease in activity has been attributed to either decreased energy availability (102,110), or inhibition by free fatty acids which accumulate during ischemia (115). A measurable decrease in the activity of the Na^+/K^+ ATPase was demonstrated in crude homogenates from rabbit myocardium after only 10 minutes of zero flow ischemia (113). Longer periods of ischemia in the same preparation were associated with increased inhibition of Na^+/K^+ ATPase with a maximal inhibition of 40% occurring after 30 minutes of ischemia. Winston and others (114) also found a decrease in the Na^+/K^+ ATPase activity in pig myocardium subjected to 30 minutes of complete coronary occlusion using an enzyme histochemical technique that measures enzyme activity in situ. In the same study an immunohistochemical technique was used to demonstrate that the decrease in activity was not due to redistribution or loss of the Na^+/K^+ ATPase from the membrane (114).

The importance of energy supply in maintaining the activity of the Na^+/K^+ ATPase during ischemia has also been demonstrated. Substrate free low flow anoxia resulted in

an increase in intracellular Na^+ in perfused rat hearts which was prevented by the provision of glucose and subsequent preservation of ATP during anoxia (102). Since the beneficial effect of glucose in preventing the increase in Na^+ during low flow anoxia was blocked by ouabain, the investigators concluded that the activity of the Na^+ -K ATPase during ischemia is dependent on energy production by anaerobic glycolysis (102).

In a second study, a strong relationship was found between the decrease in the free energy of ATP hydrolysis and the increase in intracellular Na^+ during 30 minutes of anoxia in perfused rat hearts (110). Furthermore, the investigators were able to demonstrate close agreement between measured membrane gradients for Na^+ and K^+ , and gradients predicted by assuming that the Na^+ - K^+ ATPase was in thermodynamic equilibrium during hypoxia or ischemia. They concluded that the Na^+ - K^+ pump, and therefore the transmembrane gradients for Na^+ and K^+ , may be controlled directly by myocardial energy availability during hypoxia and ischemia (110). Therefore, the ability of the Na^+ - K^+ ATPase to maintain the Na^+ gradient should be compromised as high energy phosphates are depleted during hypoxia and ischemia.

The decrease in oxygen supply during ischemia promotes an increase in lactate and H^+ production via increased anaerobic glycolysis. Lazdunski and others (109) were the first to propose that elimination of excess H^+ by myocytes through Na^+ - H^+ exchange might play a key role in producing an increase in intracellular Na^+ during ischemia. These investigators demonstrated that Na^+ - H^+ exchange was the primary mechanism by which cardiac myocytes recovered from transient acidification with NH_4^+ , and they concluded that Na^+ - H^+ exchange is the most important mechanism for myocardial pH regulation (109). The results of other studies have confirmed that Na^+ - H^+ exchange is the primary mechanism for pH regulation in the heart (116,117), and have indicated that Na^+ - H^+ may

be important in the recovery of intracellular pH following ischemia (118). The process of $\text{Na}^+\text{-H}^+$ exchange in cardiac myocytes is dependent on the transmembrane pH and Na^+ gradients (109,116,117). Therefore, removal of H^+ by $\text{Na}^+\text{-H}^+$ exchange is favored by the low intracellular pH present during ischemia. The uptake of Na^+ in exchange for intracellular H^+ should be greatest during hypoxia, low flow ischemia, or early reperfusion following zero flow ischemia when the transmembrane gradient for H^+ is preserved by vascular washout (109).

The results of a number of studies support the hypothesis that Na^+ increases during ischemia and/or reperfusion as a result of increased $\text{Na}^+\text{-H}^+$ exchange. Tani and Neely demonstrated that treatment of hearts with a range of doses of the $\text{Na}^+\text{-H}^+$ exchange inhibitor, amiloride reduced the accumulation of intracellular Na^+ in a dose dependent manner (101). Administration of amiloride before ischemia had a greater effect on the increase in Na^+ than treatment during early reperfusion (101). This observation indicated that $\text{Na}^+\text{-H}^+$ exchange was important for both the increase in Na^+ during ischemia and the increase observed during early reperfusion (101). These results are consistent with those from another lab in which treatment with amiloride blocked the increase in Na^+ during 20 minutes of zero flow ischemia in perfused rat hearts (108). Additionally, Pike and others (106) were able to block the increase in Na^+ during both zero flow and low flow ischemia in perfused rat hearts using ethylisopropylamiloride (EIPA) which is a more specific antagonist of $\text{Na}^+\text{-H}^+$ exchange. The administration of amiloride did not affect high energy phosphate concentrations, and therefore decreased Na^+ accumulation in amiloride treated hearts could not be attributed to enhanced removal of Na^+ via the $\text{Na}^+\text{-K}^+$ ATPase due to conservation of high energy phosphates (108).

In addition to inhibiting $\text{Na}^+\text{-H}^+$ exchange, amiloride (119) and EIPA (120) may

also inhibit the voltage sensitive Na^+ channels. Furthermore, the Na^+ channel blocker lidocaine has been shown to attenuate the increase in tissue Na^+ following zero flow ischemia in guinea pig hearts. Thus, there is some evidence that Na^+ influx via the Na^+ channels may contribute to the increase in Na^+ during ischemia (121). These factors suggest the possibility that the inhibition of Na^+ influx by amiloride or EIPA may have been due in part to blocking of the Na^+ channels. However, the contribution of the Na^+ channels to an influx in Na^+ during ischemia should be small due to partial depolarization of the membrane that would decrease the electrical driving force for Na^+ influx (101,122). Therefore, it is unlikely that inhibition of the Na^+ channels contributed significantly to the observed reductions in intracellular Na^+ by amiloride or EIPA.

The hypothesis that the influx of Na^+ during ischemia is due primarily to Na^+ - H^+ exchange is also supported by an experiment in which lactate and H^+ production during zero flow ischemia were reduced by preischemic glycogen depletion (101). The decrease in lactate and H^+ were associated with a decrease in the subsequent uptake of Na^+ , and thereby provided evidence that the increase in Na^+ occurred via Na^+ - H^+ exchange (101). The results of the latter study might appear to conflict with the observation by the same investigators that the provision of glucose during low flow anoxia actually prevented the increase in intracellular Na^+ that was observed in the absence of glucose (102). However, this apparent inconsistency is resolved when the effects of vascular washout during low anoxia are considered. More specifically, vascular washout during low flow anoxia reduces the accumulation of metabolites such as lactate and free fatty acids that may inhibit glycolysis (16) and the Na^+ - K^+ ATPase (115). Therefore, although greater lactate and H^+ production via anaerobic glycolysis may result in a greater influx of Na^+ via Na^+ - H^+ exchange, anaerobic energy production preserves high energy phosphates, and the influx

of Na^+ is balanced by enhanced removal of Na^+ via the Na^+/K^+ ATPase. In contrast, during zero flow ischemia the beneficial effects of increased energy production via anaerobic glycolysis are outweighed by the inhibitory effects of the greater accumulation of metabolites. More specifically, the removal of Na^+ via the Na^+/K^+ ATPase may be inhibited by metabolites of energy production and consequently unable to keep pace with the influx of Na^+ via Na^+/H^+ exchange so that the intracellular Na^+ increases. In summary, there is convincing evidence that the increase in intracellular Na^+ during ischemia is due to a combination of increased uptake of Na^+ via Na^+/H^+ exchange, and inadequate removal of Na^+ via the Na^+/K^+ ATPase.

The increase in intracellular Na^+ during ischemia or hypoxia has been proposed to promote an increase in intracellular Ca^{2+} via $\text{Na}^+/\text{Ca}^{2+}$ exchange (101,102,108,109). Elevated intracellular Ca^{2+} may, in turn, directly result in poor recovery of ventricular function following ischemia and reperfusion (101,102,108,109). Although the mechanisms by which elevated Ca^{2+} produces myocardial damage have not been clearly defined, several possible mechanisms have been proposed. Intracellular Ca^{2+} accumulation may result in decreased production of ATP due to uptake of excess Ca^{2+} by mitochondria (123,124), or depletion of ATP due to activation of ATPases by Ca^{2+} (123) and sustained cross bridge cycling (125,126). Additionally, elevated Ca^{2+} may activate phospholipases which destroy phospholipids and compromise sarcolemmal integrity (127).

The $\text{Na}^+/\text{Ca}^{2+}$ transport mechanism is capable of transporting Ca^{2+} in either direction across the sarcolemma (100). The majority of experimental data indicate that the exchanger is electrogenic with a stoichiometry of 3 or more Na^+ per Ca^{2+} (100,128,129,130). Since the direction and rate of $\text{Na}^+/\text{Ca}^{2+}$ exchange is dependent on the electrochemical gradients for Na^+ and Ca^{2+} (100), a net influx of Ca^{2+} would be

favorable by elevated intracellular Na^+ during ischemia and reperfusion.

Since there are currently no suitably specific inhibitors of Na^+ - Ca^{2+} exchange, it has been impossible to demonstrate directly that the uptake of Ca^{2+} during ischemia and reperfusion occurs via Na^+ - Ca^{2+} exchange. However, several studies have indicated that accumulation of intracellular Na^+ during ischemia or early reperfusion is associated with a subsequent increase in intracellular Ca^{2+} (101,102,108). Tani and Neely showed that $^{45}\text{Ca}^{2+}$ uptake during reperfusion following zero flow ischemia of different durations was linearly correlated with the $[\text{Na}^+]$ both at end ischemia and during the first two minutes of reperfusion (101). Additionally, the same investigators found that impaired mechanical function following ischemia and reperfusion in these hearts was correlated with the increase in both Na^+ and Ca^{2+} (101,102). Similar results were reported by Murphy and others (108) who used NMR to measure Na^+ and Ca^{2+} and demonstrated, in separate hearts exposed to the same ischemic protocol, that both Na^+ and Ca^{2+} increased during zero flow ischemia in perfused rat hearts. Increased Na^+ and Ca^{2+} in this study were again associated with depression of ventricular function following ischemia and reperfusion.

More conclusive evidence that increased Na^+ promotes an increase in Ca^{2+} and subsequent ischemic damage is provided by experiments in which the increase in intracellular Na^+ was varied during ischemia using either the Na^+ ionophore monensin (101,102), the Na^+ - H^+ exchange inhibitor amiloride (101,108), or by inhibition of the Na^+ - K^+ ATPase with ouabain or zero K^+ buffer (102). Monensin promoted a dose dependent accumulation of Na^+ , increased uptake of $^{45}\text{Ca}^{2+}$, and depressed functional recovery in isolated rat hearts subjected to either 70 minutes of low flow anoxia (102), or 15 minutes of zero flow ischemia (101). Similarly, inhibition of the Na^+ - K^+ ATPase with either ouabain,

or zero K^+ buffer during low flow anoxia produced an increase in intracellular Na^+ that was associated with increased uptake of $^{45}Ca^{2+}$, and incomplete functional recovery following reperfusion (102).

In two of the studies in which amiloride was used to attenuate the increase in Na^+ during ischemia, the decrease in Na^+ accumulation was associated with a subsequent reduction in the accumulation of Ca^{2+} during ischemia (108) or uptake during reperfusion (101). Although amiloride may also inhibit Na^+-Ca^{2+} exchange, the observation that amiloride prevented the increase in Na^+ as well as Ca^{2+} indicated that the effects of the drug were due predominately to inhibition of Na^+-H^+ exchange. Furthermore, inhibition of Na^+-H^+ exchange with dimethylamiloride (DMA) at a concentration far below the K_i for Na^+-Ca^{2+} exchange also reduced Ca^{2+} accumulation (108). Reduction in the accumulation of Na^+ and Ca^{2+} was associated with improved recovery of ventricular function. Therefore, changes in intracellular Na^+ elicited during ischemia by several different mechanisms effected changes in intracellular Ca^{2+} that are consistent with the hypothesis that Na^+-Ca^{2+} exchange contributes to Ca^{2+} overload during ischemia.

There is some disagreement concerning whether the influx of Ca^{2+} via Na^+-Ca^{2+} exchange is inhibited by the low intracellular pH during zero-flow ischemia. Tani and Neely were able to measure increased uptake of ^{45}Ca during reperfusion, but not during the period of zero flow ischemia (101). They argued that uptake of Ca^{2+} via Na^+-Ca^{2+} exchange would be inhibited by the low intracellular pH present during zero-flow ischemia (101). This argument is consistent with data from experiments with sarcolemmal vesicles which indicated that Na^+-Ca^{2+} exchange was inhibited at low pH (131). However, Murphy and others detected a substantial increase in intracellular Ca^{2+} during ischemia using NMR (108,111). Furthermore, this increase in Ca^{2+} was attenuated when the increase in

Na^+ was prevented by amiloride which suggests that the increase in Ca^{2+} during ischemia was linked to the increase in Na^+ , presumably, via $\text{Na}^+-\text{Ca}^{2+}$ exchange. These investigators proposed that the inhibitory effect of low pH on $\text{Na}^+-\text{Ca}^{2+}$ exchange was overridden by the effect of elevated intracellular Na^+ . This argument is supported by the observation that $\text{Na}^+-\text{Ca}^{2+}$ exchange could be inhibited by low pH only when the concentration of intracellular Na^+ was also low (131). Additionally, uptake of Ca^{2+} via $\text{Na}^+-\text{Ca}^{2+}$ exchange was shown to occur in myocardium treated with ouabain despite a low intracellular pH (100). These observations emphasize the importance of the Na^+ gradient as a regulator of the activity of the $\text{Na}^+-\text{Ca}^{2+}$ pump.

Therefore, there is substantial experimental evidence that supports the hypothesis that the accumulation of Na^+ during ischemia promotes an increase in Ca^{2+} via $\text{Na}^+-\text{Ca}^{2+}$ exchange and may, therefore, be a primary step in the production of ischemic dysfunction and damage in normal hearts.

B. STATEMENT OF THE PROBLEM

Hypertension is associated with myocardial hypertrophy which may develop as a compensatory response to the chronic increase in arterial pressure (1-2). Although the changes in the myocardium with the development of hypertrophy may be compensatory under basal conditions, hypertrophied hearts are more susceptible to dysfunction during ischemia, particularly diastolic, and damage following reperfusion (26,27,33-48). The causes of this exaggerated sensitivity to ischemia are unknown, but the hypertrophic process is associated with alterations in Na^+ and Ca^{2+} homeostasis (83-87,92-98) and shifts in enzyme expression (32,63,67,71) which may predispose these hearts to ischemic dysfunction and damage.

It has been hypothesized that changes in the expression of isozymes for creatine kinase and lactate dehydrogenase may increase the capacity of hypertrophied hearts to recruit anaerobic glycolysis (26,32). Although this hypothesis remains controversial (26,27), there is some evidence that hypertrophied hearts may produce more lactate during ischemia, and furthermore, that this increased lactate production may contribute to the increased sensitivity of hypertrophied hearts to ischemia (26).

Another mechanism that may contribute to the exaggerated susceptibility of hypertrophied hearts to ischemia involves altered Na^+ and Ca^{2+} homeostasis. One of the proposed mechanisms for the production of ischemic damage in normal hearts involves imbalances in cation homeostasis during ischemia. Specifically, the decrease in pH during ischemia promotes an influx of Na^+ via $\text{Na}^+\text{-H}^+$ exchange (101,102,106,108,109). This increased influx in Na^+ may be exacerbated by decreased removal of Na^+ by the $\text{Na}^+\text{-K}^+$ ATPase (102,110), and thereby produce an increase in intracellular Na^+ (101,102,106-109). The increased intracellular Na^+ concentration, in turn, is thought to promote an influx of Ca^{2+} via $\text{Na}^+\text{-Ca}^{2+}$ exchange, and a subsequent increase in the intracellular Ca^{2+} concentration (101,102,108). The increase in intracellular Ca^{2+} may directly precipitate myocardial dysfunction and damage (101,102,108,111,123-127).

There are several factors that indicate that hypertrophied hearts should have a greater propensity for ischemic dysfunction and damage via mechanisms involving disruption of cation homeostasis compared with nonhypertrophied hearts. Enhanced production of lactate and H^+ (26) in hypertrophied hearts would provide a greater stimulus for the influx of Na^+ via $\text{Na}^+\text{-H}^+$ exchange. In addition, hypertrophied hearts may have reduced $\text{Na}^+\text{-K}^+$ ATPase activity compared with control hearts (83-87) that could compromise the ability of these hearts to respond to an influx of Na^+ during ischemia.

Thus, intracellular Na^+ could accumulate to an even greater extent in hypertrophied hearts than in normal hearts. Finally, hypertrophied hearts may be characterized by a decreased capacity for Ca^{2+} sequestration by the sarcoplasmic reticulum (92-97), and greater dependence on Na^+ - Ca^{2+} exchange for normal Ca^{2+} homeostasis (86,98). This combination of factors would potentiate Ca^{2+} overload during ischemia, and perhaps result in greater ischemic dysfunction and damage compared with nonhypertrophied hearts.

There is a substantial amount of evidence that changes in the distribution of Na^+ and Ca^{2+} across the sarcolemma may indeed be important in the production of myocardial dysfunction and damage in normal hearts. The relative importance of this mechanism in promoting ischemic dysfunction and damage in hypertrophied hearts, however, has not been directly investigated. Most of the evidence for altered handling of Na^+ and Ca^{2+} by hypertrophied hearts was obtained from in vitro experiments. The significance of these changes has not, heretofore, been assessed directly in beating hearts.

C. EXPERIMENTAL DESIGN

The overall objective of the experiments that are described in the following chapters was to determine whether hypertrophied hearts do indeed exhibit a greater increase in intracellular Na^+ during ischemia, and to relate changes in intracellular Na^+ to ischemic dysfunction and recovery following reperfusion. The increase in intracellular Na^+ involves a balance between the influx of Na^+ , presumably by Na^+ - H^+ exchange, and removal via the Na^+ - K^+ ATPase which is dependent on the energetic status of the heart. Therefore, a second objective of these studies was to relate changes in intracellular Na^+

to the decrease in pH and high energy phosphate concentrations during ischemia and reperfusion.

The first study involved a series of preliminary experiments which were done using both the spontaneously hypertensive rat (SHR), which is an established model of hypertensive hypertrophy, and the borderline hypertensive rat (BHR) in which myocardial hypertrophy was previously reported, but incompletely characterized. The initial objectives were to compare the severity of hypertrophy which develops over time in the BHR fed a high salt diet with that in the SHR, and to determine whether the BHR exhibited shifts in the expression of creatine kinase isozymes that are characteristic of the SHR and other models of pressure overload hypertrophy. Creatine kinase and hypertrophy data for both the SHR and BHR were compared with data from age-matched normotensive Wistar-Kyoto (WKY) rat.

The second set of experiments with the BHR were preliminary studies using the isolated heart preparation and NMR. The primary objective of these studies was to design an ischemic protocol that could be used in combination with ^{23}Na NMR to assess the relationship between diastolic dysfunction and changes in intracellular Na^+ in hypertrophied hearts. The second objective of these studies was to determine whether hypertrophied hearts from BHR with frank hypertension exhibited evidence of greater diastolic dysfunction, or greater intracellular Na^+ accumulation during ischemia compared with nonhypertrophied BHR.

The last study was done using only the SHR as a model of hypertensive hypertrophy. The primary objective of this study was to determine whether the increase in intracellular Na^+ was greater in the hypertrophied SHR heart compared with the normotensive WKY control heart during low flow ischemia. In addition, the relationships

between the increase in intracellular Na^+ , and ischemic dysfunction and functional recovery were assessed. The increase in intracellular Na^+ was also related to changes in high energy phosphates and intracellular pH to evaluate the relative importance of these factors in promoting the increase in intracellular Na^+ in hypertrophied hearts.

CHAPTER 2

HYPERTROPHY IN THE BORDERLINE HYPERTENSIVE RAT HEART

A. INTRODUCTION

Hypertrophied hearts from hypertensive humans and animal models exhibit functional changes including impaired diastolic function and increased sensitivity to ischemic damage and dysfunction (26,27,33-48,132-134). However, it is unclear to what extent the changes in myocardial function represent the direct effects of elevated blood pressure on the heart, or the effects of the hypertrophic process per se (38,61,71). Furthermore, the relationship between the increase in blood pressure and the development of hypertrophy remains poorly understood (135). An exaggerated sensitivity to diastolic dysfunction has been reported in hearts with only minimal hypertrophy from hypertensive human patients (134) and animal models of hypertension (43). In addition, the reduction of high blood pressure with hydralazine does not mitigate left ventricular hypertrophy in hypertensive patients (61).

Hypertension develops rapidly, or at an early age, in most animal models of hypertension such as the DOCA-salt rat (33,41,42) and the Spontaneously Hypertensive Rat (SHR) (136,137). Therefore, it is not possible to distinguish the development of changes that occur in the heart as a direct result of high blood pressure from those that result from the hypertrophic process. A model of hypertension in which high blood pressure and hypertrophy develop more slowly would be valuable in understanding the relationship between pathologic changes in the heart and hypertension.

The Borderline Hypertensive Rat (BHR) is the first generation offspring of a female SHR and a male normotensive Wistar-Kyoto (WKY) rat (137). These animals normally

develop systolic blood pressures between 140 and 150 mmHg (137). However, when subjected to stress, or fed a high salt diet, the BHR develop frank hypertension (137,138). The increase in blood pressure occurs more gradually in the BHR fed a high salt diet than the pressure increase in the SHR (137).

Little is known concerning changes in the BHR heart during the development of hypertension. The hearts from 39 week old BHR that were subjected to an avoidance-avoidance conflict paradigm as a form of behavioral stress were significantly hypertrophied, and demonstrated fibrosis and myofibrillar disarray which are characteristic of pressure overload hypertrophy (138). The slower progression of hypertension in the BHR should make them a more suitable model to assess the myocardial changes that occur in hypertensive heart disease.

Myocardial hypertrophy is associated with cellular and molecular changes including altered expression of several enzymes including creatine kinase (CK). Creatine kinase is important for the synthesis, transport, and utilization of high energy phosphates, and exists as four distinct isozymes (66-67). The MM and mitochondrial isozymes normally predominate in adult myocardium, and the BB and MB isozymes are prominent in neonatal myocardium (70).

The development of myocardial hypertrophy is associated with an increase in the expression of the MB and BB isozymes, and a subsequent decrease in the expression of the MM and mitochondrial isozymes (63,67,71,72). A reduction in the total creatine kinase activity has also been reported in hypertrophied hearts (63,72). It has been hypothesized that these changes in total creatine kinase activity and isozyme expression may increase the capacity of hypertrophied hearts to use anaerobic glycolysis (26,71). The increased dependence of hypertrophied hearts on anaerobic glycolysis has been

associated with greater ischemic injury in these hearts (26).

The objectives of the preliminary experiments described in this section were to better characterize the changes in the BHR heart during the development of hypertension and hypertrophy. More specifically, the objectives were to determine whether the severity of hypertrophy increased with age and/or the duration of feeding a high NaCl diet in the BHR, and to determine whether BHR hearts developed changes in creatine kinase activity and isozyme expression that are characteristic of other models of pressure overload hypertrophy. Changes in the BHR hearts were compared with normotensive WKY and hypertensive SHR.

B. METHODS

Animals

Hearts from 4 and 12 month old male WKY, BHR, and SHR were obtained from rats studied in Dr. James Lawler's lab at the University of Tennessee, Knoxville. All BHR were bred by mating male WKY with female SHR using protocols previously described in detail (137,138). The male BHR offspring were weaned at 21 days and thereafter housed three to a cage. The WKY and SHR were purchased from Taconic farms at 4 weeks of age and housed 3 per cage. At 8 weeks of age the rats within each strain were randomly assigned to either an 8% sodium diet (HS) (ICN Nutritional Biochemicals rat chow) or a control diet (CON) of normal sodium rat chow (0.3%). One-half of the rats were maintained on these diets for 8 weeks prior to sacrifice, while the other half were maintained under these conditions for an additional 8 months.

Blood Pressure Determination and Tissue Sampling

At least two days before sacrifice, rats were anesthetized with methoxyflurane, and an indwelling teflon catheter with a polyethylene extension was surgically implanted in the femoral artery. Direct arterial pressures were obtained in awake, unsedated rats prior to sacrifice.

Rats were sacrificed by decapitation, and the hearts were rapidly removed. The left ventricle was dissected free from the other chambers, weighed, and frozen on dry ice. Left ventricular tissue samples were stored at -80°C until assayed for creatine kinase.

Creatine Kinase Assays

A 10 to 30 mg sample of left ventricular tissue was homogenized at 4°C in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EGTA, 1 mM B-mercaptoethanol and 0.1% Triton X. The final concentration of the homogenate was 5 mg/ml. Aliquots of the homogenate were used for total CK activity and isozyme analysis.

Total CK activity was assayed using a Sigma (St. Louis, MO) reagent kit. The assay uses a coupled enzyme reaction which produces NADPH as an end product (139). The change in absorbance per minute at 340nm due to the formation of NADPH, which is proportional to the CK activity, was measured using a spectrophotometer. Total CK activity was expressed as mIU/mg wet weight at 37°C. The relative distribution of CK isozymes was determined using a Beckman Paragon (Brea, CA) electrophoresis system and kit. The technique uses agarose gel electrophoresis followed by reaction of the gels to produce NADPH using an enzyme coupled reaction (140). Dithiothreitol (1mM) was added to the electrophoresis buffer to prevent oxidation of the B chain, and diadenosine pentaphosphate (10 μ M) was included in the buffer to inhibit adenylate kinase activity (67).

The peak areas were scanned at 340nm, and integrated using a Beckman (Fullerton, CA) densitometer equipped with a fluorescence detector. Isozyme activities were expressed as a fraction of the total activity.

Statistical Methods

Data were expressed as mean $\pm$ SEM. All data were analyzed using a three-way analysis of variance for the effects of strain, diet, and age. The specific comparisons performed were divided into the groups listed below. The overall one-tailed significance level for each group was 0.05. The specific one degree of freedom contrasts within each group were made using a Bonferroni correction of the overall significance level (141).

Strain within diet at 4 months of age

1. BHR-CON vs WKY-CON
2. SHR-CON vs WKY-CON
3. BHR-CON vs SHR-CON
4. BHR-HS vs WKY-HS

Strain within diet at 12 months of age

1. BHR-CON vs WKY-CON
2. SHR-CON vs WKY-CON
3. BHR-CON vs SHR-CON
4. BHR-HS vs WKY-HS

Diet

1. BHR-HS vs BHR-CON at 4 and 12 months.
2. WKY-HS vs WKY-CON at 4 and 12 months.
3. SHR-HS vs SHR-CON at 4 months.

Age 12 months vs 4 months within strain and diet:

1. WKY-CON
2. WKY-HS
3. BHR-CON
4. BHR-HS
5. SHR-CON

Strain X Diet

1. BHR-HS vs WKY-CON at 4 and 12 months.
2. SHR-CON vs BHR-HS at 4 and 12 months.

C. RESULTS

Mean Arterial Pressure

The mean arterial pressures (MAP) are shown in Table 2-1 and Figure 2-1. The MAP was higher in the BHR-CON than in the WKY-CON at both 4 and 12 months of age, but the difference was only statistically significant in 12 month old rats ($p=0.0110$). The MAP was significantly higher in the SHR-CON compared with both the BHR-CON ($p\leq 0.0002$) and the WKY-CON ($p=0.0001$) for both 4 month old and 12 month old rats. The MAP was higher in 12 month old SHR-CON compared with 4 month old SHR-CON, but the difference did not achieve statistical significance.

The high salt diet promoted an increase in the MAP in the BHR, and the MAP was significantly higher in the 4 month old BHR-HS compared with both age-matched WKY-HS ($p=0.0004$) and WKY-CON ($p=0.0004$). The MAP was significantly higher in the 12 month old BHR-HS compared with age-matched WKY-CON ($p=0.0022$), but not

Table 2-1. LEFT VENTRICULAR WEIGHTS AND ARTERIAL PRESSURES

AGE (mon)	GROUP	BW ^a (kg) (n)	LV WT ^b (g)	LV/BW ^c (g/kg)	MAP ^d (mmHg) (n)
4	WKY-CON	0.40 ± 0.01 (10)	0.91 ± 0.03	2.26 ± 0.03	119 ± 2 (9)
	WKY-HS	0.38 ± 0.01 (10)	0.92 ± 0.04	2.42 ± 0.04	120 ± 2 (10)
	BHR-CON	0.39 ± 0.01 (13)	0.93 ± 0.03	2.41 ± 0.05	131 ± 3 (13)
	BHR-HS	0.37 ± 0.01 (13)	0.98 ± 0.03	2.68 ± 0.06 ^{egi}	144 ± 3 ^{ei} (12)
	SHR-CON	0.28 ± 0.01 ^{ef} (10)	0.88 ± 0.02	3.14 ± 0.06 ^{efj}	157 ± 6 ^{ef} (10)
	SHR-HS	0.24 ± 0.01 ^g (8)	1.02 ± 0.07	4.22 ± 0.21 ^g	190 ± 14 ^g (8)
12	WKY-CON	0.63 ± 0.02 ^h (9)	1.31 ± 0.04 ^h	2.10 ± 0.04	116 ± 4 (8)
	WKY-HS	0.58 ± 0.02 ^h (8)	1.49 ± 0.07 ^{eh}	2.58 ± 0.05 ^g	124 ± 5 (8)
	BHR-CON	0.56 ± 0.01 ^{eh} (9)	1.31 ± 0.05 ^h	2.35 ± 0.07	134 ± 6 ^e (8)
	BHR-HS	0.48 ± 0.01 ^{eghi} (10)	1.37 ± 0.04 ^h	2.82 ± 0.04 ^{eg}	138 ± 4 ^e (10)
	SHR-CON	0.39 ± 0.01 ^h (10)	1.33 ± 0.06 ^h	3.41 ± 0.13 ^{efjk}	174 ± 5 ^{efj} (9)

Notes: Data are mean ± SEM. Probabilities are one-tailed.

^a BW is body weight.

^b LV WT is left ventricular wet weight.

^c LV/BW is the left ventricular wet weight to body weight ratio.

^d MAP is direct mean arterial pressure.

^e p ≤ 0.0028 vs WKY-CON at the same age.

^f p ≤ 0.0002 vs BHR-CON at the same age.

^g p ≤ 0.0036 vs control diet for the same age and strain.

^h p ≤ 0.0001 vs 4 month old rats of same strain and diet.

ⁱ p ≤ 0.0071 vs WKY-HS at same age.

^j p = 0.0001 vs WKY-HS

^k p ≤ 0.0098 vs SHR-CON at 4 months of age.

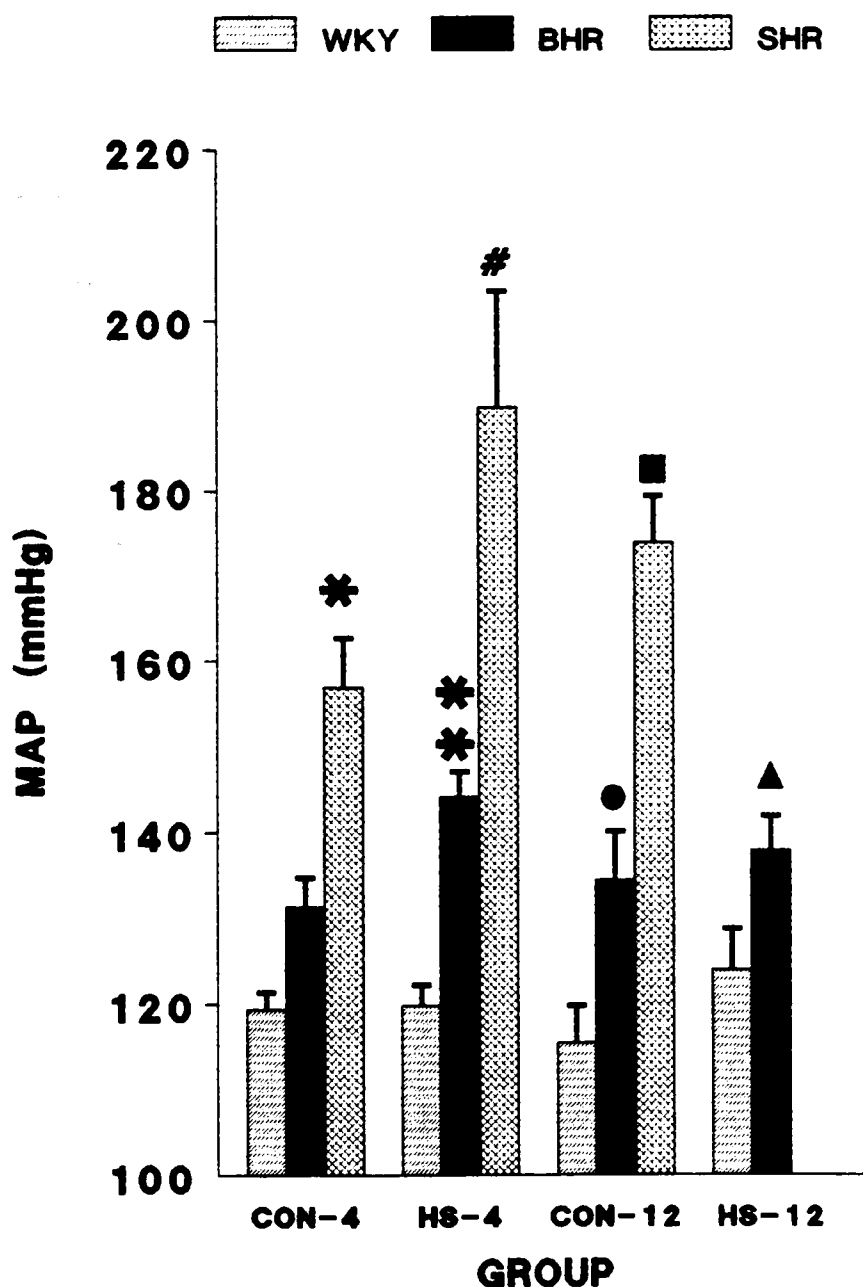


Figure 2-1. Mean Arterial Pressure. Bars are mean $\pm$ SEM for direct mean arterial pressure (MAP). The group abbreviations are defined as follows: CON-4 are 4 month old rats fed a control diet; HS-4 are 4 month old rats fed an 8% Na⁺ diet; CON-12 are 12 month old rats fed a control diet; HS-12 are 12 month old rats fed an 8% Na⁺ diet. * p= 0.0001 vs WKY-CON and p=0.0002 vs BHR-CON at 4 months. ** p=0.0004 vs WKY-CON and WKY-HS at 4 months. # p= 0.0001 vs SHR-CON at 4 months. • p= 0.011 vs WKY-CON at 12 months. ■ p= 0.0001 vs WKY-CON and BHR-CON at 12 months. ▲ p=0.0022 vs WKY-CON and p=0.0001 vs SHR-CON at 12 months.

compared with the WKY-HS or the BHR-CON. The MAP was not significantly higher in the 12 month old BHR-HS compared with the 4 month old BHR-HS. In fact, the mean pressure in the 12 month old BHR-HS was actually somewhat lower than for the 4 month old rats. The MAP in the 12 month old BHR-HS was also significantly lower compared with age matched SHR-CON ($p=0.0001$). The MAP was significantly higher in the 4 month old SHR-HS rats compared with the SHR-CON at the same age ($p=0.0001$). An insufficient number of SHR-HS rats survived for the evaluation of the effects of the high salt diet in 12 month old SHR. The high salt diet did not significantly increase MAP in WKY rats at either 4 or 12 months of age.

Severity of Hypertrophy

The severity of left ventricular hypertrophy was assessed in this study by comparing the left ventricular wet weight to body weight ratios (LV/BW). The LV/BW data are presented in Table 2-1 and Figure 2-2. Left ventricular weights and body weights are also shown in Table 2-1. Both 4 and 12 month old BHR-CON tended to have higher LV/BWs than age matched WKY-CON, but these differences were not statistically significant. The LV/BW was significantly higher in both the 4 and 12 month old SHR-CON compared with age matched BHR-CON ($p=0.0001$) and WKY-CON ($p=0.0001$). The LV/BW was significantly greater in 12 month old SHR-CON compared with 4 month old SHR-CON ($p=0.0098$) indicating that the severity of hypertrophy increased with age in this strain. In contrast, the severity of LV hypertrophy was not greater in 12 month old BHR-CON or BHR-HS compared with the younger rats.

The high salt diet did not significantly affect the LV/BW in the 4 month old WKY, but the LV/BW was significantly higher in the 12 month old WKY-HS compared with 12

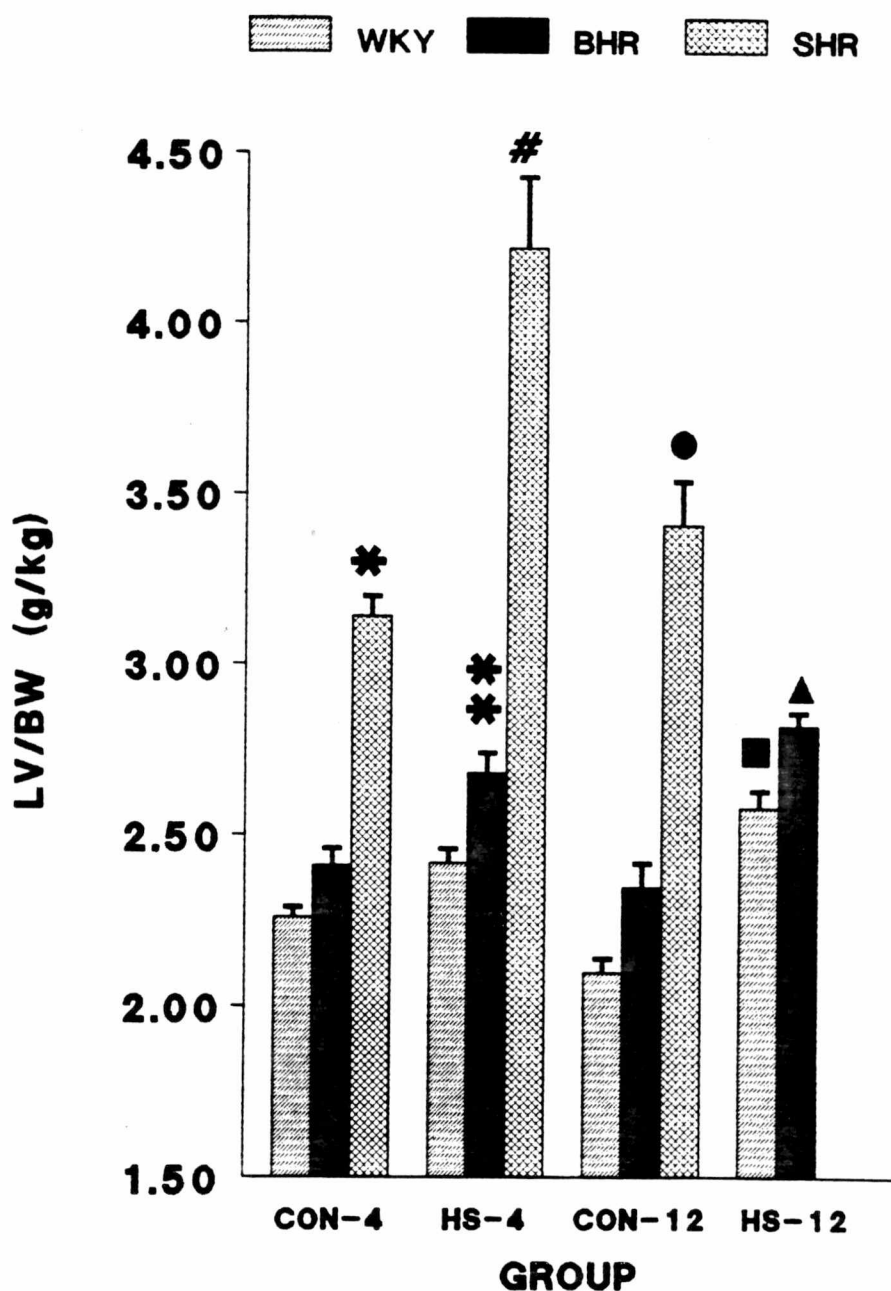


Figure 2-2. Comparison of the Severity of Left Ventricular Hypertrophy. Bars are mean $\pm$ SEM for the left ventricular wet weight to body weight ratio. See Figure 1 for definition of group abbreviations. * $p = 0.0001$ vs WKY-CON and BHR-CON at 4 months. ** $p = 0.0001$ vs WKY-CON and SHR-CON at 4 months, $p = 0.0036$ vs BHR-CON at 4 months and $p = 0.0071$ vs WKY-HS at 4 months. # $p = 0.0001$ vs SHR-CON at 4 months. • $p = 0.0001$ vs WKY-CON and BHR-CON at 12 months and $p = 0.0098$ vs 4 month old SHR-CON. ■ $p = 0.0001$ vs WKY-CON at 12 months. ▲ $p = 0.0001$ vs WKY-CON, BHR-CON and SHR-CON at 12 months.

month old WKY-CON ($p=0.0001$). The greater LV/BW in the WKY-HS compared with the WKY-CON was due primarily to significantly greater LV mass ($p=0.0028$) rather than to lower body weights in the WKY-HS (Table 2-1). The LV/BW was significantly higher in the BHR-HS at both 4 and 12 months of age compared with age matched BHR-CON ($p\leq 0.0036$) and WKY-CON ($p=0.0001$). The severity of hypertrophy was greater in the BHR-HS compared with the WKY-HS at 4 months of age ($p=0.0071$), but not at 12 months. The LV/BW was significantly higher in the 4 month old SHR-HS compared with SHR-CON of the same age ($p=0.0001$).

Creatine Kinase Activity and Isozyme Distribution

The total CK activities, and isozyme distributions are presented in Table 2-2. A sample electrophoretogram is shown in Figure 2-3. Since the separation of the MM and mitochondrial isozymes was incomplete, the sum of the two activities (MM+mito) was used in all analyses. Additionally, for simplicity, the BB and MB isozyme distributions were also represented as the sum of the two isozymes (BB+MB).

There were no significant differences in the total CK activity between strains of rats fed a control diet at 4 months of age (Figure 2-4). The total CK activity was significantly lower in 12 month old rats compared with 4 month old rats in all three strains ($p\leq 0.0003$). The 12 month old SHR had significantly lower CK activity compared with age-matched WKY-CON ($p=0.0031$). The total CK activity was not significantly lower in the 12 month old BHR-CON compared with WKY-CON at the same age.

There was no significant effect of the high salt diet on CK activity in 4 month old rats for any of the three strains (Figure 2-4). The total CK activity tended to be lower in the WKY-HS at 12 months of age compared with the WKY-CON, but the difference was

Table 2-2. CREATINE KINASE ACTIVITY AND ISOZYME DISTRIBUTION

AGE (month)	STRAIN	DIET	CK (mIU/mg) (n)	MM+MITO (%) (n)	BB+MB (%) (n)
4	WKY	CON	1733 $\pm$ 39 (10)	81.2 $\pm$ 2.3 (8)	18.8 $\pm$ 2.2 (8)
		HS	1615 $\pm$ 40 (9)	80.3 $\pm$ 1.4 (7)	19.7 $\pm$ 1.5 (7)
	BHR	CON	1697 $\pm$ 27 (10)	79.7 $\pm$ 4.3 (7)	20.3 $\pm$ 4.2 (7)
		HS	1768 $\pm$ 37 (10)	83.5 $\pm$ 1.6 (8)	16.5 $\pm$ 1.6 (8)
	SHR	CON	1644 $\pm$ 33 (10)	83.9 $\pm$ 1.9 (6)	16.1 $\pm$ 1.8 (6)
		HS	1536 $\pm$ 61 (8)	77.0 $\pm$ 1.8 (8)	23.0 $\pm$ 1.7 (8)
12	WKY	CON	1468 $\pm$ 61 ^a (8)	82.0 $\pm$ 1.1 (5)	18.0 $\pm$ 1.4 (5)
		HS	1323 $\pm$ 32 ^a (8)	79.7 $\pm$ 3.8 (7)	20.3 $\pm$ 3.8 (7)
	BHR	CON	1391 $\pm$ 56 ^a (8)	78.0 $\pm$ 2.8 (7)	22.0 $\pm$ 2.5 (7)
		HS	1422 $\pm$ 54 ^a (9)	79.4 $\pm$ 1.4 (8)	20.6 $\pm$ 1.4 (8)
	SHR	CON	1270 $\pm$ 81 ^{ab} (10)	71.3 $\pm$ 2.6 ^{ace} (9)	28.7 $\pm$ 2.6 ^{ade} (9)

Notes: Data are mean $\pm$ SEM. Probabilities are one-tailed. CK is total creatine kinase activity; n is the number of observations. MM+Mito is the sum of the MM and mitochondrial isozymes of creatine kinase. BB+MB is the sum of the BB and MB isozymes of creatine kinase.

^a p $\leq$ 0.0003 vs same strain and diet at 4 months of age.

^b p=0.0031 vs 12 month old WKY-CON.

^c p=0.0023 vs 12 month old WKY-CON.

^d p=0.0028 vs 12 month old WKY-CON.

^e p $\leq$ 0.0066 vs 12 month old BHR-HS

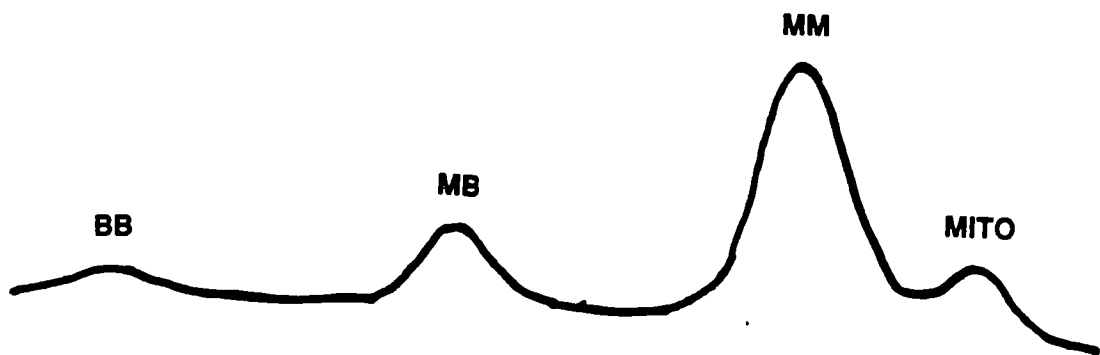


Figure 2-3. Agarose Gel Electrophoretogram of Left Ventricular Creatine Kinase. The peaks corresponding to the bands for the four CK isozymes are labeled BB, MB, MM, and Mito (mitochondrial CK). The cathode is to the right.

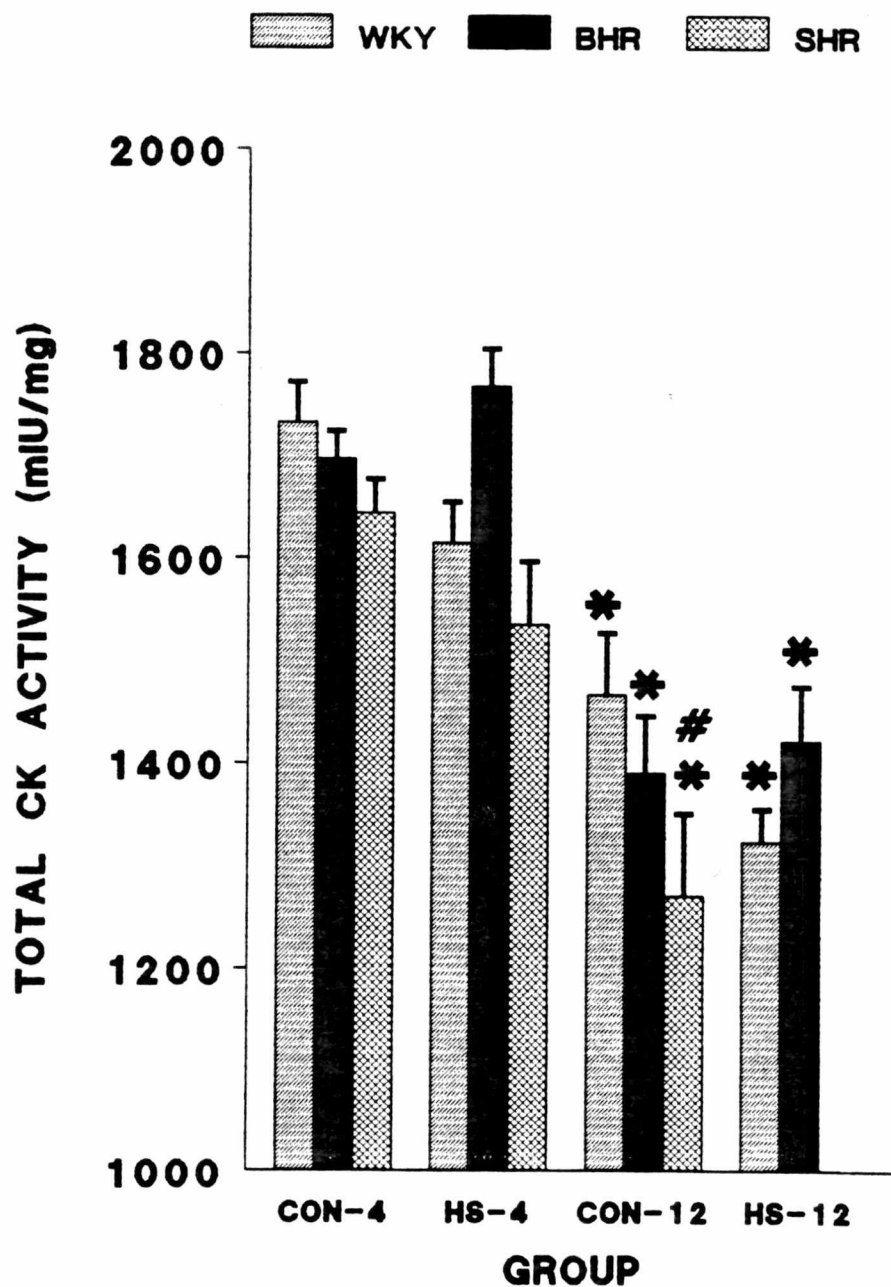


Figure 2-4. Total Creatine Kinase Activity. Bars are mean $\pm$ SEM for myocardial creatine kinase activity (CK). See Figure 1 for definition of group abbreviations. * $p < 0.0003$ vs 4 month old rats of the same strain and diet. # $p = 0.0031$ vs 12 month old WKY-CON.

not statistically significant. There was no significant difference in CK activity in the 12 month old BHR-HS compared with the 12 month old BHR-CON or WKY-CON. The total CK activity was significantly lower in 12 month old rats compared with 4 month old rats for all three strains of rats fed a high salt diet ($p=0.0001$).

There were no significant differences in the relative distribution of CK isozymes between strains of rats on a normal diet at 4 months of age (Figure 2-5,2-6). The isozyme profiles for the WKY-CON and BHR-CON were also similar in 12 month old rats, and the isozyme profiles in these strains did not change with age. In contrast, the percentage of BB+MB isozymes increased significantly in the 12 month old SHR-CON compared with 4 month old SHR-CON ($p=0.0003$), and the percentage of MM+mito isozymes decreased proportionally ($p=0.0003$). Additionally, the percentage of BB+MB isozymes was significantly higher ($p\leq 0.0064$), and the percentage of MM+mito isozymes was lower ($p\leq 0.0066$) in the 12 month old SHR-CON compared with both age matched WKY-CON and BHR-HS. The high salt diet did not significantly affect the CK isozyme distribution at either age in any strain.

D. DISCUSSION

Mean Arterial Pressure

The development of frank hypertension in BHR fed a high NaCl diet has been established previously by Lawler and others (137,138). The MAP in the 4 month old BHR-HS in the present study was significantly higher compared with age matched WKY-CON and WKY-HS. Although the MAP in 12 month old BHR-HS was still elevated significantly compared with the WKY-CON, the MAP was not higher in the 12 month old BHR-HS

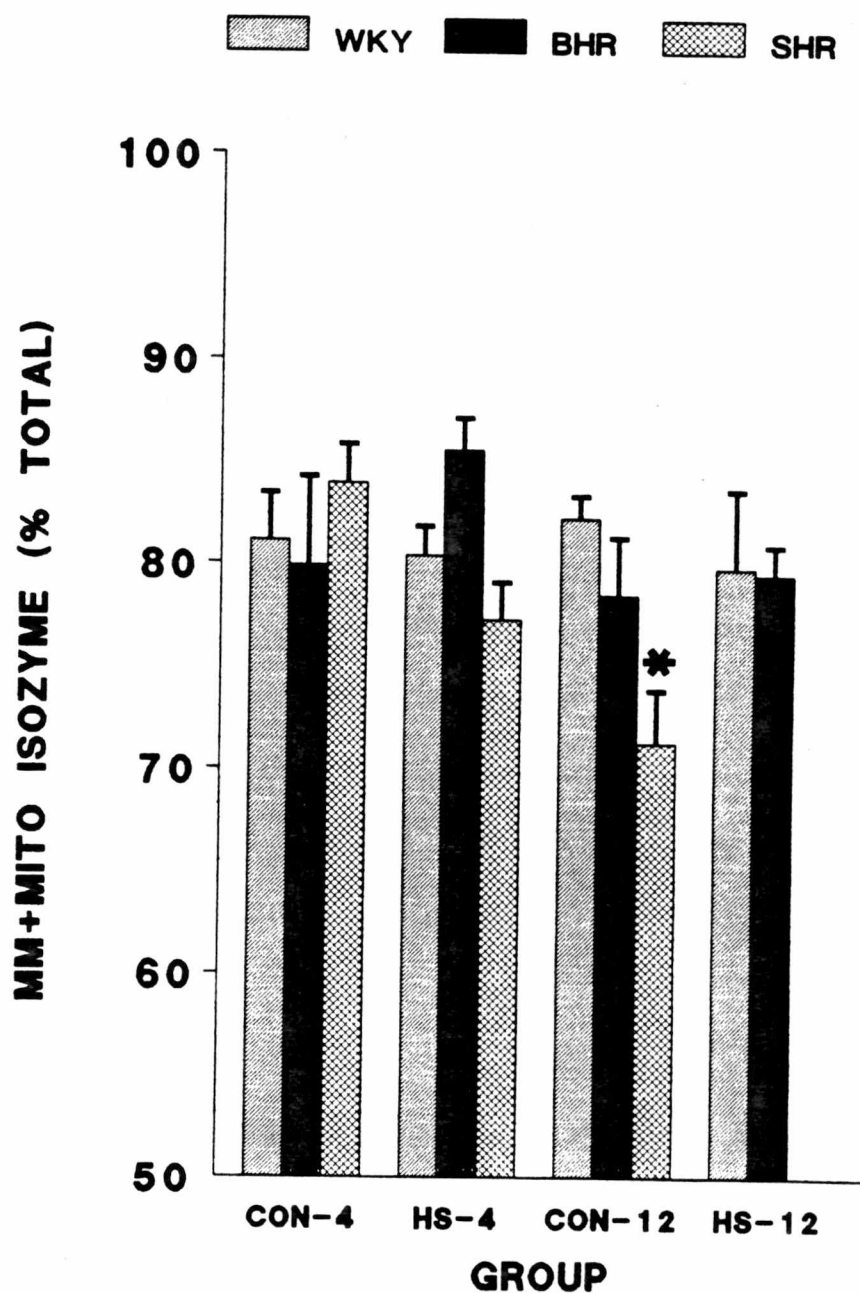


Figure 2-5. Expression of MM and Mitochondrial Isozymes of Creatine Kinase. Bars are mean $\pm$ SEM for the sum of the MM and mitochondrial isozymes (MM+mito) of creatine kinase. See Figure 1 for definition of group abbreviations. * $P=0.0003$ vs 4 month old SHR-CON, $p=0.0023$ vs 12 month old WKY-CON and $p=0.0066$ vs 12 month old BHR-HS.

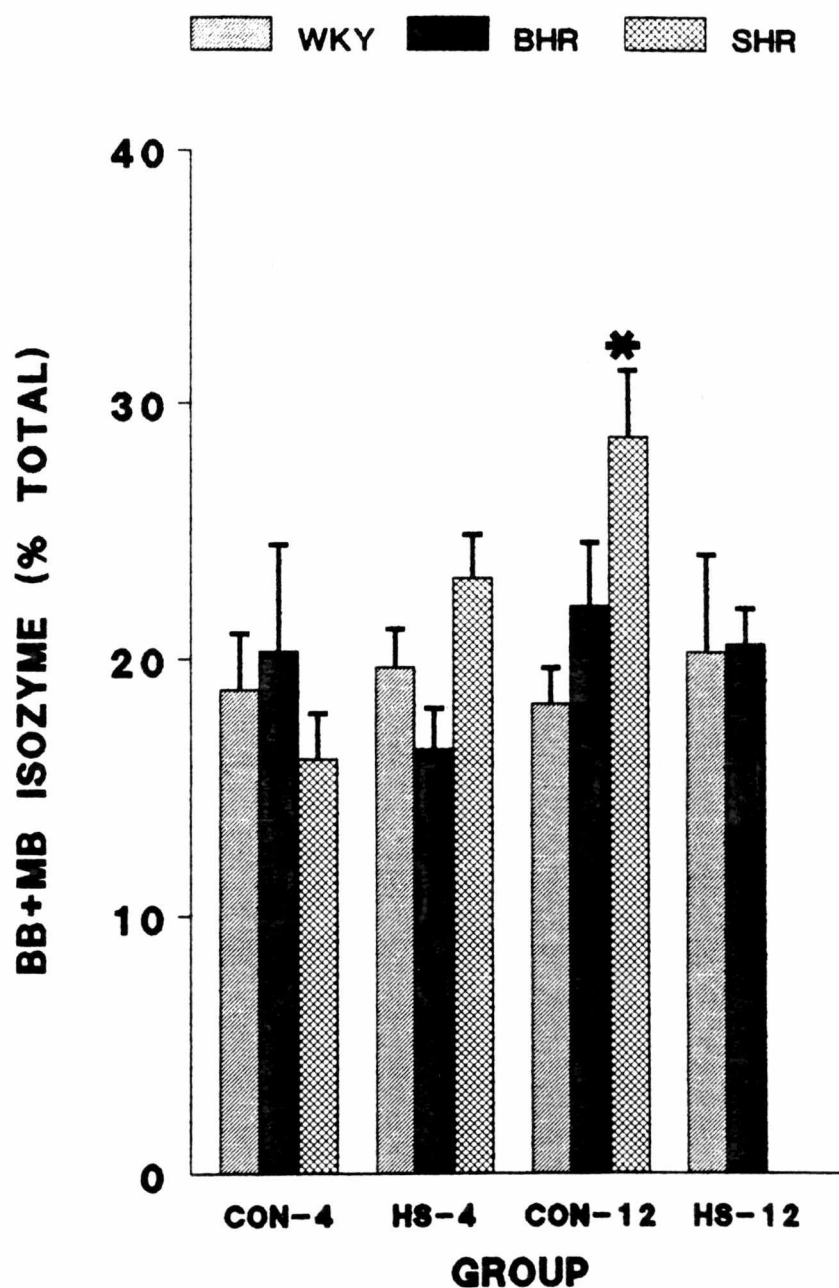


Figure 2-6. Expression of BB and MB Isozymes of Creatine Kinase. Bars are mean $\pm$ SEM for the sum of the BB and MB isozymes of creatine kinase (BB+MB). See Figure 1 for a definition of group abbreviations. * $p = 0.0003$ vs 4 month old SHR-CON, $p = 0.0028$ vs WKY-CON and $p = 0.0064$ vs 12 month old BHR-HS.

compared with the 4 month old BHR-HS. In addition, the MAP was significantly lower in the BHR-HS compared with the SHR-CON at 12 months of age. These observations indicate that the severity of hypertension did not increase with age in the BHR-HS, and that the elevation in blood pressure was less severe in the older BHR-HS compared with SHR on a normal sodium diet. Thus, the elevation in arterial pressure in the older BHR-HS remained moderate compared with the severity of hypertension observed in other rat models of hypertension including the SHR (2,61,137) and DOCA-salt rat (27,41).

Left Ventricular Hypertrophy

Lawler and others (138) had previously observed significant hypertrophy in 39 week old BHR subjected to behavioral stress. The results of the present study indicate significant hypertrophy was also induced in BHR in which hypertension was induced with a high NaCl diet. Myocardial hypertrophy was detectable in BHR-HS as young as 4 months old, and after 8 weeks on the 8% NaCl diet. Significant hypertrophy was maintained in 12 month old BHR-HS, but the severity of the hypertrophy was not increased significantly with age in this strain.

The severity of hypertrophy was significantly greater in the SHR-CON compared with the WKY-CON, BHR-CON and BHR-HS at both 4 and 12 months of age. Additionally, unlike the severity of hypertrophy in the BHR, the LV/BW in the SHR-CON did increase significantly with age. The LV/BW was 62% higher in the 12 month old SHR-CON hearts compared with age matched WKY-CON. The severity of hypertrophy in these SHR hearts is similar to that reported previously for 12 month old SHR (61).

An interesting, and unexpected, finding was that the 12 month old WKY-HS developed significant hypertrophy compared with WKY-CON. The LV/BW was 23% higher

in the WKY-HS compared with WKY-CON at 12 months of age. This was comparable to the amount of hypertrophy observed in the 4 month old BHR-HS. The significant increase in the LV/BW in the 12 month old WKY-HS was due primarily to a significantly greater left ventricular weight rather than to a lower body weight. This finding indicates that ingestion of a high NaCl diet for a prolonged period may produce left ventricular hypertrophy independently of the development of hypertension.

Ventricular weight has been shown to be proportional to body weight (142). Furthermore, ventricular weight referenced to body weight was shown to be a superior predictor of ventricular hypertrophy compared with wall thickness, ventricular weight alone, or ventricular weight referenced to body surface area or body length in hearts at necropsy (142). These observations are the basis for using the LV/BW as an index of left ventricular hypertrophy in the present study. However, it should be noted that the LV/BW may overestimate the severity of hypertrophy when hearts are compared between groups of animals with systematically different body weights (143-144).

The observed increase in LV/BW in the rats fed a high salt diet was due to both greater left ventricular weights and lower body weights in these rats. Lower body weights were particularly important in the 4 month old SHR-HS and the 12 month old BHR-HS in which the high salt diet produced significantly lower body weights compared with control rats of the same strain. The lower body weights in the rats fed the high salt diet may represent caloric restriction in these rats relative to controls, presumably due to either reduced palatability of the high salt rat chow, or reduced appetite in the hypertensive rats.

Previous studies have indicated that the heart weight to body weight ratio may increase during severe caloric restriction because the weight of the heart is better

preserved than whole body weight (143-144). Therefore, these data suggest that the LV/BW may overestimate the severity of hypertrophy in rats in which the high salt diet resulted in reduced body weights. However, the caloric restriction in the studies described above promoted severe decreases in body weight of 26-42% (143-144) that are large compared with the maximal difference of 14% observed in the body weights of the high salt rats compared with controls in the present study. The importance of the effect of caloric restriction on the estimation of the severity of hypertrophy using LV/BW in the rats fed a high salt diet would require additional studies in which the control rats were subjected to the same degree of caloric restriction. Although the importance of lower body weights on the estimation of the severity of hypertrophy should be considered, the observation that the LV/BW was significantly greater in the 4 month old BHR-HS which had body weights similar to the control rats indicates that the high salt diet did induce left ventricular hypertrophy.

Creatine Kinase

There were no significant differences in either total CK activity or the distribution of CK isozymes in the BHR-CON or BHR-HS compared with WKY-CON and WKY-HS at 4 and 12 months of age. In contrast, the total CK activity was significantly lower in 12 month old SHR-CON compared with age matched WKY-CON. Similar reductions in creatine kinase activity have previously been reported in hypertrophied human hearts (63), aortic banded rat hearts (72) and hearts from rats with renal hypertension (71). The observed decrease in creatine kinase activity with age in all groups has also been observed previously (145).

The relative increase in the sum of MB and BB isozymes of CK and decrease in

MM and mitochondrial isozymes in 12 month old SHR-CON is consistent with earlier studies using the SHR (67) and other models of pressure overload hypertrophy (63,67,71,72). It has been proposed that the increased expression of the MB and BB isozymes of CK may represent one of the changes that signals an increased dependence on, and perhaps capacity to recruit, anaerobic glycolysis by hypertensive and hypertrophied hearts (26,71).

In conclusion, the BHR developed significant, but minimal, hypertrophy at 4 months of age after 2 months on a high salt diet. The severity of hypertrophy did not increase significantly with age in these rats. Furthermore, decreases in CK activity and shifts in the isozyme distribution that are characteristic of other models of pressure overload hypertrophy did not occur in the BHR hearts studied. Therefore, the BHR-HS may not be an appropriate model for studying the progression of hypertrophy from the early developmental stage to stages of severe hypertrophy and failure. The BHR may, however, prove to be a good model for studying the early stages of hypertrophy, and the effects of mild, stable hypertension on heart function.

CHAPTER 3

PRELIMINARY PERFUSION STUDIES WITH THE BHR

A. INTRODUCTION

Hypertrophied hearts from hypertensive human patients and animal models of hypertension have an increased sensitivity to ischemic dysfunction and injury (26,27,33-48). Hypertrophied hearts are particularly susceptible to diastolic dysfunction during ischemia and hypoxia (27,33,41-43). Lorell and others (43) were able to detect a greater increase in left ventricular stiffness during ischemia in hearts from hypertensive rabbits with only minimal hypertrophy. Similarly, hypertensive human patients often exhibit diastolic dysfunction including abnormal left ventricular filling rates (132,133). Impaired diastolic function has been proposed as an early indicator of cardiac involvement in hypertension (134).

Diastolic dysfunction may result from either changes in passive diastolic properties which affect chamber compliance, or from changes in myocardial relaxation (41). Myocardial relaxation requires removal of calcium from the cytoplasm and the dissociation of cross-bridges (51). The cytoplasmic calcium level may be reduced by the energy dependent transport of calcium into the sarcoplasmic reticulum or across the sarcolemma by distinct Ca^{2+} -ATPases at these sites (51,99). Alternatively, calcium can be removed from the cell via Na^{+} - Ca^{+2} exchange (100).

There is substantial evidence that an increase in intracellular Na^{+} occurs during myocardial ischemia (101,102,106,109). The increase in Na^{+} may subsequently promote increased cellular uptake of Ca^{2+} , presumably via Na^{+} - Ca^{2+} exchange, and result in Ca^{2+} overload (101,102,108). Since myocardial relaxation is dependent on the reduction

of cytoplasmic Ca^{2+} , diastolic function should be particularly sensitive to a Ca^{2+} overload during ischemia.

Hypertrophied hearts may have a decreased capacity for Ca^{2+} sequestration by the sarcoplasmic reticulum (92-97), and a greater dependence on Na^{+} - Ca^{2+} exchange for normal Ca^{2+} homeostasis (86,98). Sodium-calcium exchange functions to transport Na^{+} and Ca^{2+} in both directions across the sarcolemma depending on the electrochemical gradients for Na^{+} and Ca^{2+} (100). Therefore, an increased dependence on Na^{+} - Ca^{2+} exchange for Ca^{2+} removal would be detrimental during ischemia because elevated intracellular Na^{+} would inhibit the removal of Ca^{2+} via Na^{+} - Ca^{2+} exchange, and perhaps even promote increased Ca^{2+} uptake by this mechanism. Therefore, changes in Na^{+} and Ca^{2+} handling may potentiate the development of a Ca^{2+} overload during ischemia, and predispose hypertrophied hearts to greater ischemic dysfunction and injury. Diastolic dysfunction during ischemia may be a particularly sensitive indicator of altered cation homeostasis in hypertrophied hearts.

The primary objective of the preliminary studies described in this section was to develop an ischemic protocol that could be used to determine whether increased diastolic dysfunction in hypertrophied hearts is related to a greater increase in intracellular Na^{+} during ischemia compared with control hearts. More specifically, the objective was to develop a protocol that would promote an increase in left ventricular end diastolic pressure under ischemic conditions, and would also permit measurement of intracellular Na^{+} . Regulation of intracellular Na^{+} levels by the Na^{+} - K^{+} ATPase, and maintenance of normal Ca^{2+} transients by the Ca^{2+} -ATPase in the sarcoplasmic reticulum are both energy dependent processes. Therefore, high energy phosphates were also monitored. A second objective of these studies was to determine whether there was evidence that

hypertrophied hearts from Borderline Hypertensive Rats (BHR) fed an 8% NaCl diet exhibited greater diastolic dysfunction during demand ischemia than nonhypertrophied hearts from BHR fed a normal diet.

B. METHODS

Rats

The BHR rats were bred by mating male WKY with female SHR using protocols previously described in detail by Lawler and others (137,138). The male BHR offspring were weaned at 21 days and thereafter housed two to a cage. At 8 weeks of age rats were randomly assigned to either a high NaCl (8%) diet (BHR-HS) or a control diet of normal (0.3%) NaCl rat chow (BHR-CON). Isolated heart experiments were done using 4 or 9 month old BHR-CON and BHR-HS. Two days prior to the isolated heart experiment, rats were anesthetized with methoxyflurane, and an indwelling catheter was surgically placed in the femoral artery. Direct blood pressures were measured in awake, unsedated rats on the day before the isolated heart experiment.

Isolated Heart Preparation

The isolated, isovolumic heart preparation was used in all preliminary protocols. A schematic of the apparatus used for constant flow perfusion of hearts during collection of functional and NMR data is shown in Figure A-1 of the Appendix. Prior to isolation of the heart, rats were injected with heparin (100 units/Kg IP) and anesthetized with pentobarbital (60mg/Kg IP). Hearts were rapidly excised, arrested with cold isotonic saline (4°C), and cannulated via the aortic root for retrograde coronary perfusion. Hearts

were perfused using a non-recirculating system at a constant coronary flow of approximately 15 mls/min/g wet weight. Coronary perfusion pressure (CPP) was monitored through the aortic cannula using a P23XL pressure transducer (Spectramed, Oxnard CA).

All hearts were initially perfused with a modified Krebs-Henseleit buffer with the following composition: 118 mM NaCl, 24 mM NaHCO₃, 4.7 mM KCl, 1.2 mM MgSO₄, and 0.5 mM Na₂EDTA. The perfusate also contained CaCl₂ and energy substrates at concentrations that were modified for the 4 different protocols. The free [CaCl₂] was approximately 1 mM for protocols 1 and 2, and 1.5 mM for protocols 3 and 4. The perfusate for protocols 1 and 2 contained 11 mM glucose and 2 mM pyruvate; the perfusate for protocol 3 contained 11 mM glucose, and the perfusate for protocol 4 contained 5.5 mM glucose and 1 mM lactate. The perfusate for all protocols was equilibrated with 95% O₂ and 5% CO₂ to produce a pH of 7.4 to 7.45.

For the hearts in protocols 1 and 2, the perfusate was changed to buffer containing 5 mM of the ²³Na NMR shift reagent Na₅Tm(DOTP) immediately after the hearts were placed in the magnet. The Na₅Tm(DOTP) buffer was identical to the original perfusate except that the NaCl was decreased to maintain the total [Na⁺] at 142 mM, and the total [Ca²⁺] was increased to 6.5 mM to maintain the free [Ca²⁺] at approximately 1 mM in the presence of significant chelation of Ca²⁺ by the shift reagent. Measurement of the perfusate free [Ca²⁺] using an ion selective electrode in a few of the hearts was used to establish that the free [Ca²⁺] was 1 mM with the addition of 6.5 mM total Ca²⁺.

To facilitate pacing, the right atrium was removed, and the AV node was crushed. Platinum pacing electrodes were attached to the right ventricle, and connected to a constant voltage stimulator (Stoelting, Chicago IL) via a KCl-agar salt bridge (protocols

1-2) or insulated wire (protocols 3-4). Hearts were initially paced at 4 Hz using a voltage just above the threshold voltage.

A drain was placed in the apex of the LV to allow drainage of the thebesian veins, and a small fluid filled latex balloon was placed in the left ventricle through the mitral valve. The balloon was connected to a P23XL pressure transducer (Spectramed, Oxnard CA) via polyethylene tubing for measurement of left ventricular pressure. Left ventricular peak systolic, and end diastolic pressures were recorded continuously using an analog physiologic recorder (Astromed, West Warwick RI; Gould, Cleveland Ohio). Left ventricular developed pressure (LVDEVP) was calculated as the difference between left ventricular peak systolic pressure and left ventricular end diastolic pressure (LVEDP). Hearts subjected to protocols 1 and 2 were submerged in buffer inside a 20mm NMR tube which was connected to the perfusion apparatus via a water-jacketed umbilical that allowed hearts to be perfused at 37°C while inside the magnet. No NMR data were collected for hearts subjected to protocols 3 and 4. These hearts were perfused using a similar perfusion apparatus except that hearts were submerged in a water jacketed chamber instead of an NMR tube, and the tubing connecting the left ventricular balloon to the pressure transducer was shortened to 15 cm.

NMR Spectroscopy

The NMR spectra were obtained using an 8.5 Tesla vertical bore superconducting magnet and a Bruker AM-360 spectrometer (Billerica MA). The ^{23}Na and ^{31}P spectra were acquired using a single home-built, temperature controlled probe. The probe design included two orthogonal saddle coils on separate circuits that were connected to separate dedicated preamplifiers. One coil was tuned to 145.78 MHz for ^{31}P and the

second coil simultaneously tuned to 95.25 MHz for ^{23}Na . The magnet was shimmed prior to data acquisition to optimize field homogeneity using the ^{23}Na signal.

The ^{23}Na spectra were obtained by averaging 336, 90° excitation pulses which were collected using an acquisition time of 0.154 seconds, with no additional relaxation delay, and a dead time of 96.3 μsec . Data were accumulated over a one minute period using 2K data points and a spectral width of 6667 Hz. The recycle time was more than 5 times greater than the T_1 (20-30 ms), estimated by inversion recovery, for both the intracellular and extracellular Na^+ resonances. Therefore, the pulsing conditions did not produce saturation of the ^{23}Na resonances. The shift reagent $\text{Tm}(\text{DOTP})^{5-}$ was used to shift the extracellular Na^+ resonance and allow resolution of the intracellular and extracellular Na^+ peaks (146). The free-induction decays (FIDs) were multiplied by a Gaussian function to enhance resolution of the intracellular resonance. The Gaussian function was introduced using a standard Bruker Gaussian multiplication (GM) subroutine with gaussian broadening (GB) = 0.1 Hz and line broadening (LB) = -10 Hz. The area of the intracellular ^{23}Na resonance was determined using a hand-held digitizer.

The ^{31}P spectra were obtained by accumulating a total of 56, 60° excitation pulses which were collected using an acquisition time of 0.145 seconds, and an interpulse delay of 1.85 seconds over a two minute period. Data were accumulated using 2K data points and a spectral width of 7042 Hz. Exponential multiplication of the FID corresponding to a line broadening of 40 Hz was used to improve the signal-to-noise ratio. Peak areas for phosphocreatine (PCr), adenosine triphosphate (ATP), and inorganic phosphate (P_i) were multiplied by 1.32, 1.10 and 1.27, respectively, to correct for the saturating effects of repetitive pulses. These saturation factors were determined by comparing peak resonances acquired using the experimental relaxation delay with fully

relaxed resonances acquired using a relaxation delay of 10 seconds. The intracellular pH was determined from the shift of the P_i with respect to the PCr resonance (144). The ^{31}P spectra were interleaved automatically with ^{23}Na such that the first minute of each 3 minute interval was used to acquire one ^{23}Na spectrum, and the last two minutes were used to acquire one ^{31}P spectrum. The ^{31}P resonances were curve fit to a Lorentzian lineshape, and automatically integrated using an NMR-1TM (New Methods in Research INC, Syracuse NY) software package.

Demand Ischemia Protocols

All hearts were studied using one of the four protocols described below. Protocols 1 and 2 were compared to assess the effects of a greater reduction in flow during demand ischemia. Protocol 3 was done to assess the effects of increasing the free extracellular $[\text{Ca}^{2+}]$ from 1 to 1.5 mM. Protocol 4 was done to evaluate the effects of a combination of higher extracellular Ca^{2+} and a lower glucose concentration.

Protocol 1: The perfusate free $[\text{Ca}^{2+}]$ was approximately 1 mM, and contained 11 mM glucose and 2 mM pyruvate as energy substrates. Hearts were perfused for a 20 minute control period at a coronary flow of 15 mls/min/g, and were paced at a heart rate of 240 bpm. The heart rate was increased to 360 bpm for 6 minutes, and then the flow was decreased to 20% of the control flow at the higher heart rate for 22 minutes of demand ischemia. Demand ischemia was followed by 20 minutes of reperfusion at the control heart rate and flow.

Protocol 2: The free $[\text{Ca}^{2+}]$, glucose and pyruvate concentrations were the same as those used in protocol 1. However, the coronary flow was decreased to 10% of the control flow, and the duration of demand ischemia was also reduced to 11 minutes. The

protocol was otherwise identical to protocol 1.

Protocol 3: The free CaCl_2 was increased to 1.5 mM, and the perfusate contained only 11 mM glucose as an energy substrate. The protocol was otherwise the same as protocol 1.

Protocol 4: This protocol was identical to protocol 3 except that the perfusate glucose concentration was reduced to 5.5 mM and supplemented with 1 mM lactate.

Data Analysis

Data were expressed as mean $\pm$ SEM. Insufficient data were collected to allow statistical comparisons of changes in Na^+ , high energy phosphates and pH between protocols. The increase in LVEDP was compared between protocols using a one-way analysis of variance. The effects of age and the high salt diet on mean arterial pressure, left ventricular weight, body weight, and left ventricular weight to body weight ratio were determined using a two-way analysis of variance with diet and age as factors.

C. RESULTS

Left Ventricular Function

The actual perfusion conditions for each protocol are given in Table 3-1. The mean increase in LVEDP during demand ischemia and reperfusion for each of the 4 protocols are presented in Figure 3-1. Changes for individual hearts are shown in Table 3-2. The decrease in flow during demand ischemia resulted in an initial decrease in the LVEDP at the beginning of ischemia due to a loss of turgor in the coronary arteries. Therefore, changes in LVEDP at the end of demand ischemia were determined with reference to the

Table 3-1. PERFUSION CONDITIONS FOR PRELIMINARY PROTOCOLS

PR #	n	HR-CON (bpm)	HR-ISC (bpm)	Q-CON ^a ml/min/g	Q-ISC ^a ml/min/g	TIME-ISC (mins)	PER
1	4	240	360	15.1 ± 1.5	3.6 ± 0.5	22	1
2	6	240	360	14.6 ± 0.8	1.8 ± 0.2	11	1
3	4	240	360	14.4 ± 1.0	3.0 ± 0.7	22	2
4	8	240	360	18.0 ± 1.1	4.0 ± 0.8	22	3

NOTES: PR is protocol. N is the number of hearts. HR-CON is the control heart rate. HR-ISC is the ischemia heart rate. Q-CON is the control flow. Q-ISC is the ischemia flow. Time-ISC is the duration of demand ischemia. PER is the perfusate energy substrate and Ca²⁺ composition. PER1 contained 11 mM glucose, 2 mM pyruvate and 1 mM free Ca²⁺. PER2 contained 11mM glucose and 1.5 mM free Ca²⁺. PER3 contained 5.5 mM glucose, 1 mM lactate and 1.5 mM Ca²⁺.

^a Data are mean ± SEM.

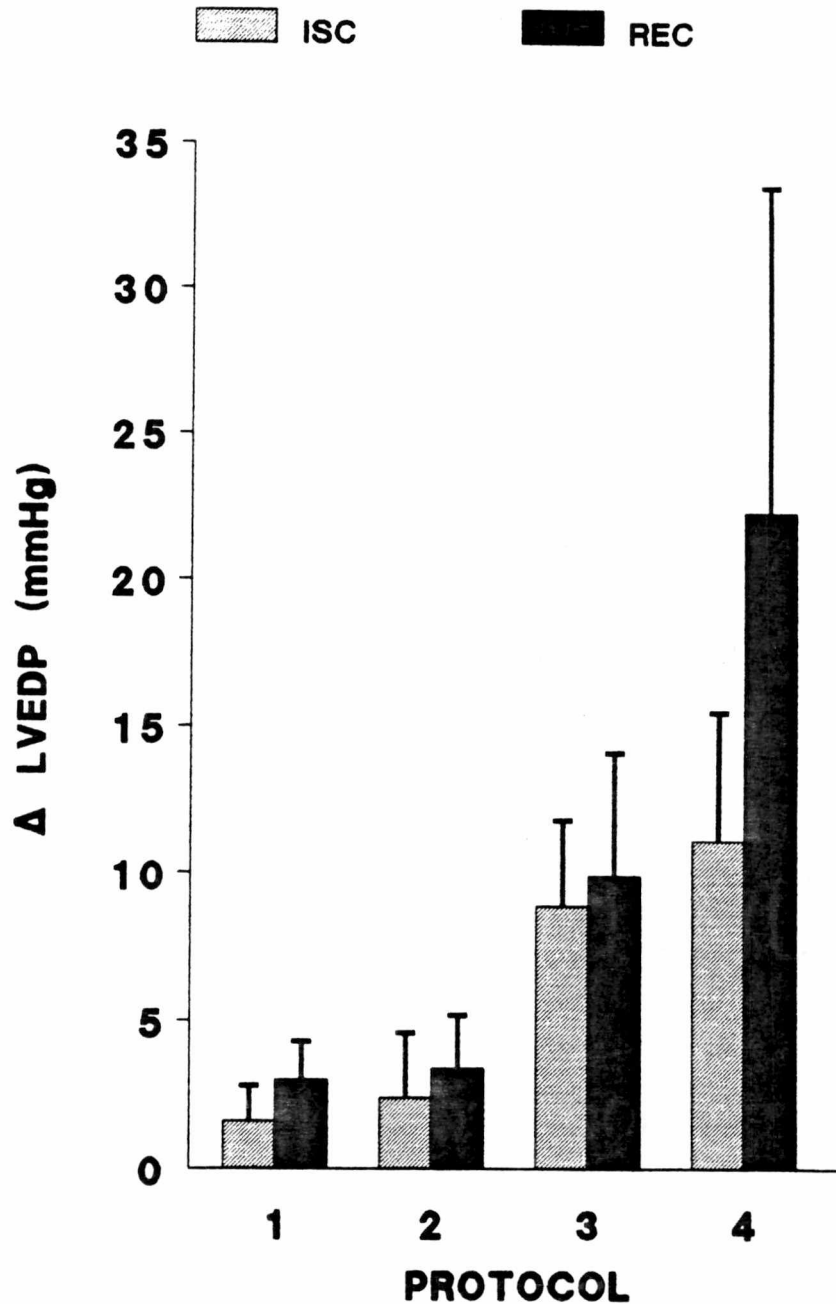


Figure 3-1. Increase in Left Ventricular End Diastolic Pressure (LVEDP) During Demand Ischemia and Reperfusion. Bars are mean $\pm$ SEM for the increase in LVEDP at end ischemia (ISC) and end reperfusion (REC). The control LVEDP was 14 ± 2.3 , 14 ± 2.6 , 10 ± 0.9 and 8 ± 0.7 for protocols 1-4, respectively. The increase in LVEDP at end reperfusion was calculated with respect to the control pressure. Since the loss of coronary turgor at the onset of ischemia promoted an initial decrease in LVEDP, the increase at end ischemia was calculated with respect to the LVEDP during the initial minute of ischemia. See text for a description of protocols.

Table 3-2. INDIVIDUAL HEART DATA FOR PRELIMINARY EXPERIMENTS

PR #	RAT #	DIET	AGE weeks	MAP mmHg	LV/BW g/kg	Δ LVEDP mmHg	PCr %con	Na %con	pH
1	15	HS	16	-	0.54	+ 1.5	62	-	7.08
1	16	CON	16	117	0.48	0	32	-	7.01
1	65	CON	16	136	0.45	+ 5.0	46	135	6.87
1	64	HS	16	143	0.56	0	41	134	7.08
2	50	CON	16	125	0.43	+33.0	20	-	6.93
2	42	HS	24	138	0.61	+ 6.0	22	-	6.73
2	63	HS	16	125	0.53	0	0	124	6.81
2	251	HS	16	130	0.49	+ 5.0	4	192	6.82
2	72	CON	16	131	0.43	0	0	184	6.55
2	252	HS	16	144	0.52	+ 1.0	20	165	6.89
3	60	CON	36	128	0.45	+10.0			
3	61	CON	36	129	0.42	+ 5.5			
3	20	HS	36	-	0.45	+ 3.5			
3	21	HS	36	-	0.46	+16.5			
4	57	CON	35	122	0.43	+ 6.0			
4	58	CON	36	118	0.48	+17.0			
4	59	CON	36	126	0.40	+11.0			
4	52	HS	37	-	0.48	+ 4.0			
4	56	HS	35	144	0.54	+15.0			
4	62	HS	35	-	0.45	+13.5			
4	70	HS	36	165	0.51	+ 9.5			
4	71	HS	34	154	0.47	+12.5			

NOTES: PR is protocol. MAP is mean arterial pressure. LV/BW is the ratio of left ventricular dry weight to body weight. Δ LVEDP is the maximal change in LVEDP during demand ischemia. PCr is the lowest value of phosphocreatine content during demand ischemia. Na^+ is the maximal value of intracellular Na^+ content during demand ischemia. pH is the minimal value of pH during demand ischemia. CON is control diet. HS is 8% NaCl diet.

LVEDP measured during the first minute of ischemia. Changes in LVEDP at end reperfusion were determined with respect to the control value.

The maximal increase in LVEDP during demand ischemia was small for protocols 1 and 2 (Figure 3-1). Data for individual hearts in Table 3-2 show that several hearts subjected to either protocol 1 or protocol 2 exhibited no increase in LVEDP during demand ischemia. The BHR-HS and BHR-CON responded similarly to the two protocols. Therefore, protocols 1 and 2 were not successful in producing a consistent increase in LVEDP during ischemia. Note that one heart from protocol 2 (#50) exhibited a large increase in LVEDP during demand ischemia of 33 mmHg, and the LVEDP continued to increase during reperfusion in this heart. There was no change in coronary perfusion pressure or any other variable to account for the greater increase in LVEDP in this heart. Since heart #50 was an extreme observation, the LVEDP data for the heart were not used to calculate the mean change during ischemia and reperfusion for protocol 2.

In contrast with protocols 1 and 2, the protocols which utilized a higher perfusate $[Ca^{2+}]$ (protocols 3 and 4) did promote a moderate increase in LVEDP during demand ischemia (Figure 3-1). The data in Table 3-2 indicate that there was a detectable increase in LVEDP in every heart subjected to either protocol 3 or 4. There was no significant difference in the increase in LVEDP in protocol 4 compared with protocol 3. The mean increase in LVEDP for the hearts in protocols 3 and 4 was significantly higher than the mean increase for the hearts in protocols 1 and 2 ($p=0.0004$). The increases in LVEDP for hearts in protocols 3 and 4 were averaged in order to determine whether diastolic dysfunction was related to the development of hypertrophy or hypertension. The mean increase in LVEDP was similar in the BHR-CON (9.9 ± 2.1 mmHg) and the BHR-HS (10.6 ± 2.0) subjected to protocols 3 and 4.

There was a measurable sustained increase in LVEDP at the end of reperfusion for all four protocols (Figure 3-1). However, protocols 3 and 4 promoted a much greater sustained increase in LVEDP than either protocol 1 or protocol 2. The sustained increase in LVEDP was greatest for the protocol (#4) that combined a higher perfusate $[Ca^{2+}]$ with a lower glucose concentration.

The mean LVDEVPs at the end of ischemia and end reperfusion are shown in Figure 3-2. The decrease in developed pressure was substantial for all protocols. The decrease in LVDEVP was most severe in hearts perfused at the lowest coronary flow (protocol 2) during demand ischemia. The recovery of LVDEVP was complete, or nearly complete, for hearts in all protocols except for protocol 4.

Intracellular Na^+

The changes in intracellular Na^+ content at the end of demand ischemia and at end reperfusion with respect to control are shown in Figure 3-3 for hearts subjected to protocols 1 and 2. The higher perfusate $[Ca^{2+}]$ used for protocols 3 and 4 precluded the use of the ^{23}Na shift reagent $Tm(DOTP)^{5-}$. Therefore, no Na^+ data were obtained during these protocols.

There was a measurable increase in intracellular Na^+ content during ischemia in all hearts for which Na^+ data were obtained (Table 3-2). There was no detectable change in Na^+ with the increase in heart rate prior to the decrease in flow. The increase in Na^+ was apparently somewhat higher in hearts subjected to protocol 2 in which the coronary flow during demand ischemia was approximately half that used in protocol 1 (Figure 3-3). However, Na^+ data were measured in an insufficient number of hearts to allow meaningful statistical comparisons. There was no apparent relationship between diet and

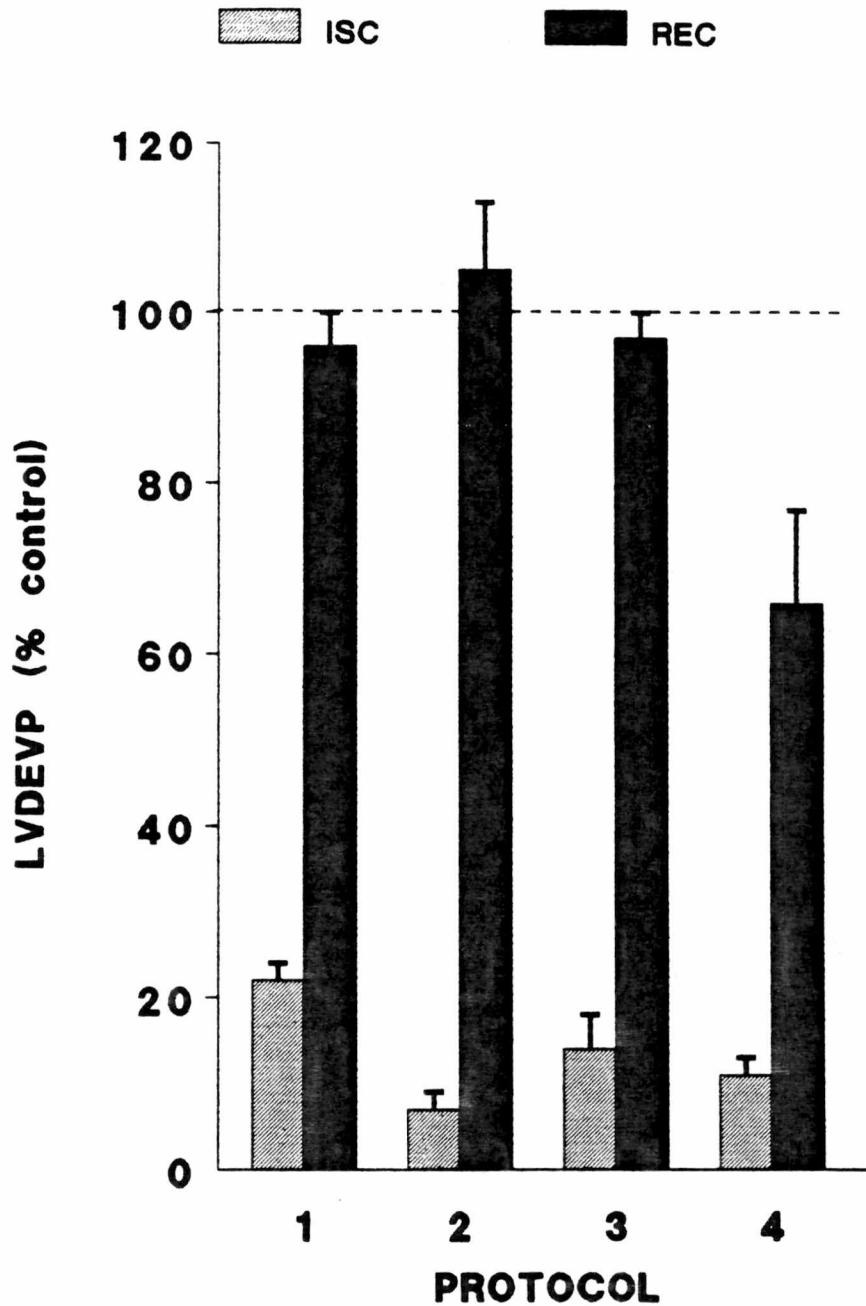


Figure 3-2. Changes in Left Ventricular Developed Pressure (LVDEVP) During Demand Ischemia and Reperfusion. Bars are mean $\pm$ SEM at end ischemia (ISC) and following reperfusion (REC). The dashed, horizontal line represents the control value. The control LVDEVP was 52 ± 10 , 57 ± 9 , 127 ± 12 , and 136 ± 5 for protocols 1-4, respectively. See text for a description of protocols.

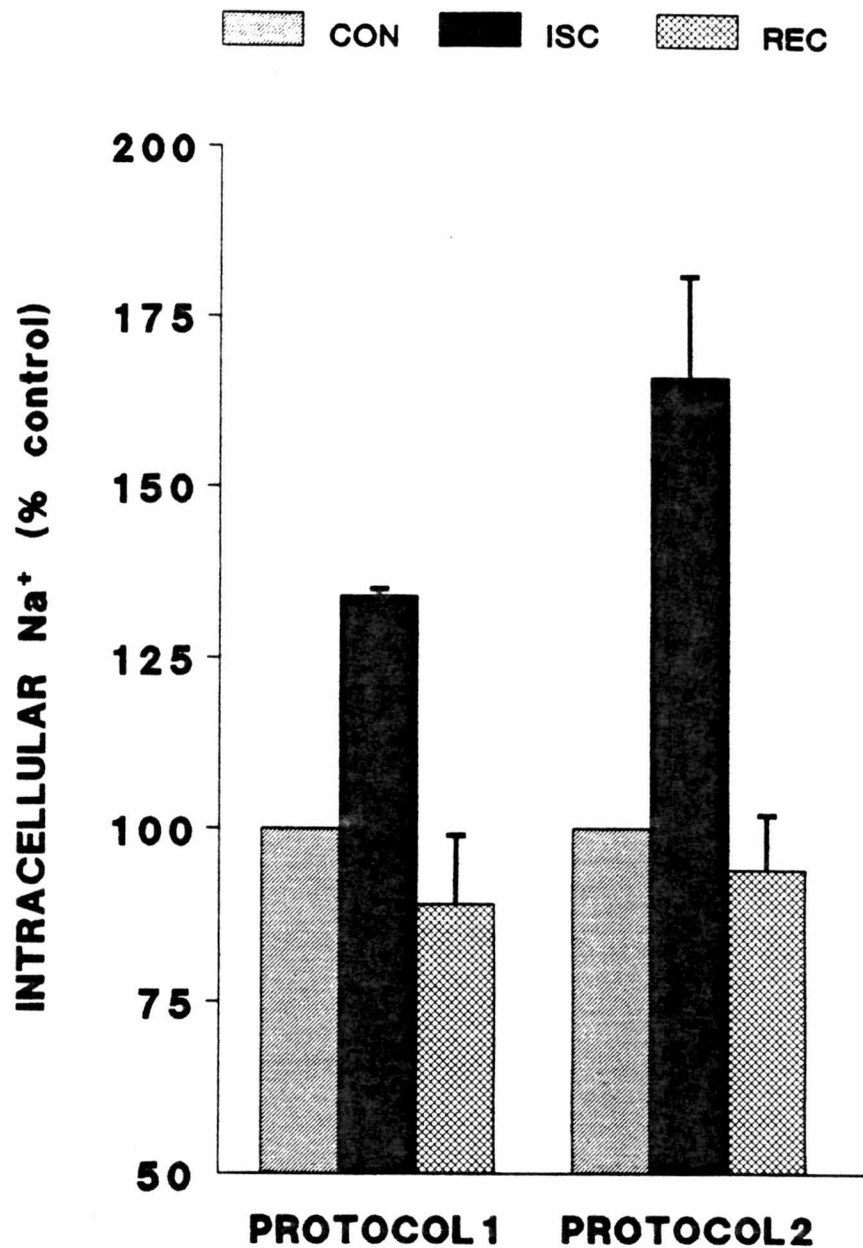


Figure 3-3. Changes in Intracellular Na⁺ During Demand Ischemia and Reperfusion. Bars are mean $\pm$ SEM for control (CON), end ischemia (ISC), and end reperfusion (REC). See text for a description of protocols.

the magnitude of the increase in Na^+ for the 6 hearts in which Na^+ was measured (Table 3-2). The sodium recovered rapidly to the control value during reperfusion in all hearts. The mean intracellular Na^+ content at the end of reperfusion was lower than the control value for both protocol 1 and protocol 2.

PCr and pH

The mean phosphocreatine (PCr) contents at the end of demand ischemia and at end reperfusion are shown in Figure 3-4 for protocols 1 and 2. No ^{31}P NMR data were collected during protocols 3 and 4. End ischemia PCr data for individual hearts are shown in Table 3-2. Both protocols produced a substantial decrease in the PCr content. The decrease in PCr, like the increase in Na^+ , tended to be greater in hearts subjected to the lower ischemic flow during protocol 2. At the onset of reperfusion, the PCr content increased rapidly towards control values, and the recovery of PCr was complete or nearly complete for the hearts in both protocols.

The mean intracellular pH during control perfusion, at the end of demand ischemia, and at the end of reperfusion are shown in Figure 3-5. The end ischemic pH data are presented in Table 3-2 for individual hearts. The decrease in pH during demand ischemia was greater for protocol 2 in which the coronary flow during ischemia was half that used in protocol 1. The pH returned to the control value during reperfusion for both protocols.

Characteristics of Hearts

The mean arterial pressure (MAP) and left ventricular weight to body weight ratio (LV DRY/BW) are given in Table 3-3 for the BHR used in the studies described above.

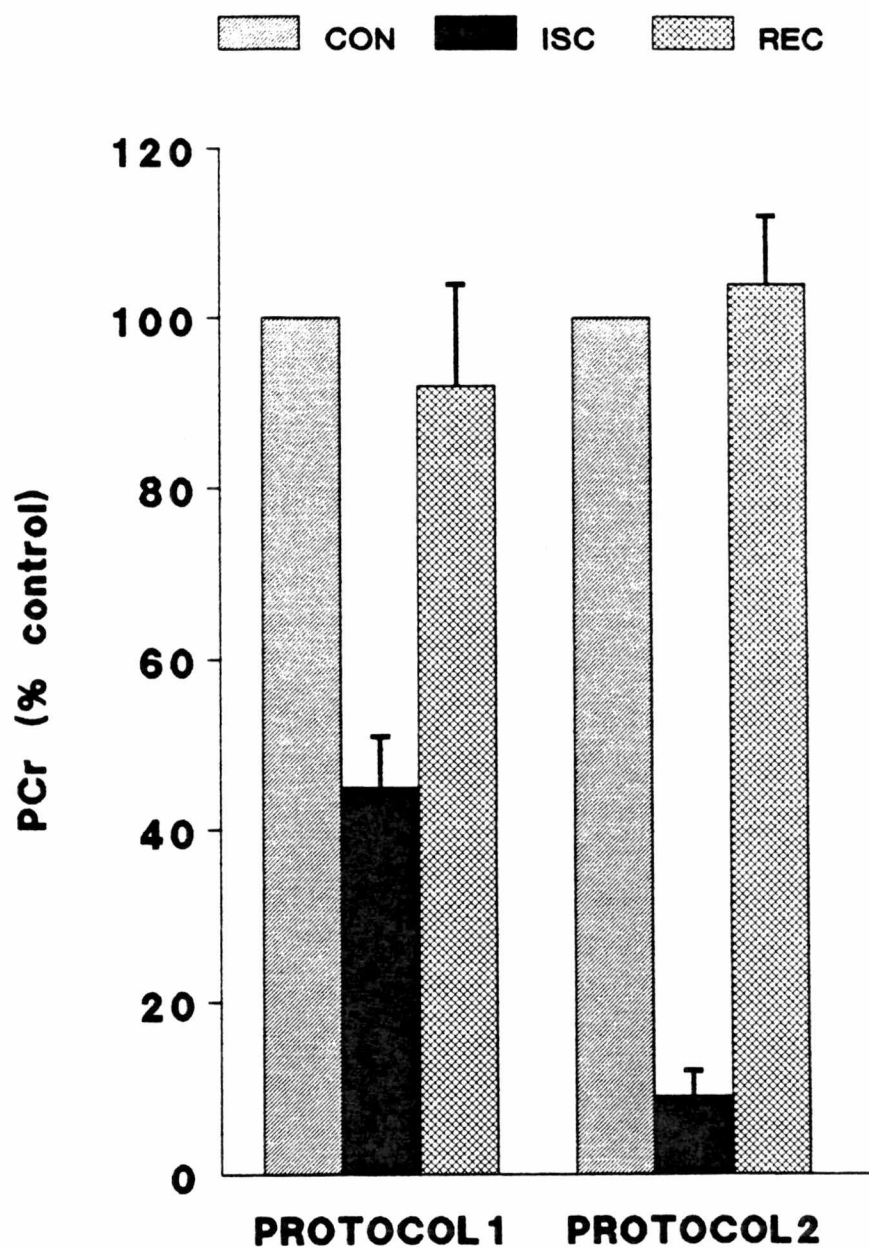


Figure 3-4. Changes in Phosphocreatine Content (PCr) During Demand Ischemia and Reperfusion. Bars are mean $\pm$ SEM for control (CON), end Ischemia (ISC), and end reperfusion (REC). See text for a description of protocols.

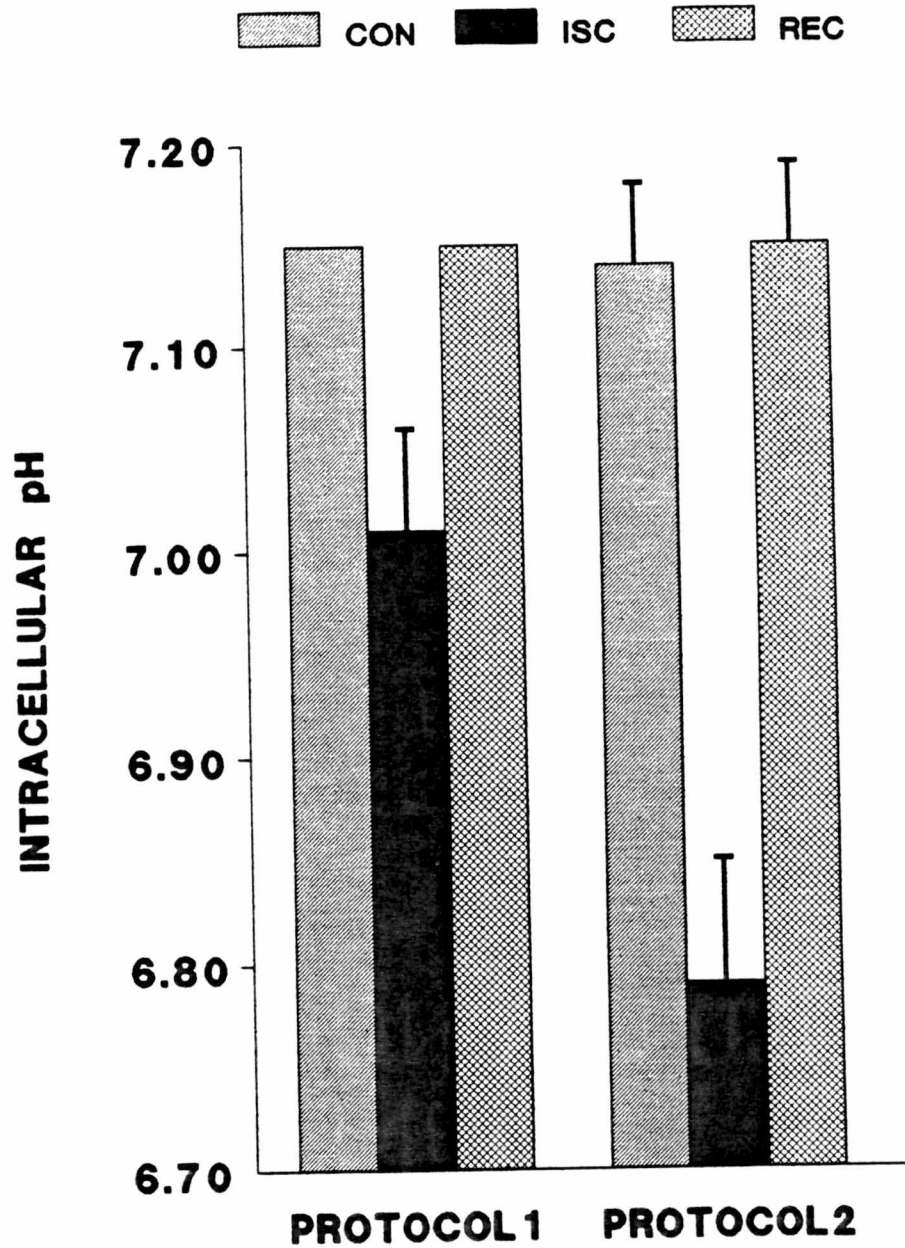


Figure 3-5. Changes in Intracellular pH During Demand Ischemia and Reperfusion. Bars are mean $\pm$ SEM at control (CON), end ischemia (ISC), and end reperfusion (REC). See text for a description of protocols.

Table 3-3. ARTERIAL PRESSURES AND LEFT VENTRICULAR WEIGHTS

AGE (month)	DIET	MAP ^a (mmHg)	n ^b	BW ^c (kg)	n ^d	LV WT ^e (g)	LV DRY/BW ^f (g/kg)
4	CON ^g	127 ± 4	4	0.38 ± 0.01	4	0.17 ± 0.01	0.45 ± 0.01
4	HS ^h	136 ± 5	4	0.35 ± 0.01 ^k	5	0.18 ± 0.01 ^k	0.53 ± 0.01 ^k
9	CON	125 ± 2	5	0.47 ± 0.02 ^k	5	0.21 ± 0.01 ^k	0.44 ± 0.01
9	HS	154 ± 6 ^{i,j}	3	0.44 ± 0.01 ^j	7	0.21 ± 0.01 ^j	0.48 ± 0.01 ⁱ

Notes: Data are mean ± SEM. Probabilities are one-tailed.

^a mean arterial pressure

^b number of rats for the MAP measurement.

^c body weight

^d number of rats for the BW, LV WT, and LV DRY/BW measurements.

^e left ventricular dry weight

^f ratio of left ventricular dry weight to body weight.

^g CON is control diet (0.3% NaCl).

^h HS is high salt (8% NaCl).

ⁱ $P \leq 0.0215$ vs 9 month old control

^j $p \leq 0.0066$ vs 4 month old HS

^k $p \leq 0.0462$ vs 4 month old control

The 4 month old BHR were used in protocols 1 and 2, and the 9 month old rats were used in protocols 3 and 4. The MAP was higher in both 4 and 9 month old BHR-HS compared with the BHR-CON, but the difference only achieved statistical significance ($p=0.0006$) in the 9 month old rats. Both 4 month old ($p=0.0095$) and 9 month old ($p=0.0215$) BHR-HS were significantly hypertrophied compared with age-matched BHR-CON.

The MAP was significantly higher in the 9 month old BHR-HS compared with the 4 month old BHR-HS. In contrast, the LV DRY/BW was actually lower in the 9 month old BHR-HS compared with the 4 month old BHR-HS. Therefore, although the MAP was higher in 9 month old BHR-HS, the severity of hypertrophy did not increase with age in these rats.

D. DISCUSSION

The primary objective of this series of studies was to develop a protocol that would facilitate the evaluation of the relationship between increased diastolic dysfunction in hypertrophied hearts and the increase in intracellular Na^+ during ischemia. Previous studies had shown that hearts with only minimal hypertrophy exhibited increased diastolic dysfunction compared with nonhypertrophied hearts (43,134). This observation suggested that diastolic dysfunction might be a sensitive measure of the greater susceptibility of hypertrophied hearts to ischemic dysfunction and damage. The results of these preliminary studies indicate that diastolic dysfunction was not produced during demand ischemia in hypertrophied BHR hearts perfused with 1 mM extracellular free $[\text{Ca}^{2+}]$. The severity of the changes in PCr, pH and Na^+ was increased by decreasing the flow during demand ischemia by half, but there was no apparent effect on the increase in LVEDP.

Moderate increases in LVEDP were promoted during demand ischemia when the extracellular free $[Ca^{2+}]$ was increased to 1.5 mM. However, the ^{23}Na shift reagent Tm(DOTP) $^{5-}$ was sensitive to precipitation at perfusate Ca^{2+} concentrations greater than 1 mM. Therefore, it was not possible to measure changes in intracellular Na^+ using conditions that produced diastolic dysfunction during demand ischemia. The observation that hearts exhibited less diastolic dysfunction when perfused at a lower free $[Ca^{2+}]$ is consistent with previous studies that have shown that reducing the extracellular $[Ca^{2+}]$ has a protective effect on the heart during ischemia and reperfusion (23,148).

Since the BHR used in protocols 3 and 4 were older than rats used in protocols 1 and 2, age cannot be excluded as a factor that may have contributed to the greater increase in LVEDP observed during these protocols. However, the severity of hypertrophy was not greater in the 9 month old BHR-HS compared with 4 month old BHR-HS. Furthermore, the increase in LVEDP was not greater in the 9 month old BHR-HS compared with the age-matched BHR-CON. Therefore, the greater increase in LVEDP during demand ischemia in hearts subjected to protocols 3 and 4 was not due to differences in the severity of hypertrophy compared with hearts in protocols 1 and 2.

The results of an earlier study indicated that hypertrophied hearts from DOCA-salt rats exhibited a greater increase in LVEDP during hypoxia in hearts perfused with buffer containing 5.5 mM glucose and 1 mM lactate compared with hearts perfused with a much higher glucose concentration of 27.5 mM glucose in combination with insulin (27). However, our data indicate that decreasing the glucose concentration from 11 to 5.5 mM did not have a measurable effect on the observed increase in LVEDP during ischemia.

An insufficient number of hearts were studied to relate changes in Na^+ and LVEDP using statistical methods. However, it was clear from the data for individual hearts

that a substantial increase in Na^+ of up to 84% above control was not a sufficient cause for an increase in LVEDP during ischemia. It is possible that the effects of lower extracellular Ca^{2+} may have attenuated the influx of Ca^{2+} via Na^+ - Ca^{2+} exchange, or that the concurrent decrease in pH may have masked the effects of increased Ca^{2+} by decreasing the sensitivity of the myofilaments to Ca^{2+} (149,150).

The development of hypertension and left ventricular hypertrophy was similar in the BHR-HS used in these preliminary studies as that described for the BHR in Chapter 2. The BHR fed a high salt diet developed frank hypertension and significant hypertrophy that was measurable in 4 month old rats after 8 weeks on the 8% NaCl diet. These data also verified the earlier observation that the severity of hypertrophy in the BHR-HS, measured using the LV DRY/BW, does not increase with age.

In conclusion, the demand ischemia protocol required a $[\text{Ca}^{2+}]$ higher than 1 mM to induce detectable diastolic dysfunction, but higher concentrations of Ca^{2+} were not compatible with the ^{23}Na shift reagent Tm(DOTP)⁻⁵. Therefore, demand ischemia was not a useful protocol for the evaluation of the relationship between ischemic dysfunction and the increase in intracellular Na^+ during ischemia. The low flow ischemia protocol that will be described in Chapter 4 proved to be more appropriate for relating ischemic dysfunction and increases in intracellular Na^+ in hypertrophied hearts.

The BHR heart develops only mild left ventricular hypertrophy compared with other models of pressure overload. The results of these studies indicate that there were no differences in either Na^+ homeostasis or diastolic function between BHR-CON and BHR-HS that were detectable with the methods described. Therefore, hearts from Spontaneously Hypertensive Rats with more severe hypertension and hypertrophy were used in subsequent experiments.

CHAPTER 4

Na⁺ HOMEOSTASIS, pH REGULATION AND HIGH ENERGY PHOSPHATE METABOLISM IN HYPERTROPHIED SHR HEARTS DURING ISCHEMIA

A. INTRODUCTION

Myocardial hypertrophy may occur as a compensatory response to the increased pressure load in hypertensive hearts that serves to normalize wall stress and maintain normal cardiac output (1,2). However, hypertrophied hearts have been shown to have a greater susceptibility to dysfunction, particularly diastolic, during ischemia and also to injury following reperfusion (26,27,33-48). The reason for this exaggerated sensitivity to ischemia has not been identified, but the development of hypertrophy is associated with alterations in metabolism (26-32) and Na⁺ and Ca²⁺ homeostasis (83-87,92-98) that may predispose these hearts to ischemic damage and dysfunction.

Myocardial ischemia is characterized by a decrease in high energy phosphates and pH, the accumulation of metabolites, and imbalances in cation homeostasis (16-20). The accumulation of intracellular Na⁺ during ischemia and early reperfusion has been implicated as a primary step in the development of ischemic dysfunction and damage (101,102,106-109). The increase in Na⁺ is believed to occur due to an increased influx of Na⁺ via Na⁺-H⁺ exchange (101,102,106,108,109) that may be potentiated by a decreased capacity for Na⁺ removal by the Na⁺-K⁺ ATPase at reduced ischemic energy levels (102,110). The increase in intracellular Na⁺ has been associated with a subsequent increase in Ca²⁺ uptake presumably via Na⁺-Ca²⁺ exchange (101,102,108). The elevated intracellular Ca²⁺ is proposed to directly promote ischemic dysfunction and damage (101,102,108,111,123-127).

There are several factors that indicate that hypertrophied hearts may be more susceptible to ischemic dysfunction and damage by mechanisms involving imbalances in cation homeostasis. It has been proposed that hypertrophied hearts may have an increased dependence on, or capacity to recruit, anaerobic glycolysis (26,32). This hypothesis remains controversial (26,27), but hearts hypertrophied by aortic banding were shown to produce significantly more lactic acid during ischemia and hypoxia compared with controls (26). The increased production of lactate and protons would provide a greater stimulus for increased Na^+ influx during ischemia and early reperfusion via Na^+ - H^+ exchange.

Additionally, a number of in vitro studies have shown that hypertrophied hearts may have a lower Na^+ - K^+ ATPase activity (83-87) that would compromise the ability of these hearts to respond to an increased Na^+ influx during ischemia, and allow the intracellular Na^+ to increase to greater levels. Finally, there is evidence that hypertrophied hearts may have a decreased capacity for Ca^{2+} sequestration by the sarcoplasmic reticulum (92-97), and an enhanced ability to utilize Na^+ - Ca^{2+} exchange for normal Ca^{2+} homeostasis (86,98,100). The increase in intracellular Na^+ during ischemia would inhibit Ca^{2+} removal via Na^+ - Ca^{2+} exchange, and perhaps even potentiate the influx of Ca^{2+} by this mechanism. This additional Ca^{2+} load would be potentiated further by decreased uptake of Ca^{2+} by the sarcoplasmic reticulum in hypertrophied hearts. Therefore, hypertrophied hearts exhibit a number of alterations in Na^+ and Ca^{2+} homeostasis that may contribute to greater ischemic dysfunction and injury compared with normal hearts.

Most of the evidence for impaired Na^+ handling in hypertrophied hearts has been obtained in vitro. The primary objectives of the present study were to determine whether hypertrophied hearts exhibited greater Na^+ accumulation during ischemia, and to

determine if ischemic dysfunction and damage in these hearts was associated with the increase in intracellular Na^+ . An additional objective was to relate the increase in intracellular Na^+ with the changes in pH, and contents of high energy phosphates during ischemia in hypertrophied hearts.

B. METHODS

Experimental Preparation

Male Spontaneously Hypertensive Rats (SHR) and normotensive Wistar-Kyoto (WKY) rats were acquired from Taconic farms (Germantown NY), at either 16 weeks of age or as retired breeders. Rats were housed on a 12 hour light-dark cycle and received rat chow and water ad libitum. Isolated heart studies were done when the rats were 8 - 10 months old. One or two days before the isolated heart experiment, rats were anesthetized with methoxyflurane, and an indwelling catheter was placed surgically in the femoral artery and externalized through a dorsal cervical incision. Direct blood pressures were measured in awake, unsedated animals, in the morning, on either the day of the perfusion experiment, or one day prior to the experiment.

The isolated, isovolumic heart preparation was used to compare the response of hypertrophied SHR hearts with that of WKY controls. Prior to isolation of the heart, rats were injected with heparin (100 units/Kg IP) and anesthetized with pentobarbital (60mg/Kg IP). Hearts were rapidly excised, arrested in cold isotonic saline (4°C), and cannulated via the aortic root for retrograde coronary perfusion. During the initial preparation, the hearts were perfused at a constant pressure of 75 mmHg. After the hearts were placed in the magnet they were switched to a constant coronary flow of

approximately 15 mls/min/g wet weight. Non-recirculating systems were used for both constant flow and constant pressure perfusion. Coronary perfusion pressure (CPP) was monitored through the aortic cannula using a P23XL pressure transducer (Spectramed, Oxnard CA). A schematic of the apparatus used for constant flow perfusion of hearts during the collection of NMR and functional data is shown in Figure A-1 of the Appendix.

All hearts were initially perfused with a modified Krebs-Henseleit buffer with the following composition: 118mM NaCl, 24mM NaHCO₃, 4.7mM KCl, 1.2mM MgSO₄, 1.5mM CaCl₂, 0.5mM Na₂EDTA and 11mM glucose. Immediately after the hearts were placed in the magnet the perfusate was changed to buffer containing 4mM of the ²³Na NMR shift reagent Na₅Tm(DOTP). The shift reagent buffer was identical to the original perfusate with the following modifications. The NaCl in the buffer containing Na₅Tm(DOTP) was reduced to maintain the total [Na⁺] at 142 mM. In addition, the total [Ca²⁺] was increased to 5.5 mM to adjust for significant chelation of Ca²⁺ by the shift reagent. The mean ($\pm$ SEM) perfusate free Ca²⁺ concentration measured using a calcium specific electrode (Orion, Boston MA) was 1.01 $\pm$ 0.02 (range 0.89-1.16) mM. The perfusate was equilibrated with 95% O₂ and 5% CO₂ to produce a pH of 7.4 to 7.45.

To facilitate pacing, the right atrium was removed, and the AV node was crushed. Platinum wire (36 gauge) electrodes were attached to the right ventricular free wall, and connected to a constant voltage stimulator (Stoelting, Chicago IL) via a KCl-agar salt bridge. Hearts were paced at 4 Hz using a voltage just above the threshold voltage.

A drain was placed in the apex of the left ventricle to allow thebesian drainage, and a small fluid filled latex balloon was placed in the left ventricle through the mitral valve. The balloon was connected to a P23XL pressure transducer (Spectramed, Oxnard CA) via polyethylene tubing for measurement of left ventricular pressure. Left ventricular

peak systolic, and end diastolic pressures were recorded continuously using an analog physiologic recorder (Astromed, West Warwick RI). The left ventricular pressure data were also digitized at 10 ms intervals using an A-D converter and stored on a personal computer for later analysis. Left ventricular developed pressure (LVDEVP) was calculated as the difference between left ventricular peak systolic pressure and left ventricular end diastolic pressure (LVEDP).

The heart was submerged in buffer inside a 20mm NMR tube which was connected to the perfusion apparatus via a water-jacketed umbilical that allowed hearts to be perfused at 37°C while inside the magnet. After the hearts were placed in the magnet they were switched to perfusate containing the sodium shift reagent Tm(DOTP)^{5-} , and perfused at a constant flow of 15 mls/min/g using a Masterflex™ pump (Cole Parmer, Chicago IL). The perfusate containing Tm(DOTP)^{5-} was bubbled with 95% O_2 and 5% CO_2 and kept in an ice bath. The perfusate was circulated through a 25ft coil of silastic tubing bathed in fluid aerated with 95% O_2 and 5% CO_2 at 37°C to provide temperature and gas equilibration. The coil of silastic tubing emptied into a 50 ml jacketed condenser that was also aerated with 95% O_2 and 5% CO_2 . The perfusate was pumped from the bottom of the condenser to the heart, and overflow from the top of the condenser was returned to flask containing cold perfusate. The condenser was necessary to maintain the flow constant because gas bubbles were formed in the silastic tubing as the perfusate was warmed to 37°. The perfusate was filtered using in-line 5µm membrane filters.

NMR Spectroscopy

The NMR spectra were obtained using an 8.5 Tesla vertical bore superconducting magnet and a Bruker AM-360 spectrometer (Billerica MA). The ^{23}Na and ^{31}P spectra were acquired using a single home-built, temperature controlled probe. The probe design included two orthogonal saddle coils on separate circuits that were connected to separate dedicated preamplifiers. One coil was tuned to 145.78 MHz for ^{31}P and the second coil simultaneously tuned to 95.25 MHz for ^{23}Na . The magnet was shimmed prior to data acquisition to optimize field homogeneity using the ^{23}Na signal.

The ^{23}Na spectra were obtained by averaging 336, 90° excitation pulses which were collected using an acquisition time of 0.154 seconds, without an additional relaxation delay, and a dead time of 96.3 μsec . Data were accumulated over a one minute period using 2K data points and a spectral width of 6667 Hz. The recycle time was more than 5 times greater than the T_1 (20-30 ms) estimated using inversion recovery for both the intracellular and extracellular Na^+ resonances. Thus, the pulsing conditions did not produce saturation of the ^{23}Na resonances. The shift reagent $\text{Tm}(\text{DOTP})^{5-}$ was used to shift the extracellular Na^+ resonance and allow resolution of the intracellular and extracellular Na^+ peaks (107,146,151). The free-induction decays (FIDs) were multiplied by a Gaussian function to enhance resolution of the intracellular resonance. The Gaussian function was introduced using a standard Bruker Gaussian multiplication (GM) subroutine with gaussian broadening (GB) = 0.1 Hz and line broadening (LB) = -10 Hz. A standard solution containing 140mM NaCl and 4mM $\text{Tm}(\text{DOTP})^{5-}$ was included in the left ventricular balloon. A standard curve was derived for estimation of the Na^+ content of each heart by acquiring ^{23}Na spectra following the addition of known volumes of the standard solution to the balloon. The areas of the ^{23}Na resonances were determined

using a hand-held digitizer (Jandel Scientific, Sausalito CA)

The ^{31}P spectra were obtained by accumulating a total of 56, 60° excitation pulses collected using an acquisition time of 0.145 seconds with an interpulse delay of 1.85 seconds over a two minute period. Data were accumulated using 2K data points and a spectral width of 7042 Hz. Exponential multiplication of the FID corresponding to a line broadening of 40 Hz was used to improve the signal-to-noise ratio. Peak areas for phosphocreatine (PCr), adenosine triphosphate (ATP), and inorganic phosphate (P_i) were multiplied by 1.32, 1.10 and 1.27, respectively to correct for the saturating effects of repetitive pulses. These saturation factors were determined by comparing peak resonances acquired using the experimental relaxation delay with fully relaxed resonances acquired using a relaxation delay of 10 seconds. The resonance from a $10\mu\text{mole}$ (400 mM) phenylphosphonic acid standard contained in a microsphere and placed in the tube beside the heart was used to calculate the contents of PCr, ATP and P_i in the hearts. The intracellular pH was determined from the shift of the P_i with respect to the PCr resonance (147). The ^{31}P spectra were interleaved automatically with ^{23}Na such that the first minute of each 3 minute interval was used to acquire one ^{23}Na spectrum, and the last two minutes were used to acquire one ^{31}P spectrum. The ^{31}P resonances were curve fit to a Lorentzian lineshape, and automatically integrated using an NMR-1TM (New Methods in Research INC, Syracuse NY) software package.

Shift Reagent Preparation and Recovery

The $\text{Tm}(\text{DOTP})^{5-}$ was synthesized using a modification of the method of Sherry and others (146,151). The DOTP^{8-} was synthesized as described previously (151). Impurities were removed from the DOTP^{8-} by repeatedly precipitating the DOTP^{8-} from

concentrated solutions at pH 1.0, and washing with methanol. The final purity of the DOTP⁸⁻ was determined by titration with TmCl₃. Briefly, known amounts of TmCl₃ were added to an aqueous solution of DOTP⁸⁻ containing 150 mM NaCl buffered to pH 9.0 with triethanolamine, and the frequency shift was monitored using the Bruker 360-AM spectrometer. The purity of the DOTP⁸⁻ was determined on the basis that the maximal frequency shift should occur when the actual ratio of Tm³⁺ to DOTP⁸⁻ is equal to 1. The DOTP⁸⁻ of known purity was complexed with TmCl₃ using a Tm³⁺ to DOTP⁸⁻ ratio of 0.98.

The Tm(DOTP)⁵⁻ was recovered from the perfusate following each experiment. Briefly, the perfusate was filtered, 10mM Ca²⁺ was added, and the solution was warmed to 60-70°C to precipitate the Ca²⁺ salt of Tm(DOTP)⁵⁻. The Ca²⁺ salt of the shift reagent was separated by filtration. The Ca²⁺ was subsequently removed from the Tm(DOTP)⁵⁻ by decreasing the pH of an aqueous suspension of the shift reagent to 1.0 with HCL. The protonated Tm(DOTP)⁵⁻ was filtered, washed with methanol, and dried to constant weight in a desiccator.

Experimental Protocol

Hearts were placed in the magnet, switched to shift reagent buffer at a constant coronary flow of 15 mls/min/g, and allowed to stabilize for at least 20 minutes. After the initial stabilization period, the control left ventricular pressure-volume (P-V) curve was obtained. The P-V curves were acquired by increasing the volume of the intraventricular balloon in at least three 0.01 ml increments. Following acquisition of the P-V curve, the balloon was inflated to a volume which produced an end diastolic pressure of 2 to 4 mmHg, and this volume was maintained constant throughout the experiment.

Prior to acquisition of control data, a fully relaxed ³¹P spectrum was acquired for

determination of saturation factors by increasing the interpulse delay to 10 seconds. Control NMR and pressure data were collected for 12 minutes while the hearts were perfused at a flow of approximately 15 mls/min/g and paced at a rate of approximately 240 beats/minute (bpm). Hearts were subjected to low flow ischemia for 30 minutes during which the flow was reduced to 10% of the control flow, and pacing was continued at 240 bpm. Low flow ischemia was followed by 30 minutes of reperfusion at the control flow and heart rate. Left ventricular and coronary perfusion pressures were monitored continuously throughout the experiment, and ^{31}P and ^{23}Na spectra were each acquired with a time resolution of 3 minutes.

Hearts were excluded from the study if the basal left ventricular developed pressure decreased by more than 10% or the basal left ventricular end diastolic pressure increased by more than 2 mmHg after the initial equilibration. Hearts were also excluded if the coronary perfusion pressure increased above 150 mmHg during control or reperfusion.

Following the experimental protocol, the left ventricular P-V curve was repeated, and the slope was compared with the slope of the control P-V curve to assess changes in left ventricular stiffness. The mean change in the slope of the P-V curve at end reperfusion with respect to control was compared between groups to assess differences in the increase in left ventricular stiffness.

At the end of the perfusion experiment the left ventricle and septum were dissected from the right ventricle, and both chambers were blotted and weighed. Ventricular dry weights were determined after the ventricles were dried to constant weight in an oven at 100°C. The degree of myocardial hypertrophy was assessed by calculating both the left ventricular weight to body weight and right ventricular weight to body weight

ratios. Left ventricular wet weight to dry weight ratios were determined to compare the severity of LV edema between groups following the perfusion experiment.

Measurement of Lactate Production

To compare lactate production in the SHR hearts with production in WKY hearts the coronary venous effluent from the heart was collected for the 30 minute ischemic period, and for 3 minute intervals during reperfusion via an aspiration line in the NMR tube. There was no measurable lactate in the effluent during control perfusion. The lactate in the effluent from the heart was measured using a Sigma (St. Louis, Mo) reagent kit. The method is a standard enzymatic assay in which lactate and NAD are converted to pyruvate and NADH using lactate dehydrogenase. The production of NADH was measured as an increase in absorbance at 340nm using a spectrophotometer (Gilford).

Calculation of the Free Energy of ATP Hydrolysis

The free energy of ATP hydrolysis (ΔG) was calculated using the following formula: $\Delta G = \Delta G^\circ + RT \cdot \ln ([ADP] \cdot [P_i] / [ATP])$ kJ/mole, where ΔG° is the standard free energy change, R is the gas constant, and T is the absolute temperature. The [ADP]/[ATP] ratio was calculated from the equilibrium of the creatine kinase reaction:

$$K_{eq} = [ATP] \cdot [PCr] \cdot [H^+] / [ADP] \cdot [Creatine] = 1.66 \times 10^9 \text{ M}^{-1} \text{ (152)}.$$

The [PCr], $[P_i]$ and $[H^+]$ were obtained from the ^{31}P spectra for each heart. The [creatine] was determined as the difference between the mean total creatine value and the measured PCr. The mean total creatine was assumed to be equal to 68.5 $\mu\text{moles/g}$ dry wt (152). Values of PCr, P_i and creatine content in $\mu\text{moles/g}$ dry wt were converted to cytoplasmic concentrations in moles/liter using a conversion factor of 3.0 mls/g dry wt.

The conversion factor was calculated using the measured wet to dry weight ratio, and assuming that the intracellular volume was 57% of the total tissue volume (104). Values of ΔG in this paper were expressed as absolute values, but they should be understood as thermodynamically negative since ATP hydrolysis is an exergonic reaction.

Calculation of Left Ventricular Wall Stress

To correct for differences in left ventricular mass and chamber volume, left ventricular midwall stress (σ) was calculated using the following formula which is based on the assumption of spherical left ventricular geometry (153-154):

$$\sigma = (1.36 \bullet LVP \bullet VOL^{2/3}) / [VOL + (0.943 \bullet LVW)]^{2/3} - VOL^{2/3}$$

Where LVP is left ventricular peak systolic or end diastolic pressure and LVW is the wet weight of the left ventricle and septum. The VOL is the balloon volume which was determined as the sum of the volume of the balloon at 0 mmHg, determined by water displacement, and the fluid volume added to the balloon to achieve the perfusion LVEDP. Developed stress was calculated as the difference between peak systolic and end diastolic σ .

Statistical Methods

Between group differences in blood pressures, heart weight data, perfusion conditions, and recovery times were compared using a one-way analysis of variance with multiple contrasts. Group differences in the changes with respect to control at the midpoint and end of ischemia in left ventricular function, Na^+ , pH and high energy phosphates were assessed using a repeated measures analysis of variance with multiple contrasts (155). Similarly, between group differences in the maximal recovery of these

variables with respect to control following reperfusion were also assessed using a repeated measures analysis of variance. For these analyses the mean of the 12 minute control period was used as the control value. The mean of minutes 13-15 was used as the midpoint of ischemia. The mean of the last 3 minutes of ischemia was used as the end ischemia value, and the mean of the last 6 minutes of reperfusion was used as the recovery value. The Bonferroni method was used for all multiple comparisons (141). All comparisons were two-tailed unless stated otherwise.

The mean changes with respect to control across time of Na^+ , pH and high energy phosphates were assessed using a repeated measures analysis of variance by comparing the Time*Group factor for each pair of groups (156). The initial 15 minutes and the final 15 minutes of ischemia were analyzed separately.

Correlations, least squares linear regression, and multiple linear regression analyses were used to examine relationships between variables.

C. RESULTS

Group Characteristics

The ischemic protocol distinguished two groups of SHR. One subgroup of SHR, which will be referred to as the SHF ($n=7$), exhibited contractile failure and a dramatic increase in intracellular Na^+ during ischemia. A second subgroup of SHR, the SHS ($n=13$) continued to beat throughout ischemia and exhibited only a small increase in intracellular Na^+ . The differences between SHR subgroups were great enough to be detected during the experimental procedure. Therefore, all statistical comparisons were made following assignment of the SHR to one of the two separate subgroups.

The mean arterial pressure (MAP) was significantly ($p=0.0001$) higher in both the SHF and SHS than in the WKY (Table 4-1). The SHF tended to have higher pressures than the SHS, but this difference was not statistically significant. The ratio of left ventricular dry weight to body weight (LV dry/BW) was used as an index of left ventricular hypertrophy (Table 4-2, Figure 4-1). Both the SHF and SHS groups had significant ($p=0.0001$) left ventricular hypertrophy compared with the WKY. In addition, the LV dry/BW ratio was significantly greater in the SHF compared with the SHS ($p=0.0132$) indicating that the left ventricular hypertrophy was significantly more severe in the SHF. This difference could not be attributed to extreme observations in either group.

The SHF also had significant right ventricular hypertrophy compared with the WKY ($p=0.0008$) (Table 4-3). In contrast, the ratio of right ventricular dry weight to body weight (RV dry/BW) was not significantly higher in the SHS compared with the WKY.

Perfusion Conditions

Hearts were perfused using a constant flow system, and the actual coronary flows expressed per gram ventricular weight (left + right) are shown in Table 4-4. Flows were similar in all three groups. Since hearts were perfused at constant flow, coronary perfusion pressure (CPP) was proportional to coronary resistance. The control CPPs were higher in both of the SHR subgroups compared with the WKY ($p < 0.0061$) indicating greater coronary resistance in the hypertrophied hearts (Table 4-4). The CPP was higher at end reperfusion compared with the control value in all three groups, but there were no significant differences between groups in the observed increase in CPP.

To determine whether there were differences between groups in the amount of LV

Table 4-1. AGES AND ARTERIAL PRESSURES

GROUP	n	AGE (weeks)	MAP (mmHg)	DAP (mmHg)	SAP (mmHg)
SHF	7	35 ± 1.3	187 ± 3 ^b	166 ± 4 ^b	229 ± 5 ^b
SHS	13	37 ± 0.4 ^a	172 ± 5 ^b	149 ± 6 ^b	217 ± 7 ^b
WKY	8	35 ± 0.5	110 ± 3	97 ± 3	134 ± 4

Notes: Values are mean ± SEM. MAP is mean arterial pressure; DAP is diastolic arterial pressure; SAP is systolic arterial pressure.

^a p = 0.0109 vs SHF

^b p = 0.0001 vs WKY

Table 4-2. BODY WEIGHTS AND LEFT VENTRICULAR WEIGHTS

GROUP (n)	BODY WEIGHT (kg)	LV WET WEIGHT (g)	LV WET WT/BW (g/kg)	LV DRY WEIGHT (g)	LV DRY WT/BW (g/kg)
SHF (7)	0.37 ± .01 ^a	1.28 ± .02 ^b	3.4 ± .08 ^a	0.24 ± .01 ^b	0.65 ± .01 ^{ae}
SHS (13)	0.38 ± .01 ^a	1.25 ± .03 ^c	3.3 ± .07 ^a	0.23 ± .01 ^d	0.61 ± .01 ^a
WKY (8)	0.55 ± .01	1.14 ± .02	2.1 ± .03	0.21 ± .01	0.39 ± .01

Notes: Values are mean ± sem. LV is left ventricular; WT is weight; BW is body weight.

^a p = 0.0001 vs. WKY

^b p = 0.0026 vs. WKY

^c p = 0.0064 vs. WKY

^d p = 0.0156 vs. WKY

^e p = 0.0132 vs. SHS

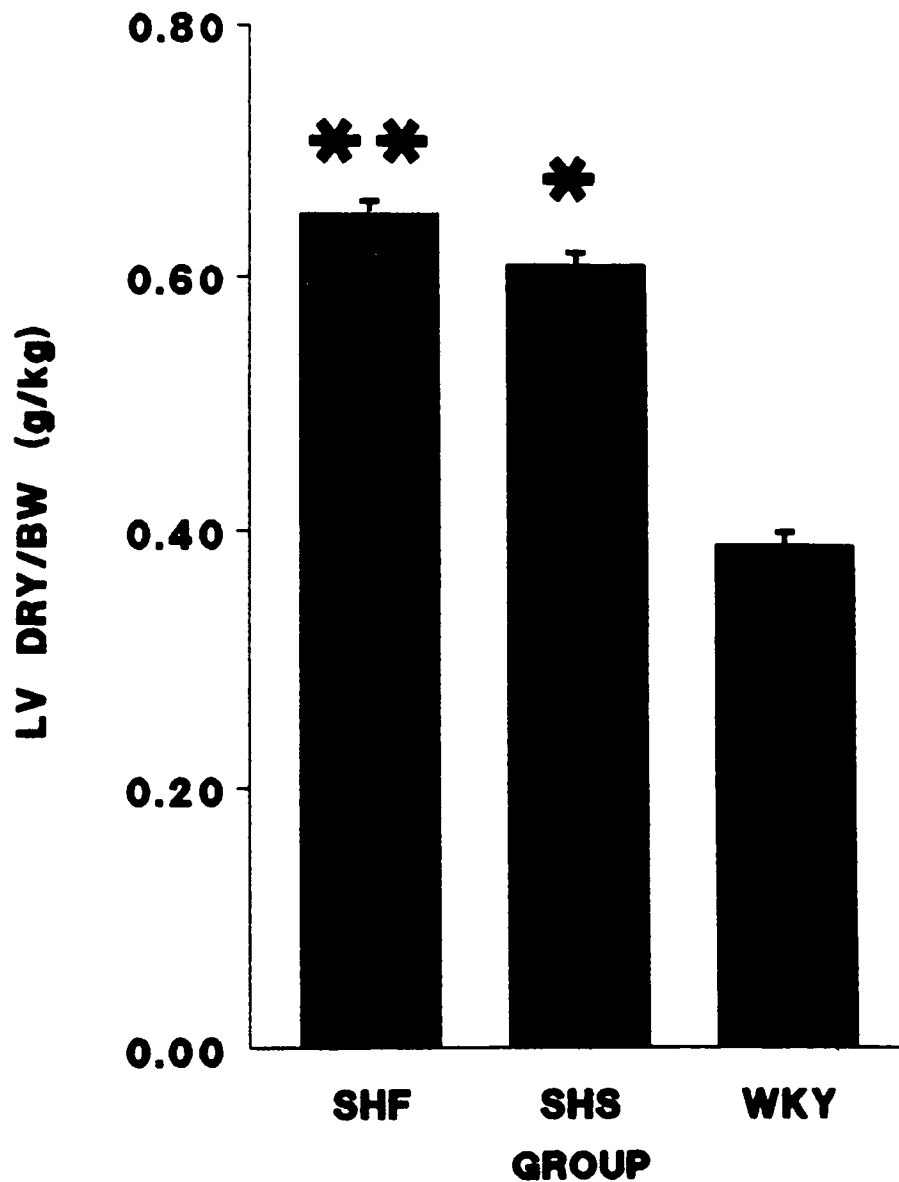


Figure 4-1. Severity of Left Ventricular Hypertrophy. Bars are mean $\pm$ SEM for left ventricular dry weight to body weight ratio. * $p = 0.0001$ vs. WKY; ** $P \leq 0.013$ vs. WKY and SHS.

Table 4-3. RIGHT VENTRICULAR WEIGHTS

GROUP (n)	RV WET WEIGHT (g)	RV WET WT/BW (g/kg)	RV DRY WEIGHT (g)	RV DRY WT/BW (g/kg)
SHF (7)	0.25 ± 0.01	0.68 ± 0.03 ^a	0.05 ± 0.004 ^c	0.13 ± 0.003 ^e
SHS (13)	0.24 ± 0.01 ^a	0.61 ± 0.02	0.04 ± 0.003 ^d	0.12 ± 0.003
WKY (8)	0.29 ± 0.01	0.53 ± 0.02	0.06 ± 0.004	0.11 ± 0.004

Values are mean ± SEM. RV is right ventricular; WT is weight; BW is body weight.

^a p = 0.002 vs. WKY

^b p = 0.0045 vs. WKY

^c p = 0.0001 vs. WKY

^d p = 0.0008 vs. WKY

Table 4-4. PERFUSION CONDITIONS

GROUP	CON FLOW ^a (mls/min/g)	ISC FLOW (mls/min/g)	CON CPP (mmHg)	ISC CPP (mmHg)	REC CPP (mmHg)
SHF	14.2 ± 0.4	1.4 ± 0.1	87 ± 5 ^b	21 ± 1 ^d	92 ± 6
SHS	14.9 ± 0.2	1.5 ± 0.1	99 ± 3 ^c	21 ± 1 ^e	100 ± 4 ^f
WKY	14.9 ± 0.3	1.6 ± 0.1	69 ± 3	17 ± 1	77 ± 5

Notes: Values are mean ± SEM. CON is control; ISC is ischemia; REC is reperfusion; CPP is coronary perfusion pressure.

^a Flows are normalized for the weight of both ventricles

^b p = 0.0061 vs. WKY

^c p = 0.0001 vs. WKY

^d p = 0.0138 vs. WKY

^e p = 0.0037 vs. WKY

^f p = 0.0012 vs. WKY

edema at the end of the experiment the LV wet weight /dry weight ratios were compared. The mean ($\pm$ SEM) LV wet wt./dry wt. ratios were 5.3 ± 0.2 , 5.4 ± 0.1 and 5.3 ± 0.1 for the SHF, SHS and WKY, respectively. There were no significant differences between groups in the development of LV edema.

The perfusion time prior to the beginning of the experimental protocol differed from heart to heart due to differences in the time required to adjust the flow, change to shift reagent, and tune and shim the probe. The mean ($\pm$ SEM) perfusion times prior to the beginning of ischemia were 1.85 ± 0.13 , 1.78 ± 0.09 , and 1.90 ± 0.06 hours for the SHF, SHS and WKY, respectively. The perfusion times were not significantly different between groups. There was also no apparent deterioration of function or decrease in high energy phosphates during control in the hearts included in these groups. Therefore, group differences were not due to differences in the viability of the isolated hearts.

Left ventricular Function

The hearts were paced throughout the experimental protocol, and the control heart rates were not different between groups (Figure 4-2). Despite continuous pacing, the heart rate became quite irregular in the SHF during ischemia and ultimately all hearts in this group stopped beating. In contrast, although intermittent arrhythmias were detected, all of the hearts in the SHS and WKY groups continued to pace throughout ischemia. A significant number of arrhythmias were exhibited by most hearts at the beginning of reperfusion, and a few of the SHS and WKY hearts arrested briefly during the initial 3 minutes of reflow. All hearts eventually returned to the paced rate with occasional arrhythmias during reperfusion.

The basal left ventricular developed pressure (LVDEVP) was significantly higher

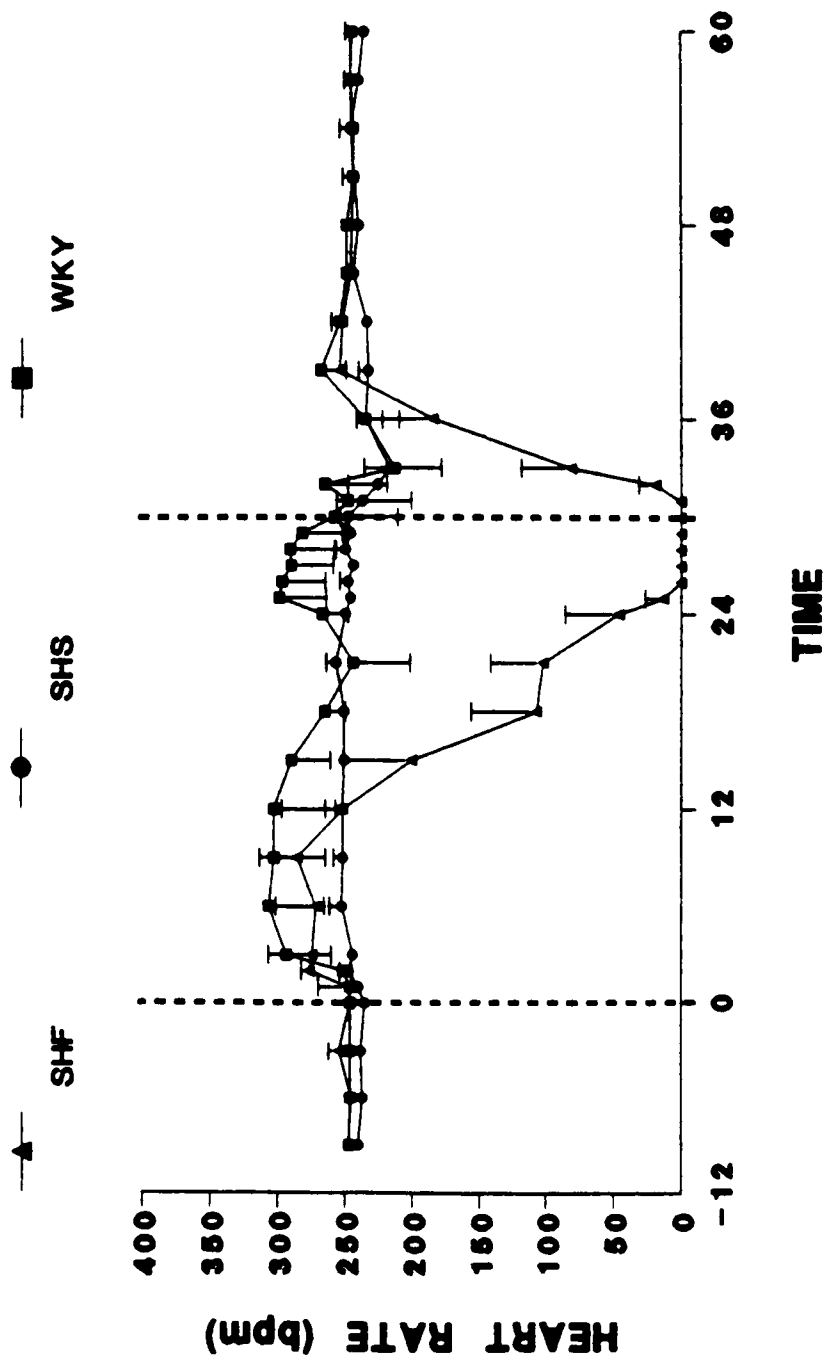


Figure 4-2. Heart Rate Scatter Plot. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the last control time point and the last ischemia time point, respectively. Note that hearts were paced throughout the experiment, but all hearts in the SHF group became refractory to pacing during ischemia and ultimately stopped beating.

in both the SHS ($p=0.0001$) and the SHF ($p=0.0049$) compared with the WKY (Figure 4-3). The LVDEVP declined rapidly and dramatically in all three groups during low flow ischemia (Figure 4-4). All of the SHF hearts exhibited contractile failure during ischemia, while all of the hearts in the SHS and WKY groups continued to develop low pressures throughout the ischemic period. The mean ($\pm$ SEM) time at which the SHF hearts initially arrested was 18 ± 1.9 minutes.

The decrease in LVDEVP was significantly greater in both the SHF ($p=0.0002$) and SHS ($p=0.0001$) groups compared with the WKY after either 15 or 30 minutes of ischemia. Since the SHF and SHS both had significantly higher LVDEVP at control compared with the WKY, the values of LVDEVP at end ischemia were compared in addition to the changes with respect to control. The pressure developed by the SHS was not significantly different from the LVDEVP in the WKY at end ischemia. Therefore, the LVDEVP in the SHS decreased from a higher control value to a value which was similar to the pressure in the WKY during ischemia. In contrast, left ventricular pressure development in the SHF decreased to zero in all hearts, and was significantly less at end ischemia than the LVDEVP in either the WKY ($p=0.0001$) or the SHS ($p=0.0001$).

The hearts in the SHF group resumed beating following 2 to 6 minutes of reperfusion, and the mean LVDEVP recovered completely to control levels during reperfusion in all three groups. The time required for the LVDEVP to recover to 80% of the control value was greater in the SHF compared with both the SHS ($p=0.0001$) and the WKY ($p=0.0001$) (Table 4-5).

The left ventricular balloon volume was adjusted at the beginning of the experiment in each heart to produce a left ventricular end diastolic pressure (LVEDP) of

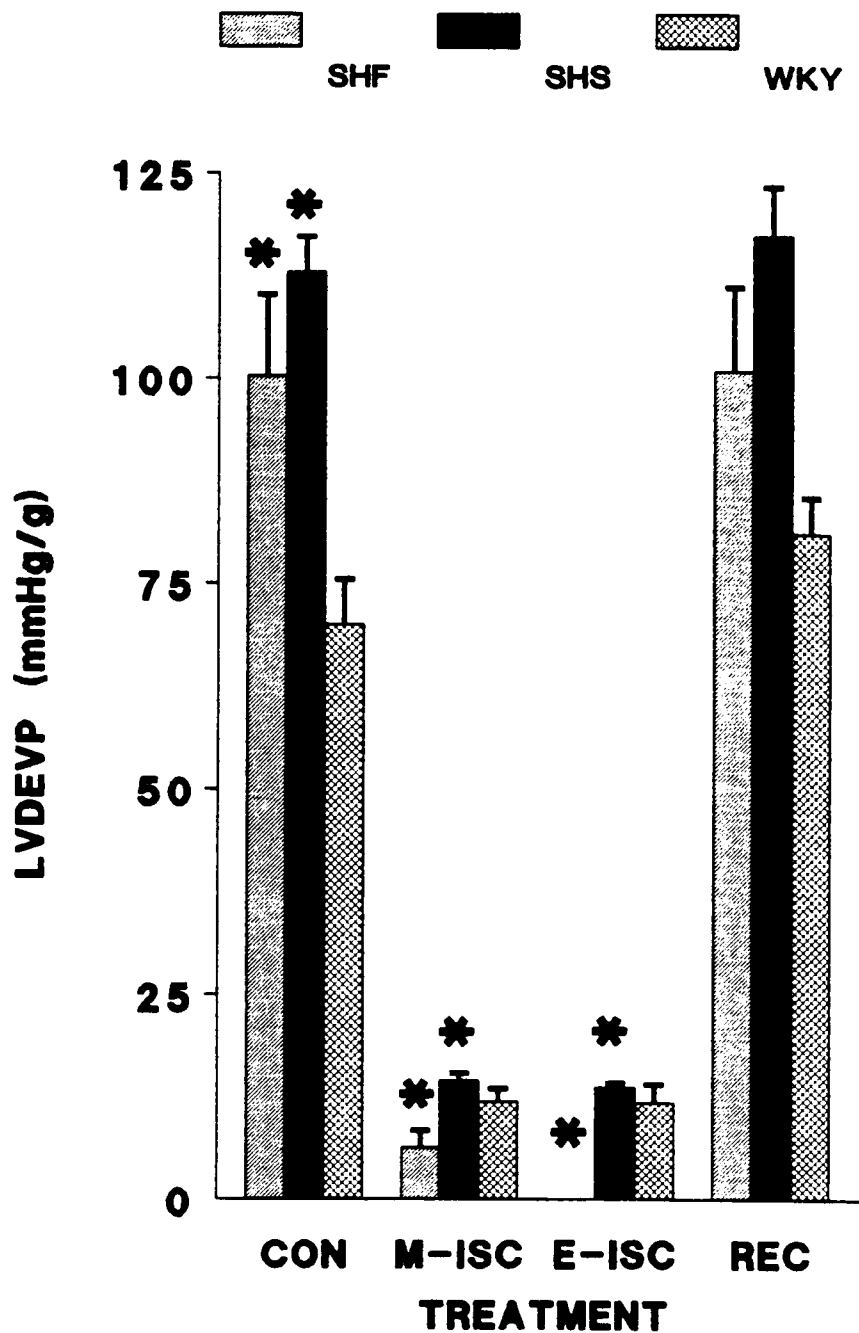


Figure 4-3. Changes in Left Ventricular Developed Pressure. Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p \leq 0.0049$ vs WKY.

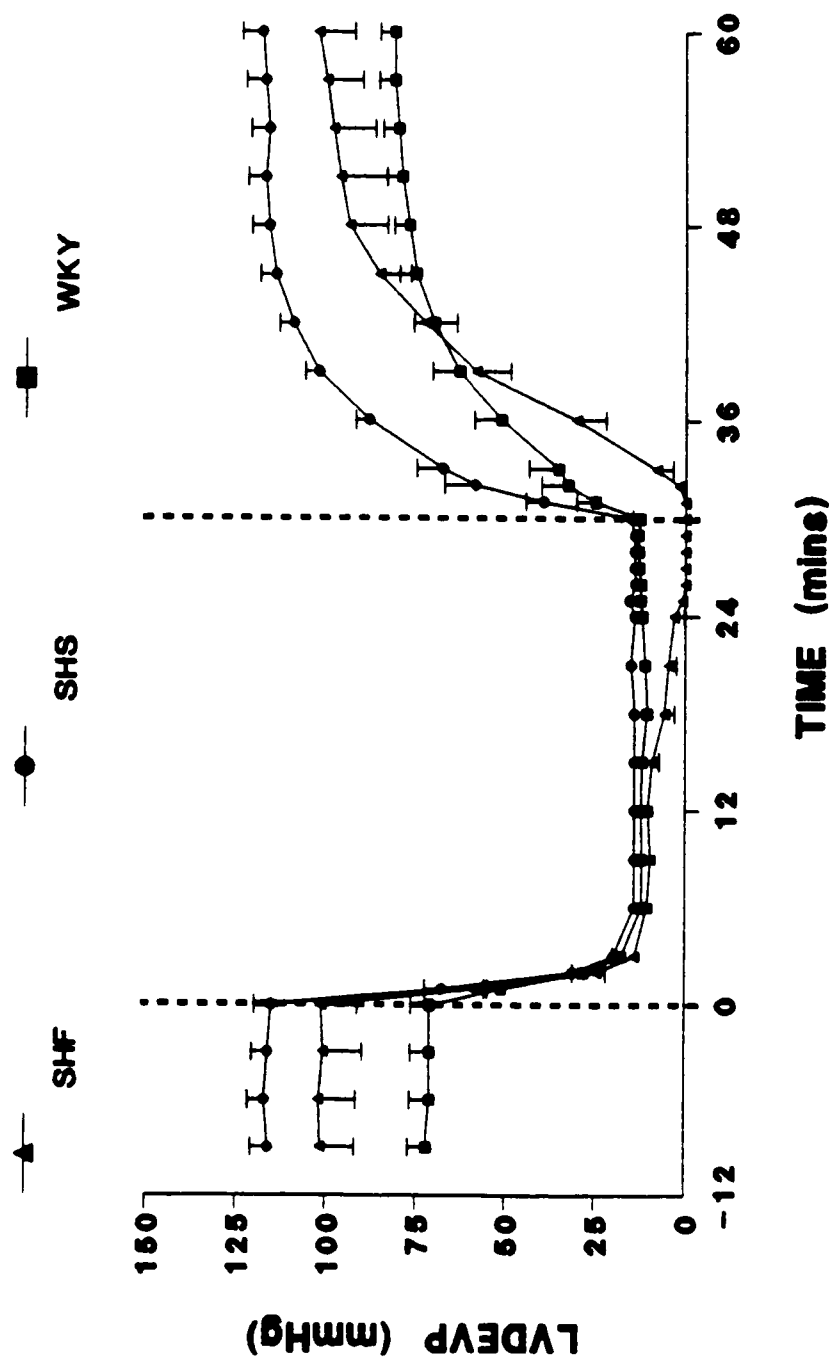


Figure 4-4. Scatter plot of Left Ventricular Developed Pressure Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final ischemia time point and the final control time point, respectively. Note that the LVDEVP required longer to recover in the SHF group compared with both the other groups ($p=0.0001$).

Table 4-5. RECOVERY TIMES FOLLOWING REPERFUSION

GROUP	REC LVDEVP (min)	REC Na ⁺ (min)	REC pH (min)	REC Δ G (min)
SHF	13 $\pm$ 0.4	7 $\pm$ 0.8 ^b	7 $\pm$ 1.1	3 $\pm$ 0
SHS	7 $\pm$ 0.8 ^a	4 $\pm$ 0.3	4 $\pm$ 0.3 ^c	3 $\pm$ 0
WKY	7 $\pm$ 0.7 ^a	7 $\pm$ 1.1 ^b	8 $\pm$ 1.4	3 $\pm$ 0

Notes: Values are mean $\pm$ SEM. REC is the recovery time. The REC for LVDEVP is the time within which each heart recovered to 80% of the pressure at control. Recovery times for LVDEVP were evaluated with respect to 80% of control because not all hearts recovered 100% of control function. The REC for Na⁺, pH and Δ G represents the time within which each heart recovered to 100% of the control value. The recovery times for Na⁺, pH and Δ G could only be estimated with a 3 minute time resolution. The REC for LVDEVP was estimated with a one minute time resolution.

^ap= 0.0001 vs SHF

^bp= 0.0075 vs SHS

^cp= 0.0029 vs WKY

less than 5mmHg. The mean ($\pm$ SEM) left ventricular volumes were 0.10 ± 0.01 , 0.08 ± 0.01 , and 0.11 ± 0.01 for the SHF, SHS, and WKY, respectively. There were no significant differences between groups in either basal LVEDP (Figure 4-5), or the left ventricular volumes at which the hearts were perfused.

The maximal increase in the LVEDP during ischemia was significantly greater in the SHF compared with the SHS ($p=0.0001$) and the WKY ($p=0.0003$) (Figure 4-5). There was no significant difference in the LVEDP between groups at the midpoint of ischemia. The greatest increase in LVEDP in the SHF hearts occurred after the hearts stopped beating, and therefore represents the development of contracture (Figure 4-6).

During the initial two minutes of reperfusion the LVEDP plateaued at the elevated end ischemic level in the SHF (Figure 4-6). The LVEDP increased transiently at the beginning of reperfusion in the SHS and WKY hearts. With continued reperfusion, the LVEDP decreased toward the control value in all groups, but the recovery of LVEDP was less complete in the SHF which had a greater sustained increase in the LVEDP at end reperfusion compared with the WKY ($p=0.0025$) (Figure 4-5).

End diastolic pressure-volume (P-V) curves were obtained and the slopes were determined as a measure of LV stiffness. The mean ($\pm$ SEM) slope of the control P-V curves were 244 ± 47 , 313 ± 60 , and 251 ± 77 mmHg/ml in the SHF, SHS, and WKY, respectively. The large variability of the slope of the P-V curves obtained in this study precluded meaningful comparisons of LV stiffness between groups. The observed variability may reflect the contribution of the compliance properties of several feet of fluid filled tubing connecting the transducer to the balloon.

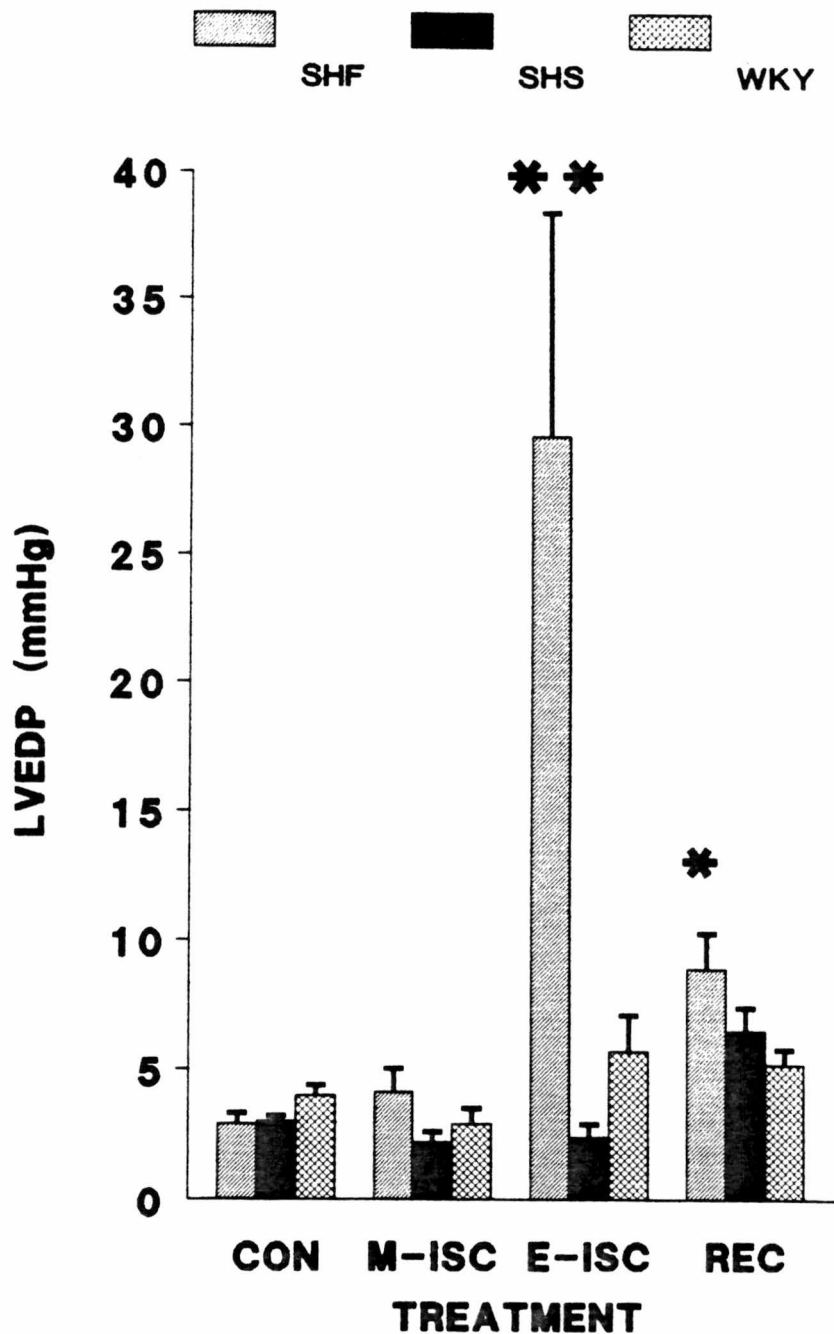


Figure 4-5. Changes in Left Ventricular End Diastolic Pressure. Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p = 0.0025$ vs. WKY; ** $p \leq 0.0003$ vs. WKY and SHS.

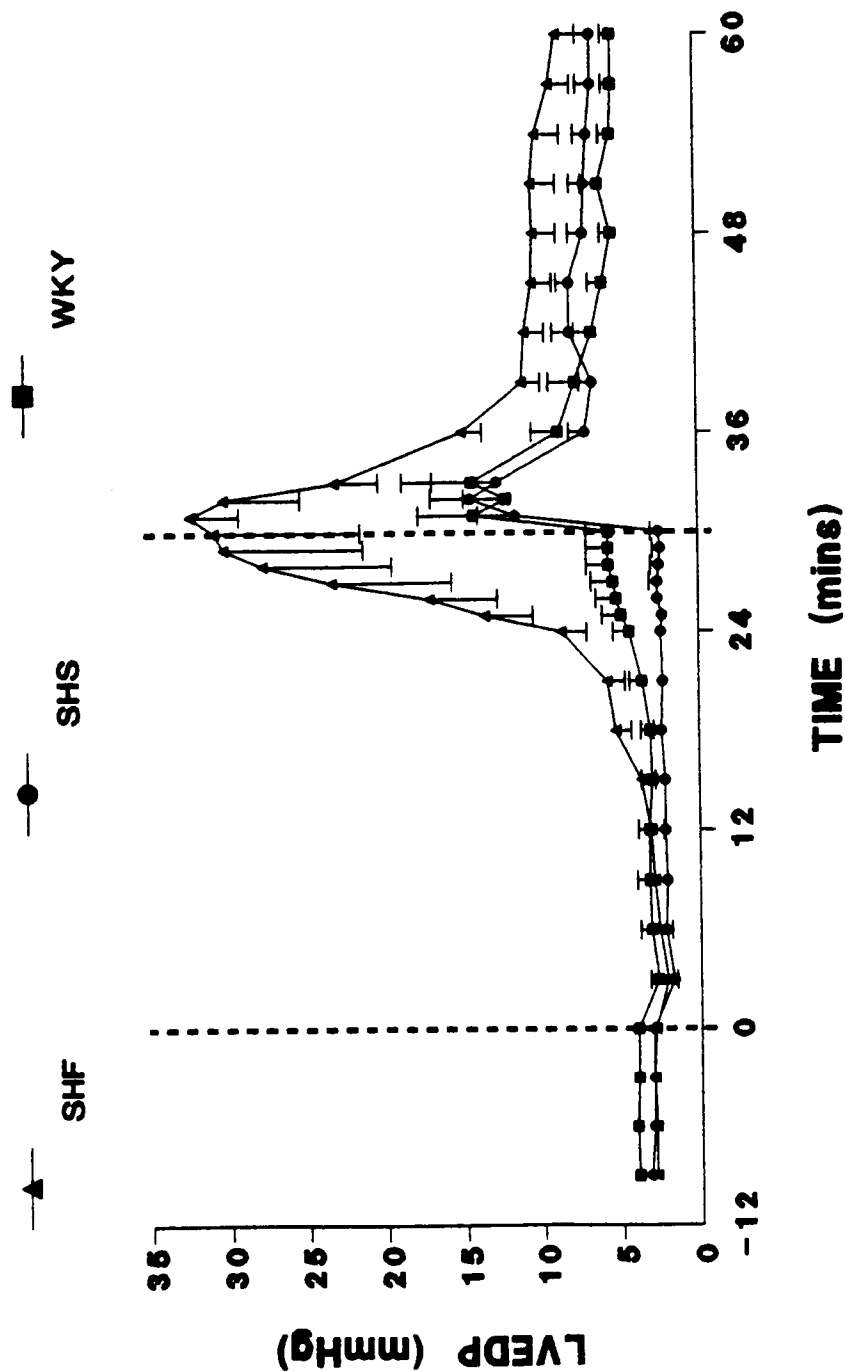


Figure 4-6. Scatter Plot of Left Ventricular End Diastolic Pressure Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point, respectively.

Left Ventricular Wall Stress

To correct for between group differences in LV volume and weight, LV pressures were used to calculate both developed, and end diastolic wall stress (Table 4-6). Developed stress ($LVDEV\sigma$), like developed pressure, tended to be higher in the SHR groups compared with the WKY, but the differences were not statistically significant. Therefore, apparent differences in control pressure development in the SHF and SHS hearts were negated when corrections were made for small between group differences in the weight and volume of the hearts.

The maximal decrease in $LVDEV\sigma$ at end ischemia was greater in the SHF compared with the WKY, but this difference did not reach statistical significance. The value of $LVDEV\sigma$ was zero in the SHF at end ischemia and was significantly lower than the $LVDEV\sigma$ in both the WKY and the SHS ($p=0.0001$). The decrease in $LVDEV\sigma$ also tended to be greater in the SHS compared with the WKY, but this difference was not statistically significant. Similarly, the value of $LVDEV\sigma$ was not lower in the SHS at end ischemia compared with the WKY. There were no significant differences between groups in the recovery of $LVDEV\sigma$.

The control end diastolic wall stress ($LVED\sigma$), unlike the $LVEDP$, was significantly lower in the SHF ($p=0.013$) and the SHS ($p=0.0017$) compared with the WKY. The increase in $LVED\sigma$ at end ischemia was significantly greater for the SHF compared with both the SHS ($p=0.0001$) and the WKY ($p=0.0006$). This greater increase in $LVED\sigma$ for the SHF resulted in an end ischemic value of $LVED\sigma$ in the SHF which was significantly greater than that for the SHS ($p=0.0001$) or the WKY ($p=0.0006$).

There was a significantly greater sustained increase in $LVED\sigma$, with respect to control, at end reperfusion in the SHF compared with the WKY ($p=0.004$). The recovery

Table 4-6. ESTIMATES OF LEFT VENTRICULAR WALL STRESS

GROUP	TREATMENT	n	DEVELOPED σ (g/cm ²)	DIASTOLIC σ (g/cm ²)
SHF	CONTROL	7	31.1 $\pm$ 4.4	0.9 $\pm$ 0.2 ^c
	ISCHEMIA	7	0.0 $\pm$ 0.0 ^{ab}	8.8 $\pm$ 2.8 ^{ab}
	REPERFUSION	7	31.1 $\pm$ 4.6	2.7 $\pm$ 0.5
SHS	CONTROL	13	31.2 $\pm$ 2.1	0.8 $\pm$ 0.1 ^d
	ISCHEMIA	13	3.9 $\pm$ 0.3	0.6 $\pm$ 0.1
	REPERFUSION	13	31.8 $\pm$ 2.4	1.7 $\pm$ 0.2
WKY	CONTROL	8	24.8 $\pm$ 2.6	1.4 $\pm$ 0.2
	ISCHEMIA	8	4.5 $\pm$ 0.6	2.0 $\pm$ 0.6
	REPERFUSION	8	28.5 $\pm$ 2.9	1.9 $\pm$ 0.3

Notes: Values are mean $\pm$ SEM. n is the number of observations.

Developed σ is LV Developed Stress; Diastolic σ is LV End Diastolic Stress.

^a p = 0.0001 vs SHS at end ischemia.

^b p = 0.0001 vs WKY at end ischemia.

^c p = 0.013 vs WKY at control.

^d p = 0.0017 vs WKY at control.

of LVED σ also tended to be less complete in the SHF compared with the SHS, but this difference did not attain statistical significance.

Therefore, the results of the comparisons of systolic and diastolic function using wall stress are consistent with those obtained by analyzing left ventricular pressures in that both indicate that systolic and diastolic function were more severely impaired during ischemia in the SHF compared with the other groups. Although the control LVDEV σ was not significantly higher in the SHR groups, the fact that the SHF and SHS developed an equivalent amount of systolic force at lower end diastolic stress is indicative of superior force development during control in both SHR groups compared with the WKY.

Intracellular Na⁺

One minute ²³Na spectra were acquired at three minute intervals throughout the experiment. Sample ²³Na spectra are shown in Figure 4-7. To control for the between heart variability in the measured values of Na⁺ content, the Na⁺ data were expressed as a fraction of the control value (Na⁺%). However, the Na⁺ data were also analyzed with Na⁺ expressed in μ moles/g dry weight to verify that conclusions made on the basis of analyses of Na⁺% were generally consistent with those using Na⁺ content.

Changes in the intracellular Na⁺ expressed as a fraction of the control value (Na⁺%) are shown in Figure 4-8. The Na⁺% increased during ischemia in all three groups, but both the rate and magnitude of the increase were greater in the SHF (Figure 4-8). The Na⁺ increased early during ischemia in the SHS and WKY to mean values less than 1.5 times the control value, and then plateaued for the duration of ischemia. In contrast, Na⁺% increased rapidly in the SHF to values greater than twice the control value, and continued to rise with no apparent plateau during the ischemic period. The mean rate of

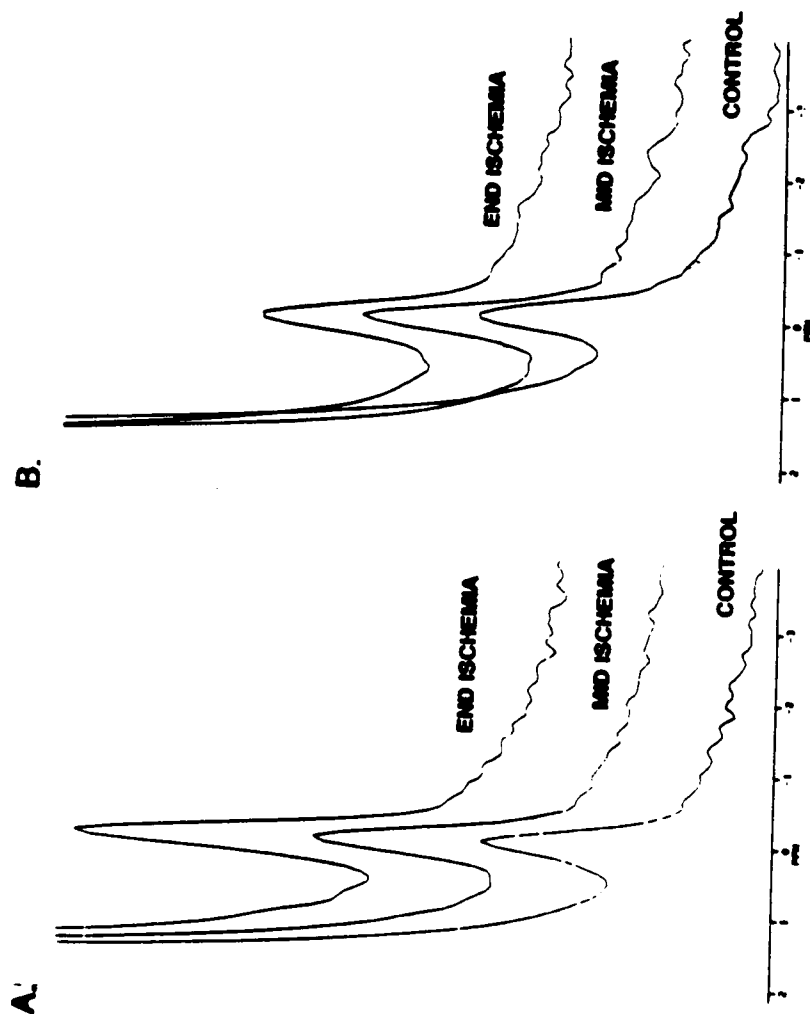


Figure 4-7. Typical ^{23}Na NMR Spectra Showing Changes in Intracellular Na^+ During Ischemia for the SHF (A) and WKY (B). Spectra for the SHF were similar to those shown for the WKY. The mid ischemia spectra were acquired during minute 13 of ischemia, and the end ischemia spectra were acquired during minute 27 of ischemia. Each spectrum is the average of 336 FIDs collected over a 1 minute period. Data were collected using 90° pulses, an acquisition time of 0.154 seconds, and a dead time of $96.3\mu\text{sec}$. Data were accumulated using 2K data points and a spectral width of 6667 Hz. The FIDs were multiplied by a Gaussian function to improve resolution.

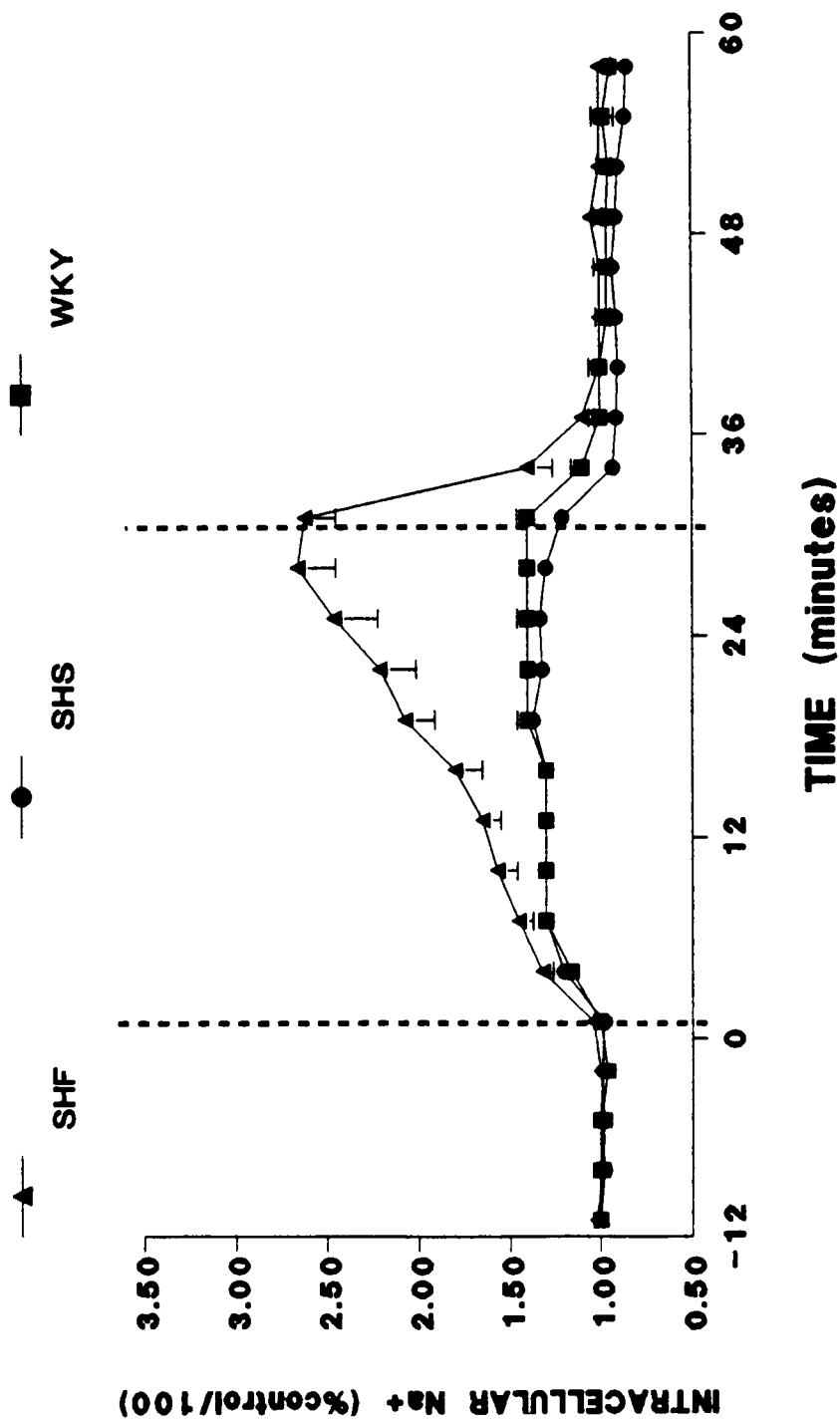


Figure 4-8. Scatter Plot of Changes in Intracellular Na⁺% Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point, respectively. Note that the mean rate of increase of Na⁺ for the last 15 minutes of ischemia was greater in the SHF compared with the SHS and WKY ($p < 0.0004$). The recovery of Na⁺ following reperfusion also required more time in the SHF compared with SHS ($p = 0.0075$).

increase for the last 15 minutes of ischemia was significantly greater in the SHF compared with both the WKY ($p=0.0004$) and the SHS ($p=0.0001$). The mean rate of increase for the initial 15 minutes was also greater in the SHF but the differences did not attain statistical significance.

The increase in $\text{Na}^+\%$ after 15 minutes of ischemia was significantly greater in the SHF compared with both the SHS ($p=0.0004$) and the WKY ($p=0.0007$) (Figure 4-9). The greater increase in intracellular $\text{Na}^+\%$ in the SHF compared with the WKY and the SHS was further magnified at end ischemia ($p=0.0001$). There were no significant differences between the SHS and WKY in the increase in $\text{Na}^+\%$ at either the midpoint or the end of ischemia. The intracellular $\text{Na}^+\%$ returned to control levels in all three groups following reperfusion. However, the time required for the Na^+ to return to the control value was less in the SHS compared with both the SHF ($p=0.0075$) and the WKY ($p=0.0075$) (Table 4-5. Figure 4-8).

Plots similar to those above are shown for intracellular Na^+ content expressed in $\mu\text{moles/g}$ in Figures 4-10 and 4-11. There were no significant differences between groups in the values of the Na^+ content at control (Figure 4-10). At the midpoint of ischemia the increase in Na^+ content was greater in the SHF compared with the WKY ($p=0.008$), but not compared with the SHS. The increase in Na^+ content at end ischemia was greater in the SHF compared with both the WKY ($p=0.0001$) and the SHS ($p=0.0001$). The actual value of Na^+ content was significantly higher in the SHF compared with the SHS ($p=0.0058$) and the WKY ($p=0.004$) at the end of ischemia, but not at the midpoint of ischemia. Normalization of Na^+ values to the control level facilitated the detection of a greater change in Na^+ in the SHF compared with the SHS at the midpoint of ischemia that was not apparent using Na^+ content, but analyses of $\text{Na}^+\%$ and Na^+ content at end

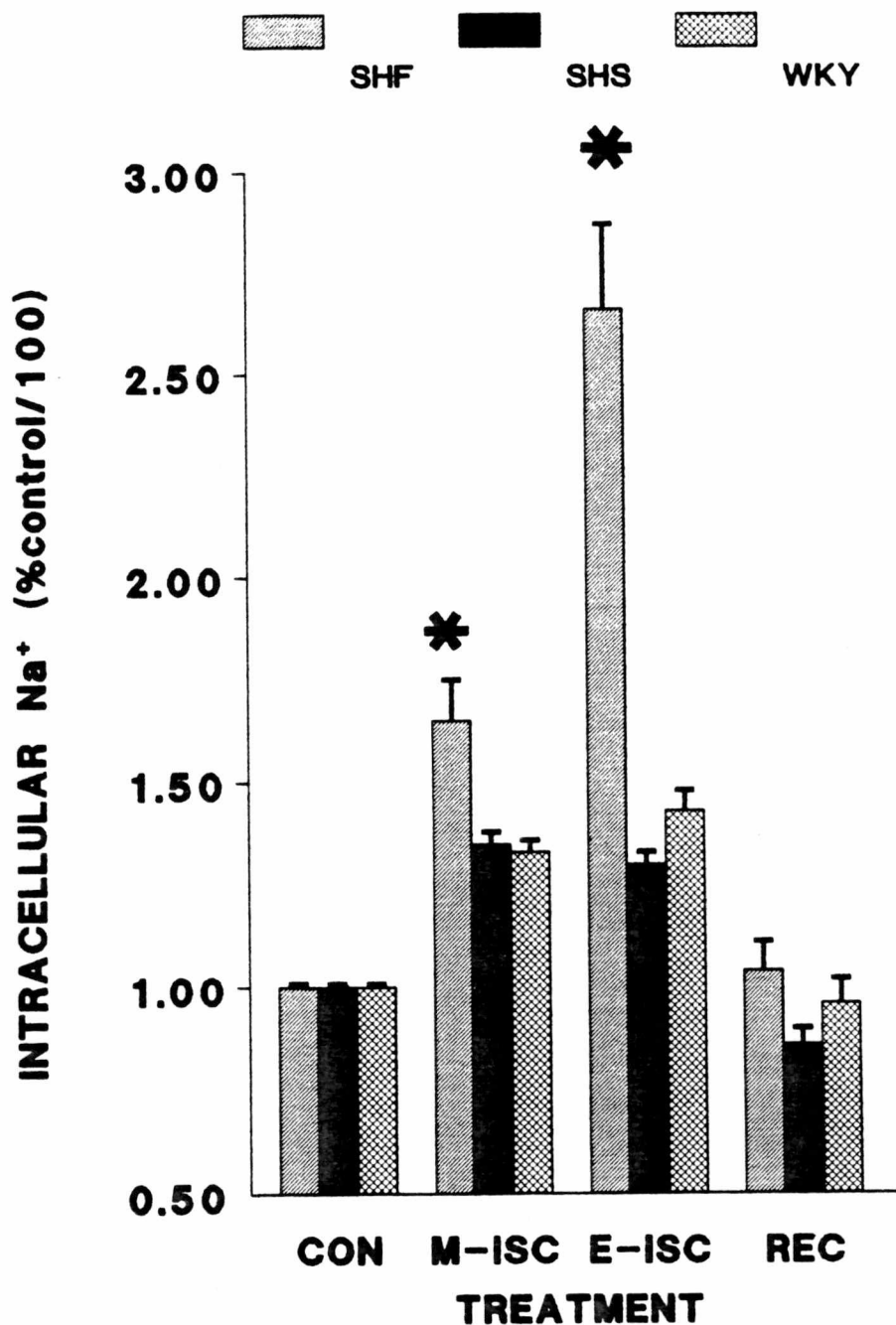


Figure 4-9. Changes in Intracellular Na^+ . Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p \leq 0.0007$ vs SHS and WKY.

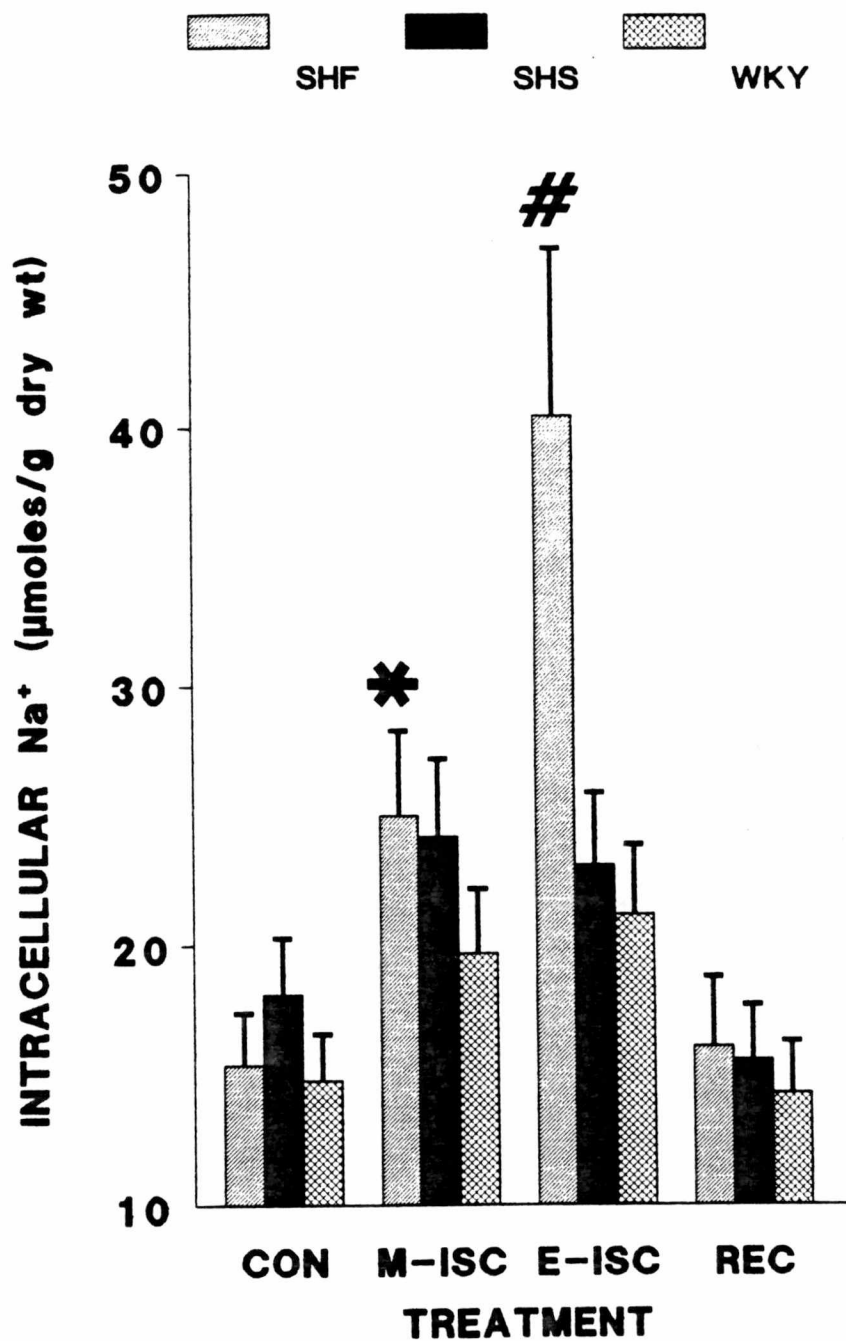


Figure 4-10. Intracellular Na^+ Content During Control, Ischemia and Reperfusion. Bars are mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p = 0.0001$ vs WKY, # $p = 0.0001$ vs SHS and WKY.

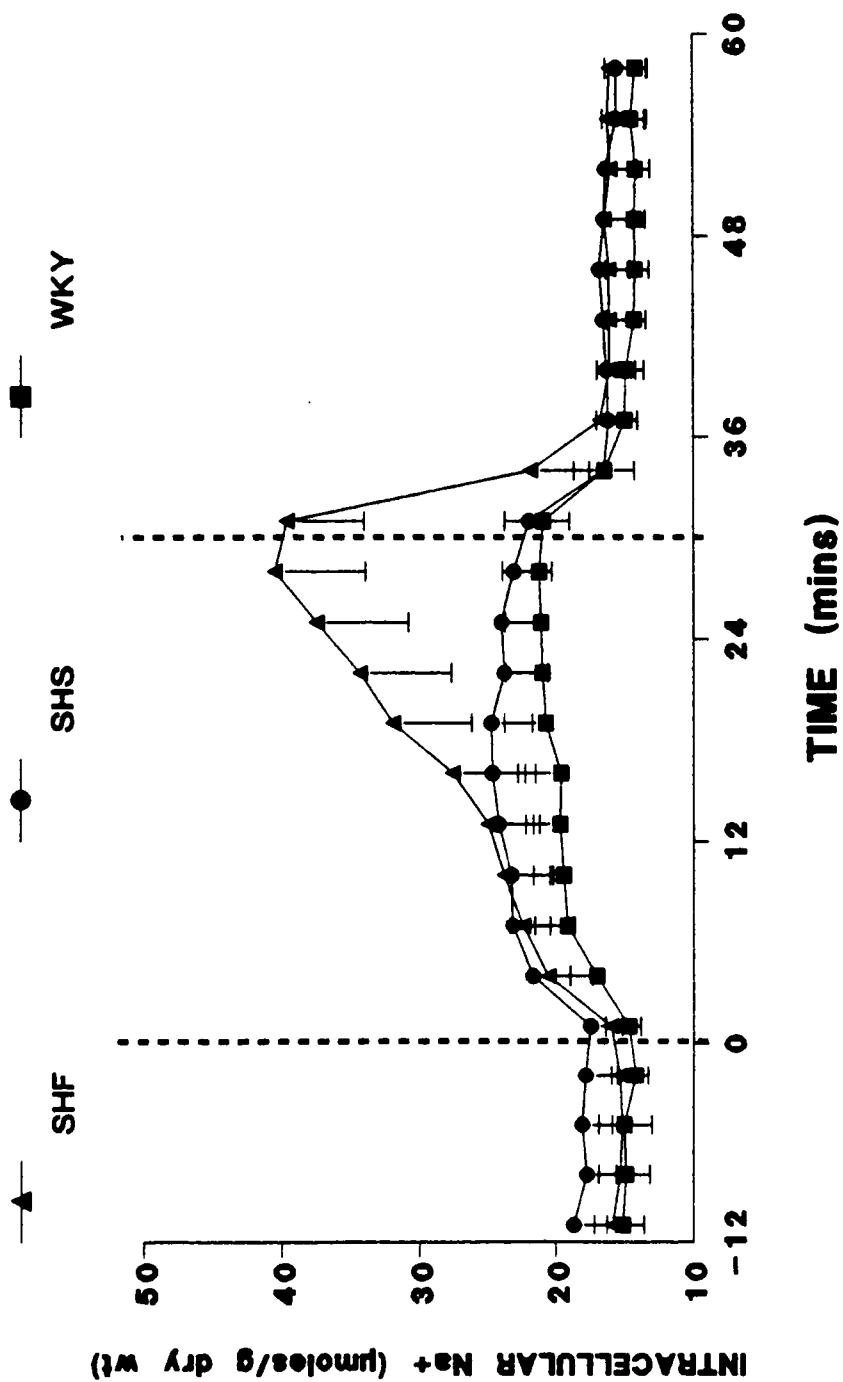


Figure 4-11. Plot of the Intracellular Na^+ Content Across Time. Symbols are the mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point, respectively. Note that this plot represents the same data presented in Figure 4-8 except that Na^+ is expressed in $\mu\text{moles/g}$ dry weight rather than as a fraction of the control content.

ischemia produced similar results.

Intracellular pH

Since one of the proposed mechanisms for the increase in Na^+ during ischemia involves $\text{Na}^+ - \text{H}^+$ exchange, it was important to monitor changes in intracellular pH. There were no significant differences in the control pH between groups (Figure 4-12). The decrease in pH was similar during the first fifteen minutes of ischemia in all three groups (Figure 4-13). However, the pH declined more rapidly during the later half of ischemia in the SHF so that the mean rate of decrease for the last fifteen minutes of ischemia was significantly greater for the SHF compared with the SHS ($p=0.017$). The mean rate of decrease in pH was also greater in the WKY, but this difference did not reach statistical significance. There was no significant difference between groups in the decrease in pH at the midpoint of ischemia. However, the maximum decrease in pH at the end of ischemia was significantly greater for both the WKY ($p=0.0057$) and the SHF ($p=0.0059$) compared with the SHS. (Figure 4-12).

The pH returned to the control value in all three groups following reperfusion, but the time required for complete recovery was significantly longer in the WKY compared with the SHS ($p=0.0029$) (Table 4-5, Figure 4-13). Since the pH was lower at end ischemia in the WKY and SHF, the time to recovery reflected the removal, or buffering, of disparate amounts of H^+ in different groups. Therefore, the increase in pH and decrease in H^+ after 3 minutes of reperfusion with respect to the end ischemic value were compared between groups to estimate the initial rate of recovery of pH or H^+ . The mean ($\pm$ SEM) recovery of pH was 0.27 ± 0.05 , 0.23 ± 0.03 and 0.10 ± 0.05 pH units in the SHF, SHS, and WKY groups, respectively. These changes in pH correspond to a

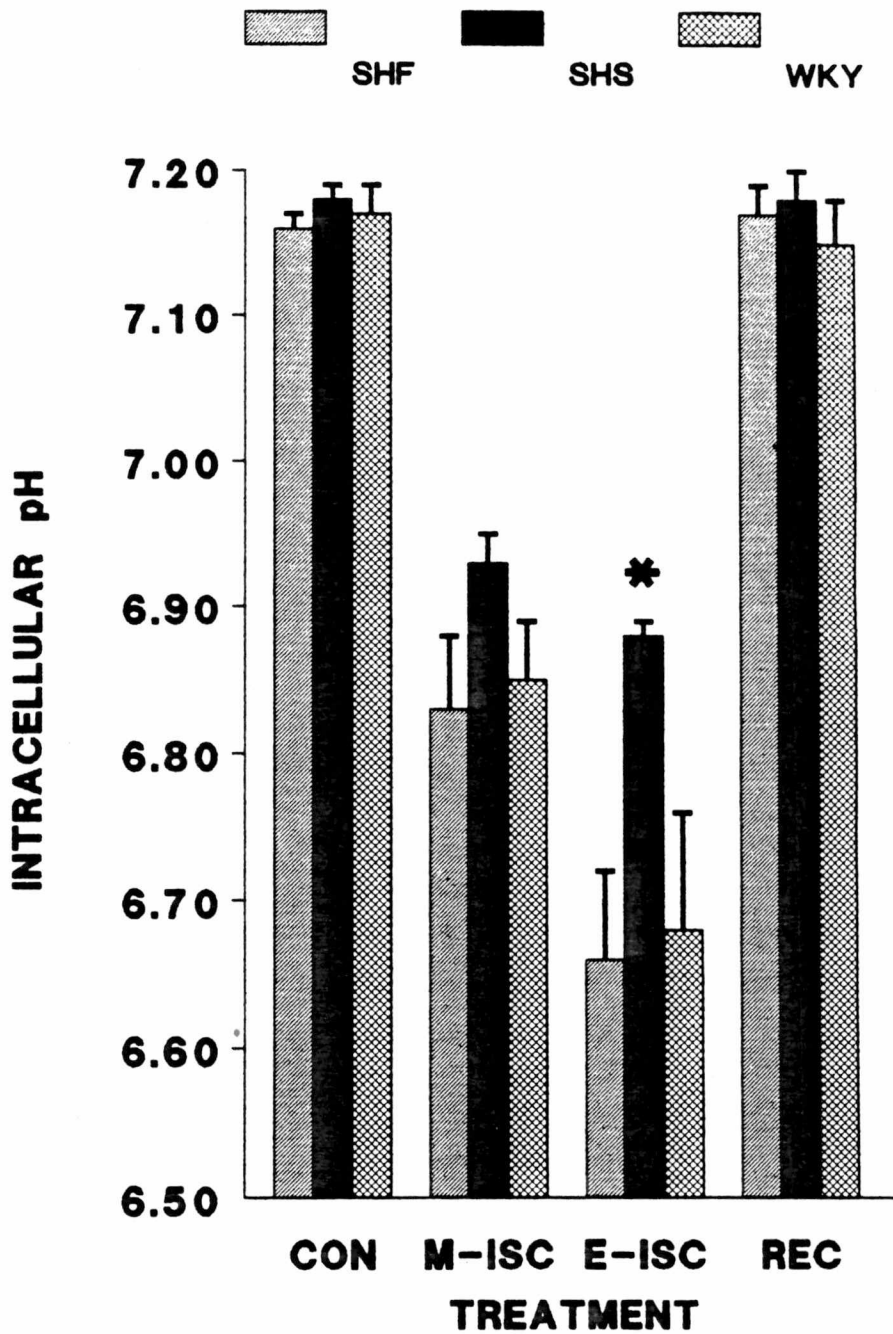


Figure 4-12. Changes in Intracellular pH. Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p \leq 0.006$ vs. SHF and WKY.

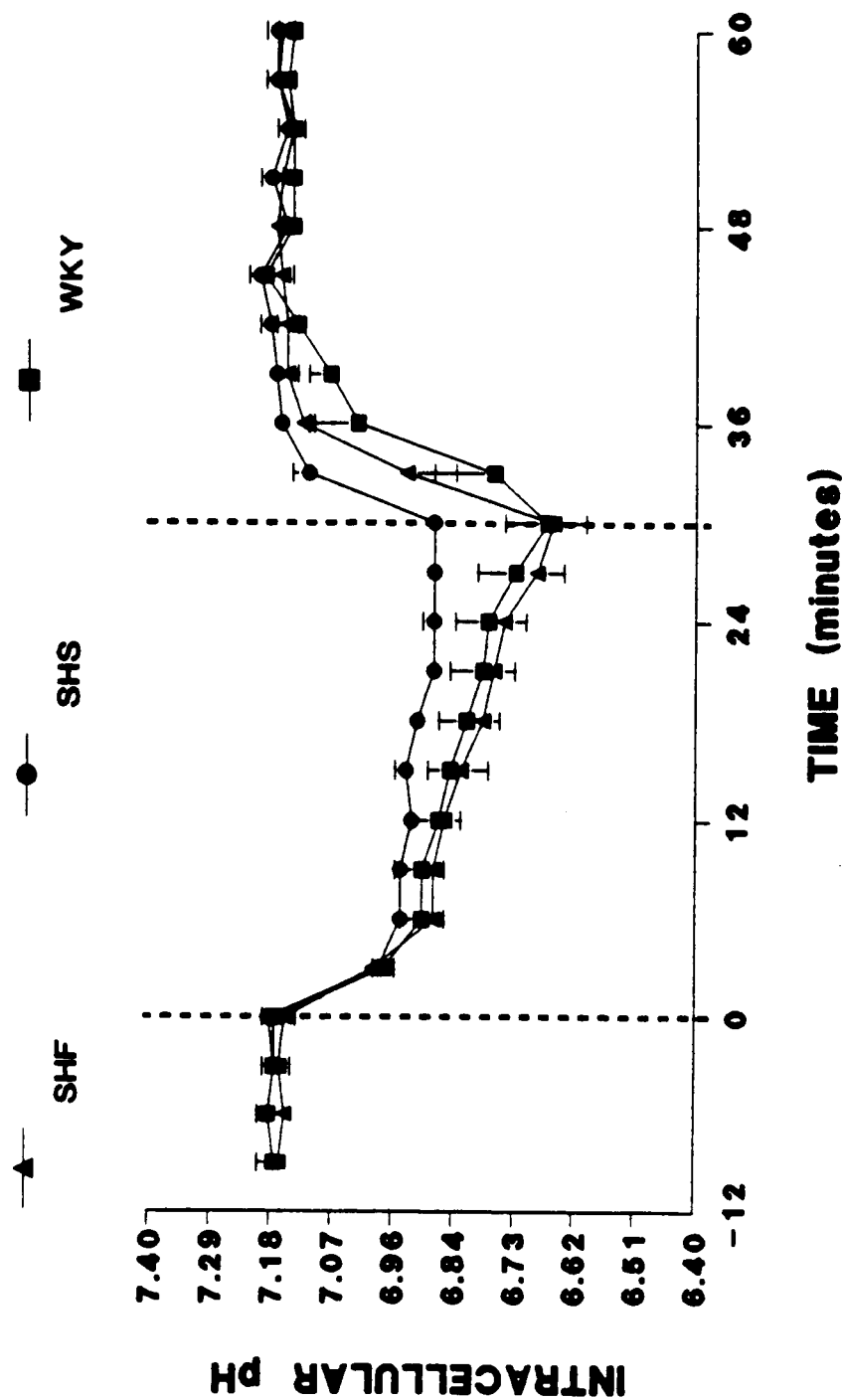


Figure 4-13. Scatter Plot of Intracellular pH Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point, respectively. Note the mean rate of decrease during the last 15 minutes of ischemia was significantly greater in the SHF compared with the SHS ($p = 0.017$). The time to recovery of pH was longer in the WKY compared with the SHS ($p = 0.0029$).

decrease in the $[H^+]$ of $0.1\mu M$, $0.05\mu M$ and $0.02\mu M$ for the SHF, SHS, and WKY, respectively, for the initial 3 minutes of reperfusion. Therefore, the initial rate of recovery of the pH (or $[H^+]$) was greater in both the SHF and the SHS compared with the WKY, and the difference was statistically significant for the SHF ($p \leq 0.0136$).

Changes in High Energy Phosphates

The net increase in intracellular Na^+ reflects the balance between the influx of Na^+ and the removal of Na^+ by the Na^+-K^+ ATPase. The removal of Na^+ via the Na^+-K^+ ATPase is energy dependent. Therefore, the energy status of the hearts in this study was monitored by measuring changes in the intracellular content of ATP, PCr and P_i . Typical ^{31}P spectra are shown in Figure 4-14. Changes in high energy phosphates were measured in all hearts, but phosphorus standards were not used in some experiments. It was possible to calculate the myocardial content of high energy phosphates in $\mu moles/g$ dry weight for 4 SHF hearts, 12 SHS hearts and 7 WKY hearts.

The control PCr content ($\mu moles/g$ dry wt) tended to be lower in both the SHS and SHF compared with the WKY, but these differences were not statistically significant (Figure 4-15). The PCr decreased rapidly in all three groups, and there were no significant differences between groups in the rate of decrease for either the initial or final half of the ischemic period (Figure 4-16). Similarly, there were no differences between groups in the magnitude of the decrease in PCr at either the midpoint or end of ischemia (Figure 4-15). The PCr content recovered to a mean value greater than the control value for all three groups, and there were no differences between groups in the time required for recovery.

There were no significant differences in the control ATP content ($\mu moles/g$ dry wt)

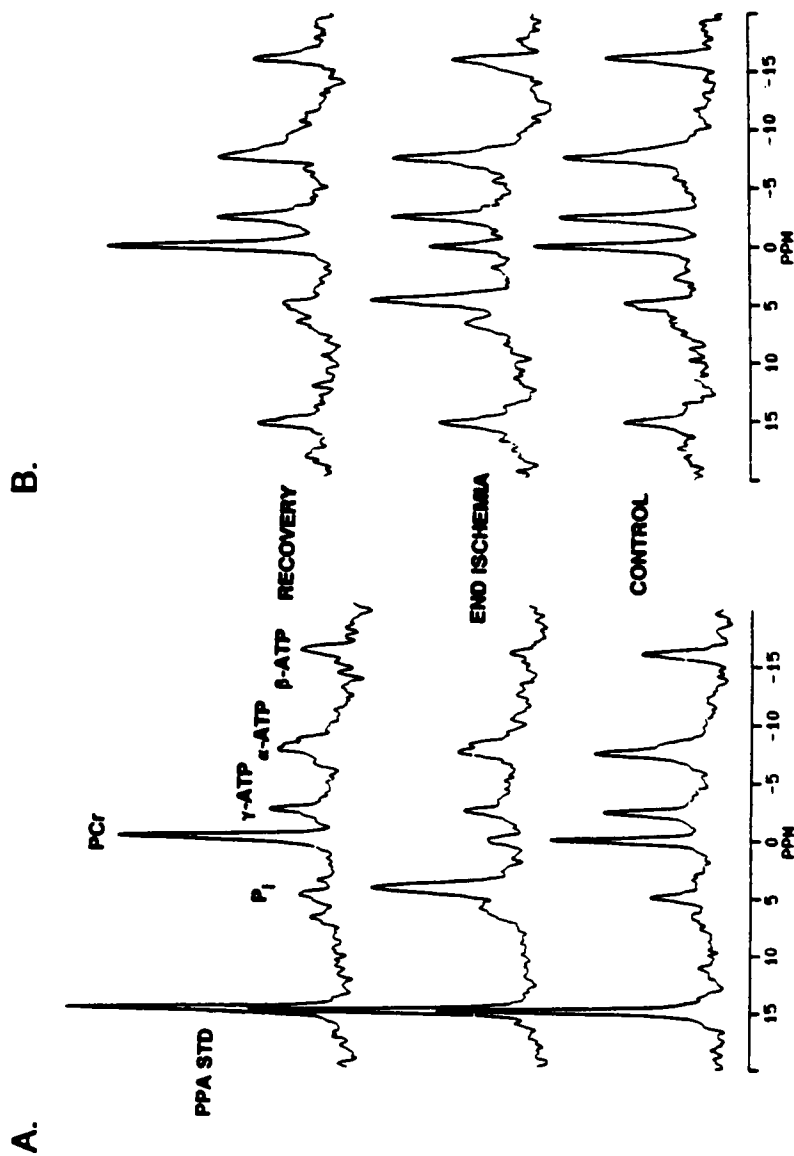


Figure 4-14. Typical ^{31}P NMR Spectra Showing the Changes in High Energy Phosphates During Ischemia for the SHF (A) and WKY (B). The control spectra were acquired during the last two minutes of control, the end ischemia spectra were acquired during the last two minutes of ischemia, and the recovery spectra were acquired during the last two minutes of reperfusion. PPA STD is phenylphosphonic acid standard. Each spectrum is the average of 56 FIDs collected over a two minute period. Data were acquired using a 60° pulse angle, an interpulse delay of 1.85 seconds, 2K data points, and a spectral width of 7042 Hz. Exponential multiplication of the FIDs corresponding to a line broadening of 40 Hz was used to improve the signal-to-noise.

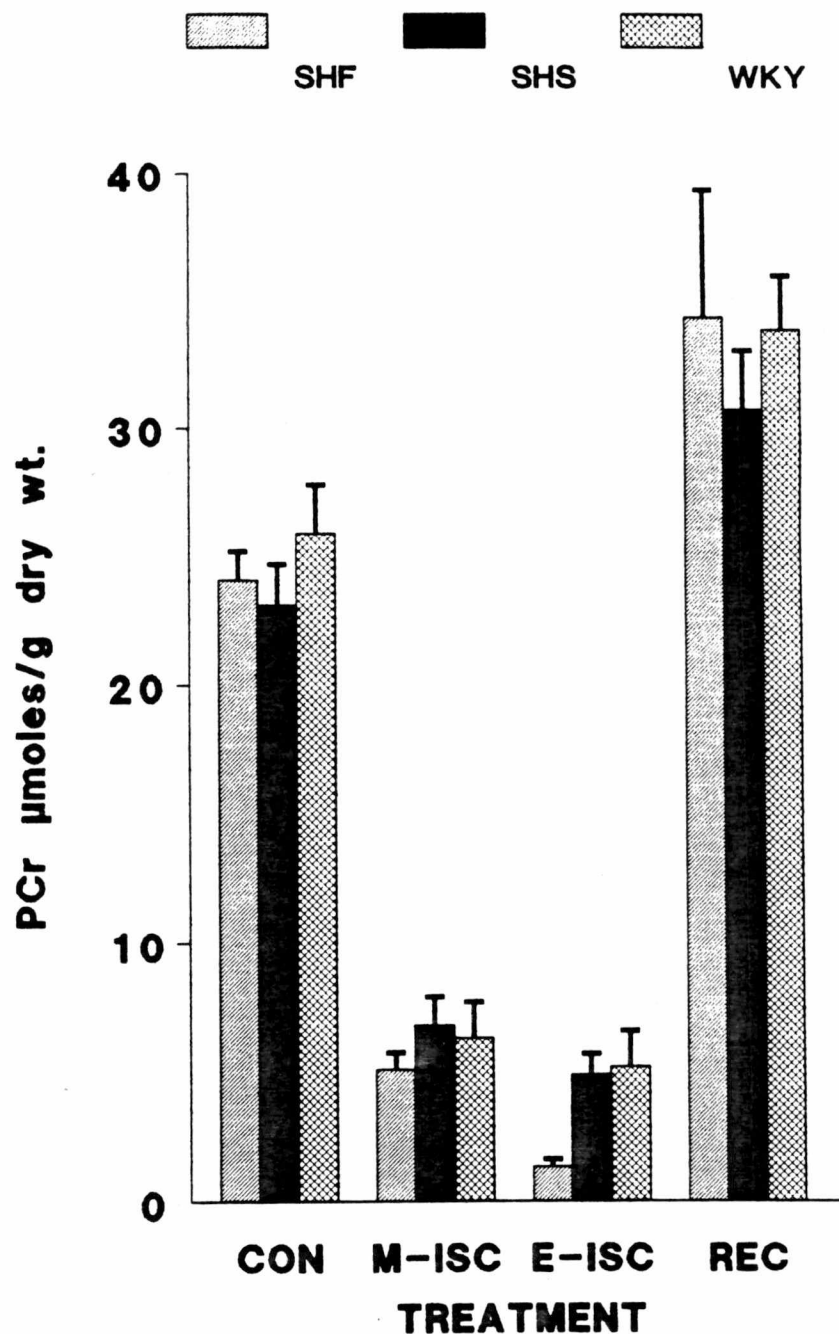


Figure 4-15. Changes in Phosphocreatine. Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details.

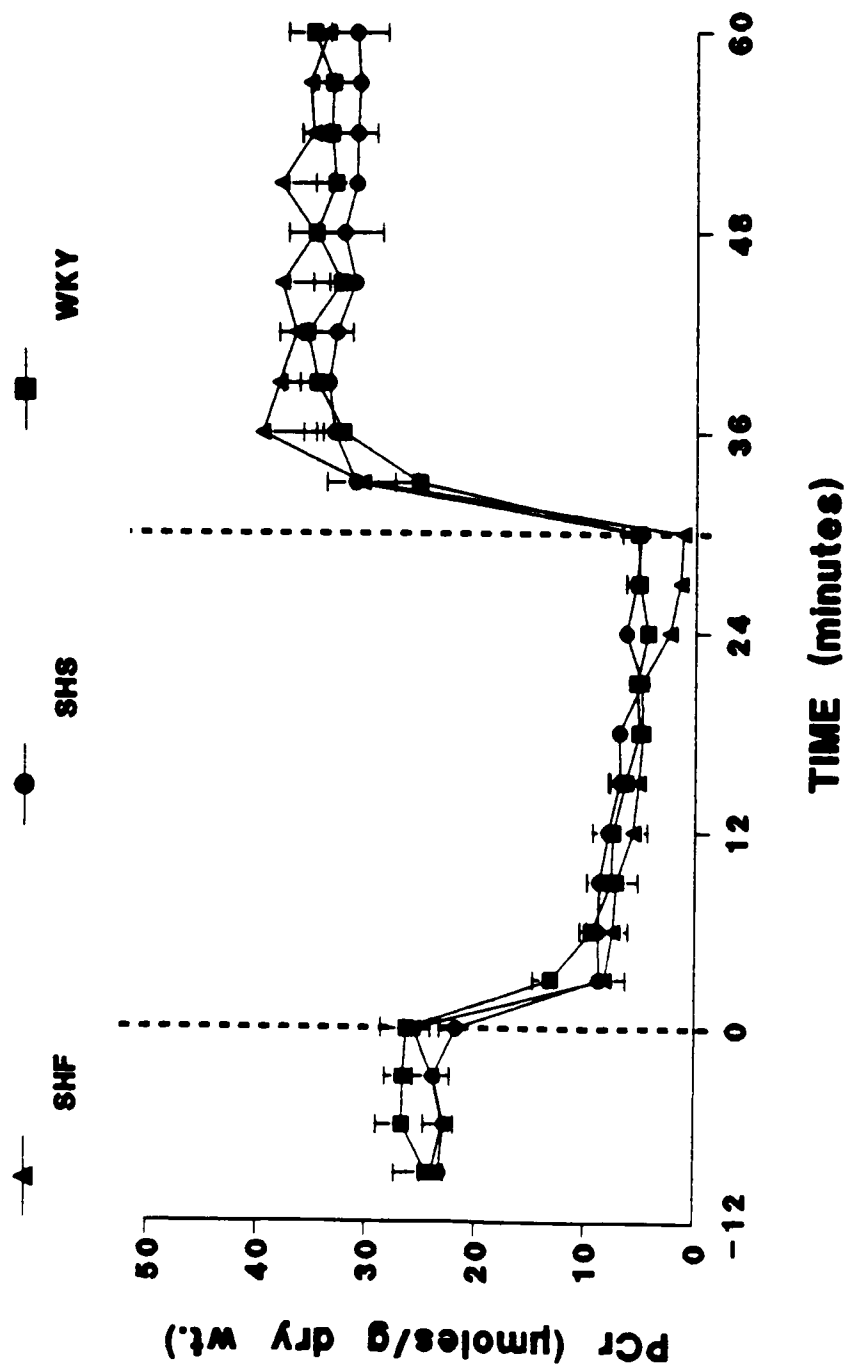


Figure 4-16. Scatter Plot of Phosphocreatine Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point, respectively. There were no significant differences between groups in the rate of decrease in PCr during ischemia or the recovery following reperfusion.

between groups (Figure 4-17). There were also no significant differences between groups in the decrease in the ATP content at the midpoint of ischemia. The mean rate of decrease in ATP during the last half of the ischemic period tended to be greater in the SHF compared with the SHS, but the difference was not statistically significant (Figure 4-18). The maximal decrease in ATP at end ischemia was significantly greater in the SHF compared with both the WKY ($p=0.0068$) and the SHS ($p=0.0023$) (Figure 4-17). Changes in ATP were similar for the SHS and the WKY. The recovery of ATP was incomplete in all three groups following reperfusion, and ATP recovery was significantly less in the SHF compared with the SHS ($p=0.0165$) (Figure 4-17).

There were no significant differences in the control P_i content between groups (Figure 4-19). The mean rate of increase in P_i for the last 15 minutes of the ischemic period was significantly less in the SHS compared with the SHF ($p=0.0025$) (Figure 4-20). The increase in P_i at the midpoint of ischemia was significantly greater in SHF compared with the SHS ($p=0.0043$). Additionally, the maximum increase in P_i at end ischemia was significantly greater in both the SHF ($p=0.0001$) and the WKY ($p=0.0032$) compared with the SHS. The P_i content recovered completely in all three groups following reperfusion.

The free energy of ATP hydrolysis (ΔG) represents energy available to do work in the cell, and is, therefore, a more elegant variable for summarizing the energy status of the heart. The control ΔG was less in both of the SHR groups compared with the WKY group, but this difference only reached statistical significance for the SHS group ($p=0.001$) (Figure 4-21). There were no differences between groups in the decrease in ΔG at the midpoint of ischemia, but the maximal decrease in ΔG was significantly greater at end ischemia in the SHF compared with the SHS ($p=0.0077$). Although the decrease in ΔG also tended to be greater in the SHF compared with the WKY this difference was

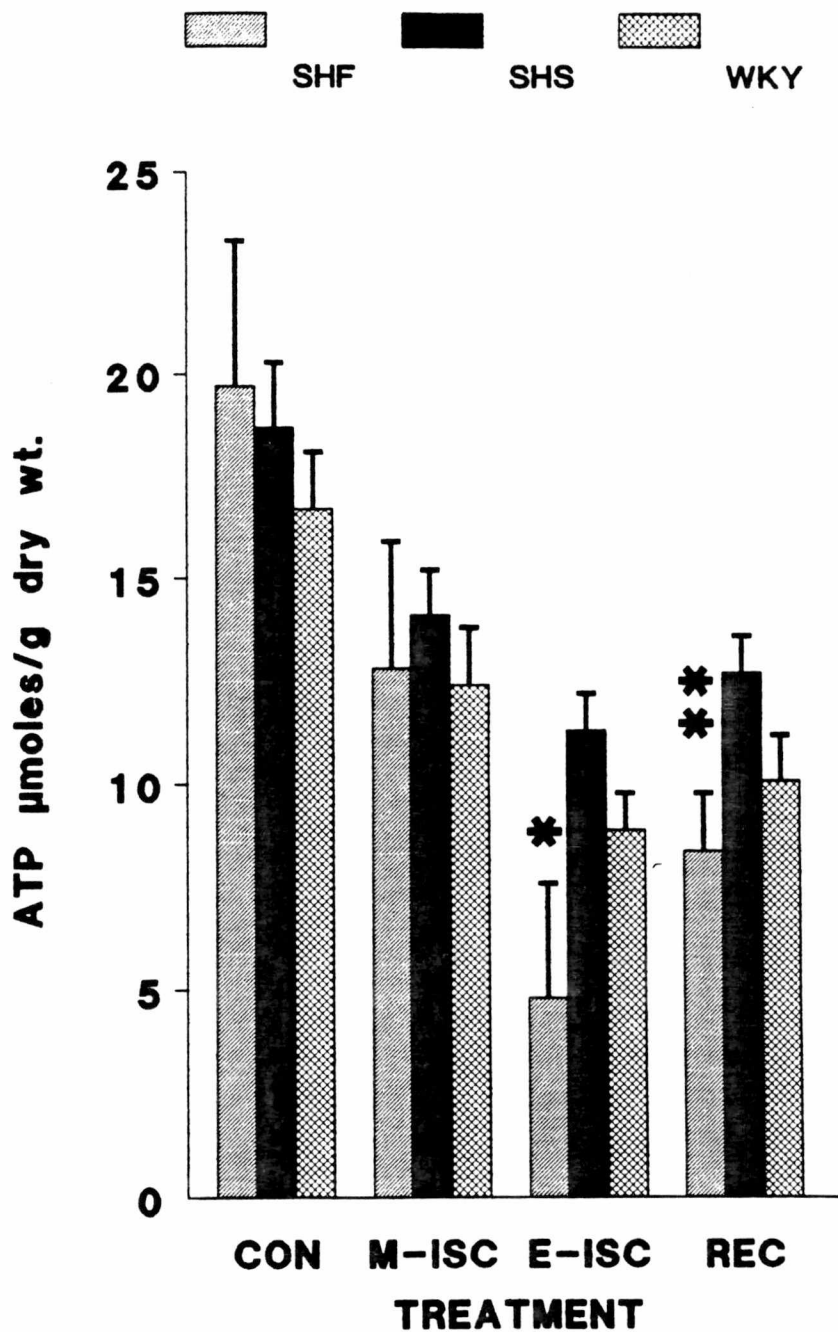


Figure 4-17. Changes in ATP. Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p \leq 0.0068$ vs WKY and SHS; ** $p = 0.0165$ vs. SHS.

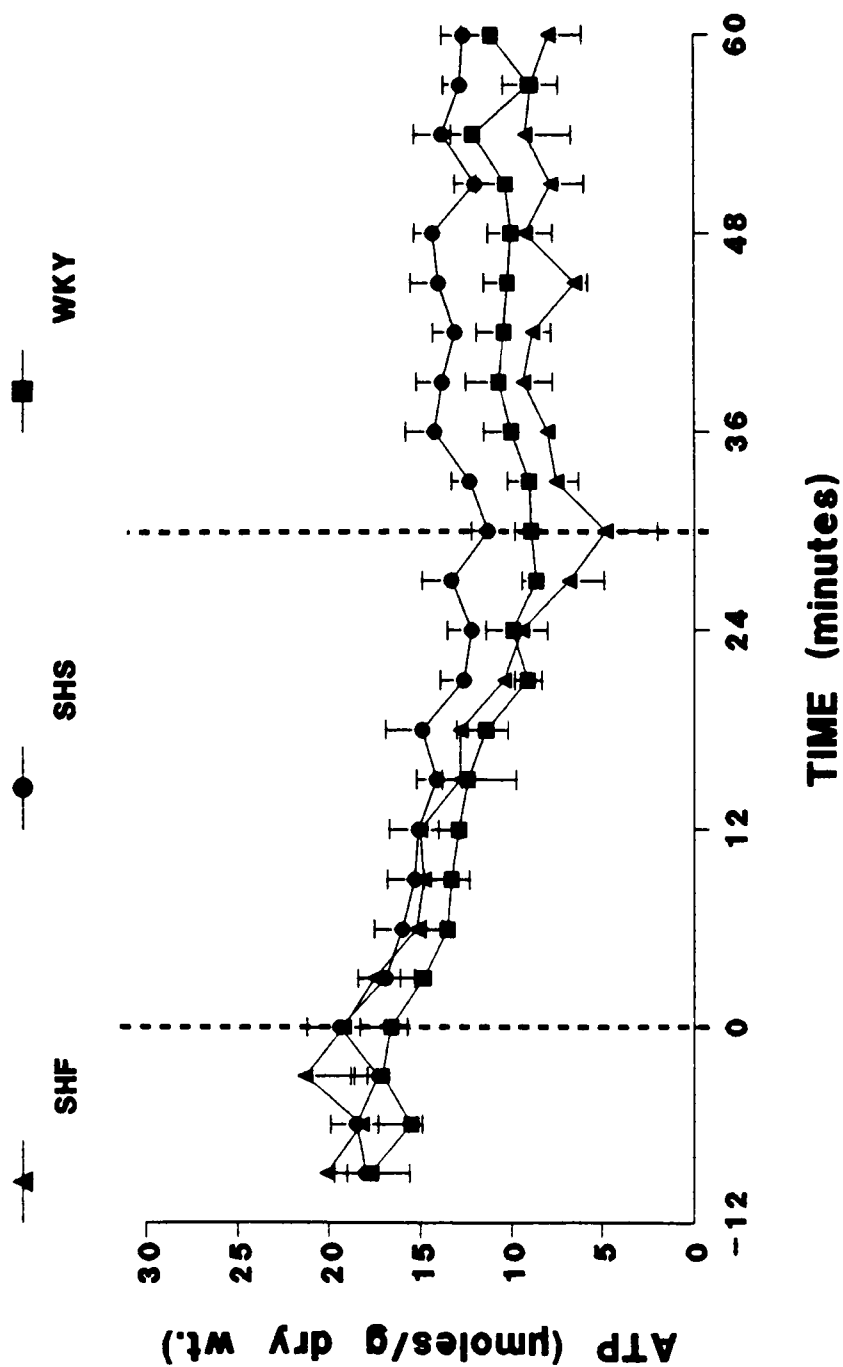


Figure 4-18. Scatter Plot of ATP Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point, respectively.

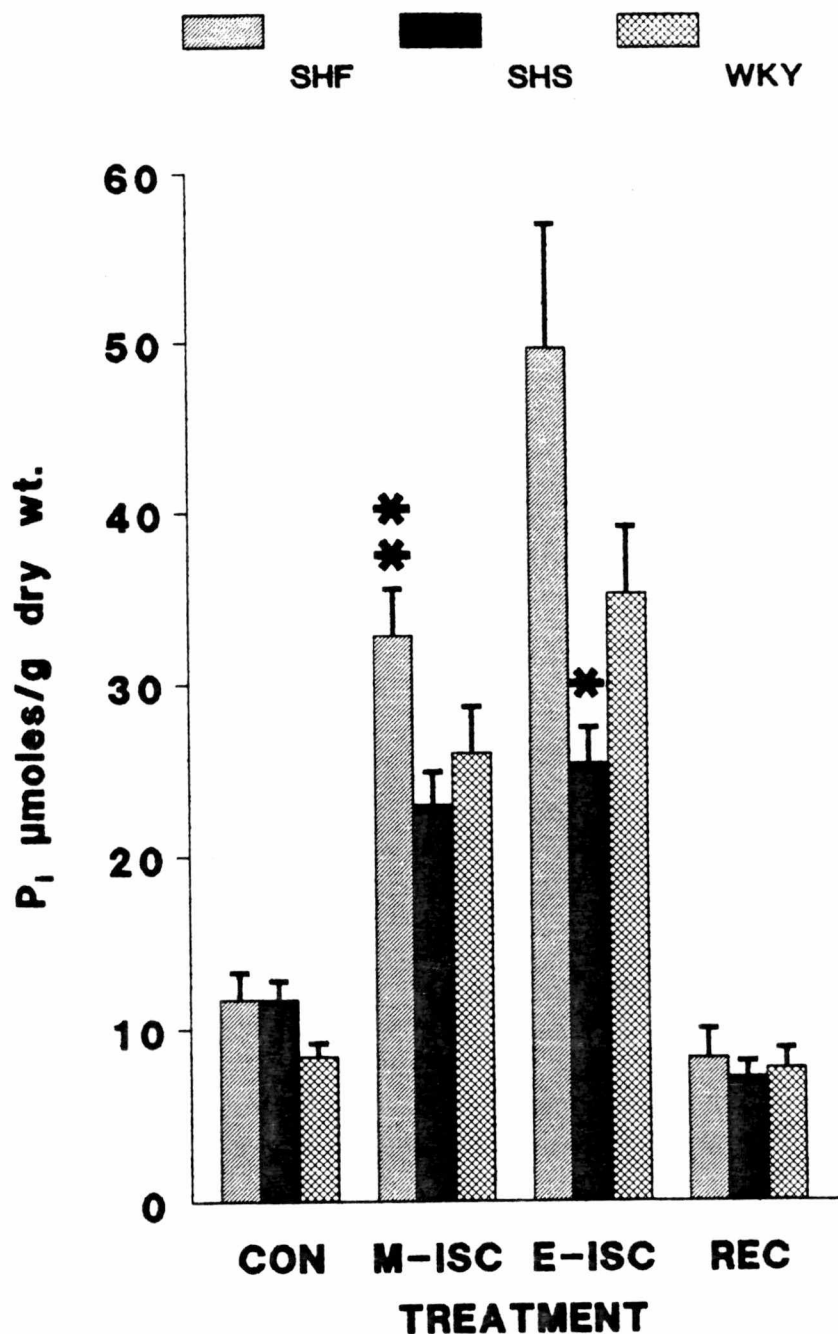


Figure 4-19. Changes in Inorganic Phosphate. Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC) and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p \leq 0.0032$ vs SHF and WKY; ** $p = 0.0043$ vs SHS.

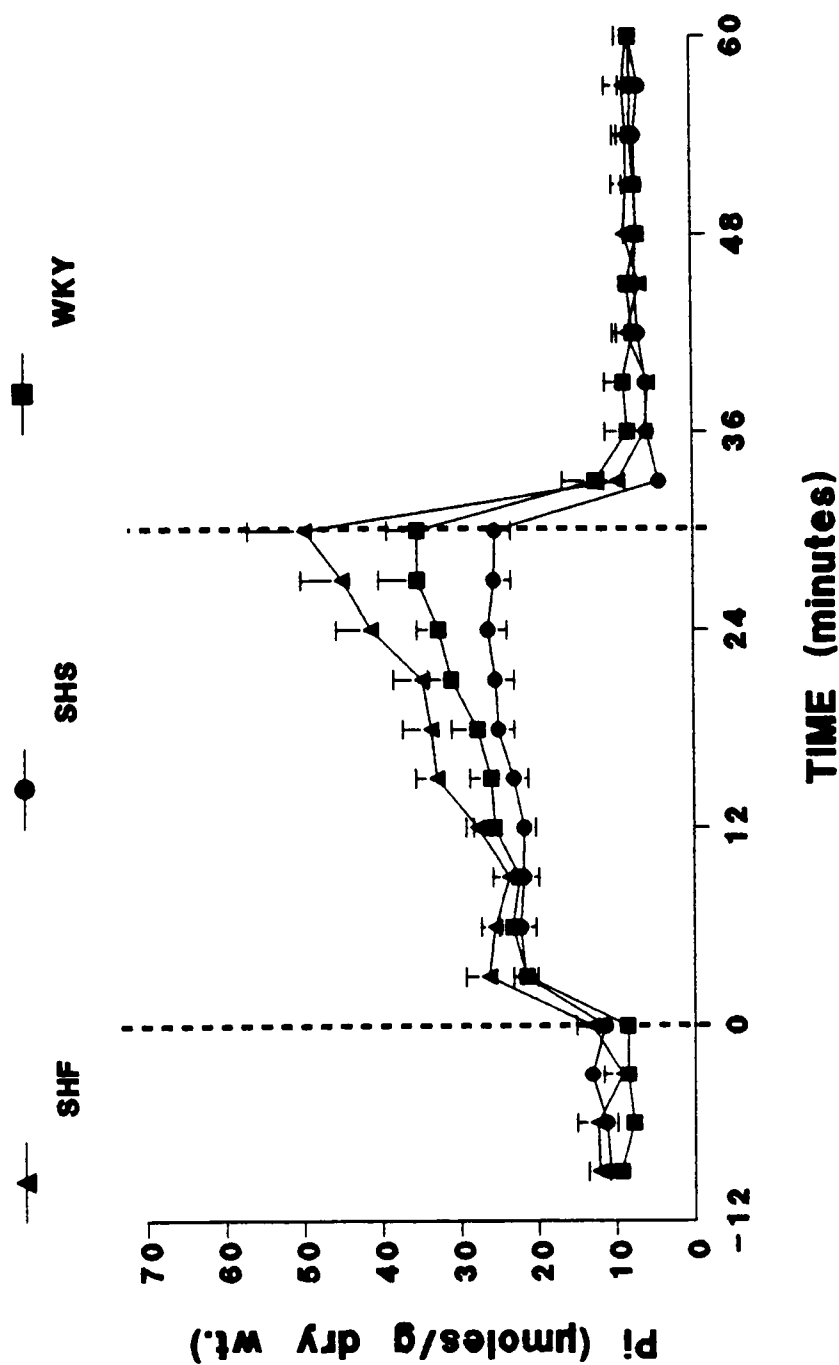


Figure 4-20. Scatter Plot of Inorganic Phosphate Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point, respectively. The mean rate of increase of P_i was greater for the SHF compared with the SHS ($p = 0.0025$) during the last 15 minutes of ischemia.

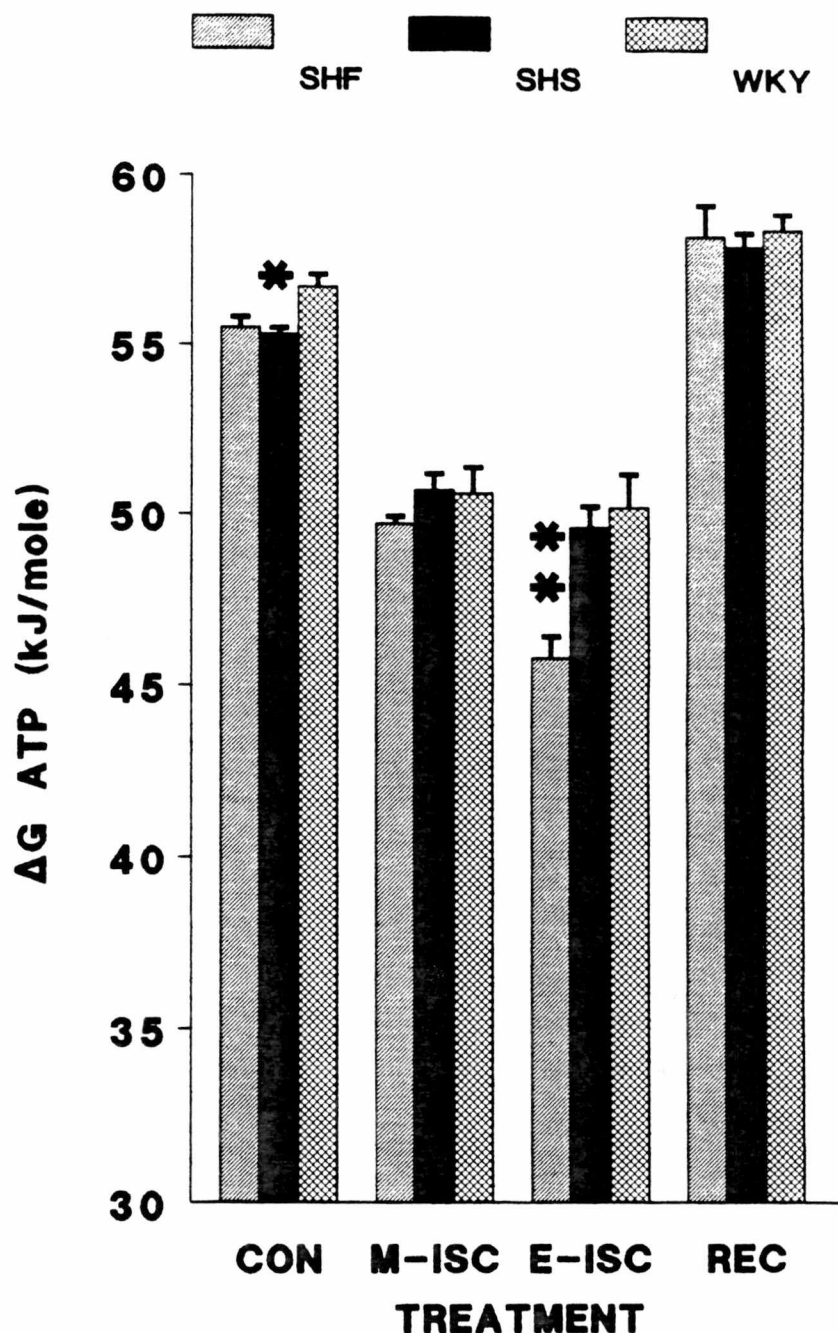


Figure 4-21. Changes in ΔG of ATP Hydrolysis. Bars represent mean $\pm$ SEM for each of the three groups for the 12 minute control period (CON), minutes 13-15 of ischemia (M-ISC), the final 3 minutes of ischemia (E-ISC), and the final 6 minutes of reperfusion (REC). Data at M-ISC, E-ISC and REC were analyzed as the mean change with respect to control. See Methods for details. * $p = 0.001$ vs. WKY; ** $p = 0.0077$ vs. SHS.

not statistically significant. Since the control ΔG was lower in the SHS and SHF hearts, the actual values of ΔG at end ischemia were also compared. The value of ΔG was significantly lower at end ischemia in the SHF compared with both the SHS ($p=0.0065$) and the WKY ($p=0.0045$).

The changes in ΔG across time are shown in Figure 4-22. The rate of change in ΔG was similar for the three groups during the first fifteen minutes of ischemia. However, ΔG decreased significantly more rapidly in the SHF during the last half of ischemia, and the mean rate of decrease in ΔG during the last half of ischemia was greater in the SHF compared with the WKY ($p=0.0115$). The rate of decrease in ΔG also tended to be greater in the SHF compared with the SHS during the last half of ischemia, but the difference was not statistically significant. The ΔG recovered to control values, or above, within the first three minutes of reflow in all hearts (Figure 4-22, Table 4-5).

Lactate Production

Total lactate production was measured in 6 SHS hearts, 2 SHF hearts and 4 WKY hearts to determine whether SHR hearts exhibited greater lactate production compared with control hearts during low flow ischemia. The results are shown in Figure 4-23. The combined lactate efflux from the heart measured during 30 minutes of ischemia, and the first 6 minutes of reperfusion was significantly higher in SHS hearts compared with WKY hearts ($p=0.0243$). The mean lactate production for the two SHF hearts was higher than that of either the SHS or the WKY, but lactate data were collected for an insufficient number of SHF hearts to allow meaningful statistical comparisons.

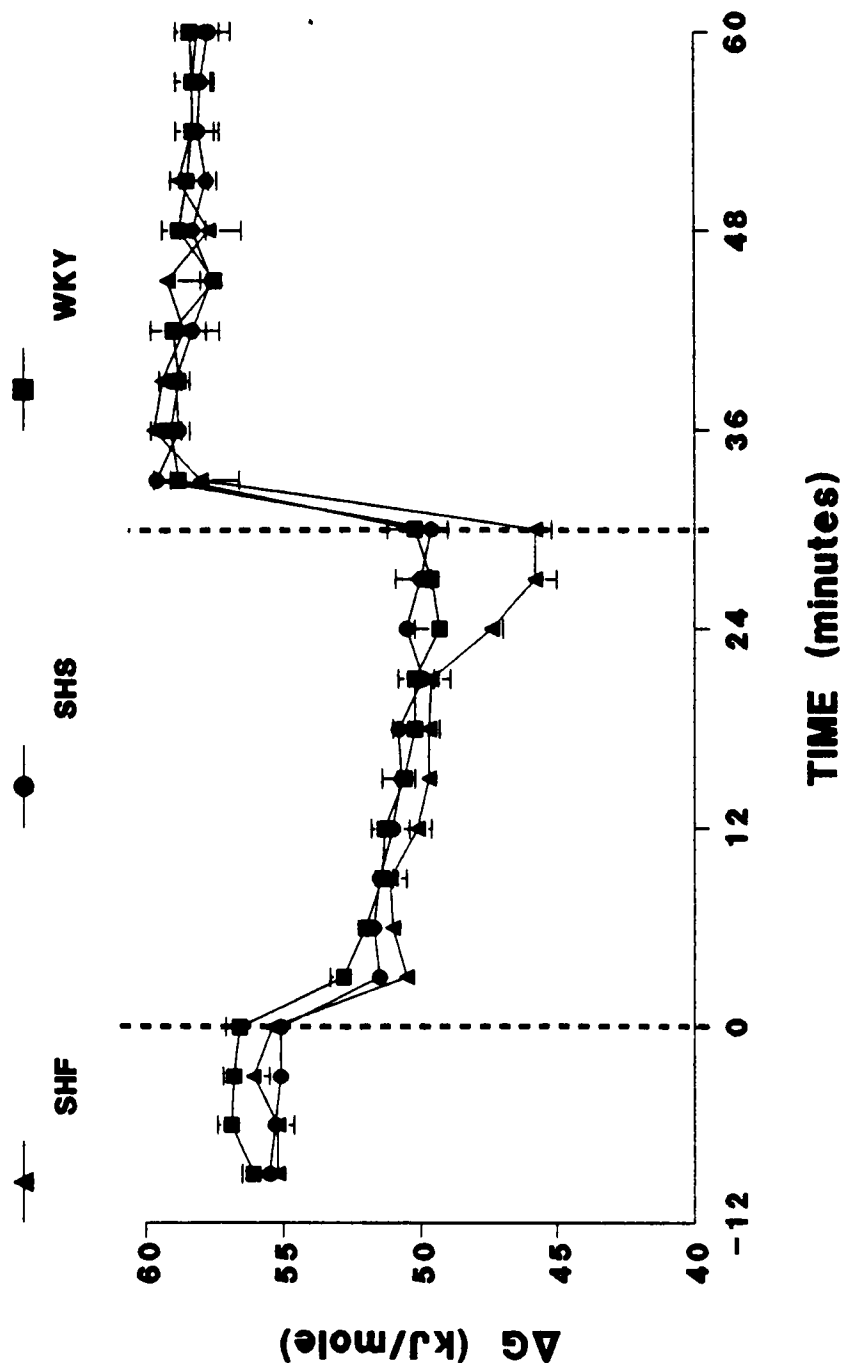


Figure 4-22. Scatter Plot of ΔG of ATP Hydrolysis Across Time. Symbols are mean $\pm$ SEM for each of the three groups as indicated. Broken vertical lines represent the final control time point and the final ischemia time point respectively. The mean rate of decrease of ΔG during the last 15 minutes of ischemia was significantly greater in the SHF compared with the WKY ($p = 0.0115$).

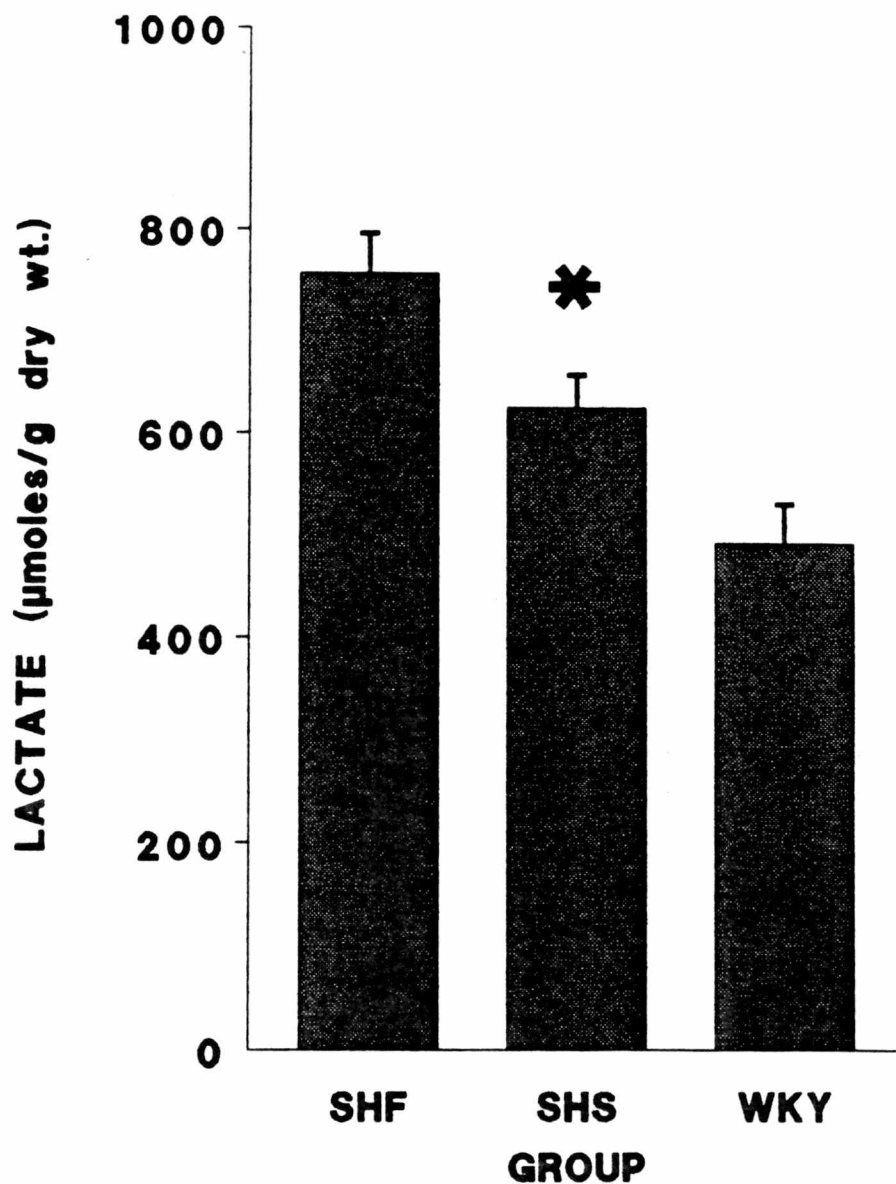


Figure 4-23. Lactate Production. Bars represent mean $\pm$ SEM for total lactate efflux from the heart during 30 minutes of low flow ischemia and 6 minutes of reperfusion. Samples taken after 6 minutes of reperfusion did not contain detectable concentrations of lactate. The number of hearts in each group were 2, 6, and 4 for the SHF, SHS, and WKY, respectively. * $p = 0.0243$ vs. WKY.

Predictors of the Increase in Na⁺

The change in Na⁺ during ischemia may, theoretically, be linked to the intracellular pH (through Na⁺-H⁺ exchange) and/or the energy status of the cell as it controls the Na⁺-K⁺ ATPase activity. Correlations of pH and high energy phosphates with Na⁺% at the midpoint of ischemia are shown in Table 4-7. The PCr/P_i ratio was included in the analysis so that high energy phosphate data for the hearts in which phosphate standards were not used could be included. At the midpoint of ischemia the Na⁺% was significantly correlated with the pH and the PCr/P_i ratio (Table 4-7). The PCr/P_i ratio was the best independent predictor of the Na⁺% at this time point. Multiple linear regression analysis indicated that the slope of the regression of Na% with pH was not significant with PCr/P_i in the model. Therefore, the PCr/P_i accounted for more of the variability in the Na⁺% than the pH after 15 minutes of ischemia.

Correlations of pH and high energy phosphates with Na⁺ content in μmoles/g are shown in Table 4-8 for comparison with analyses using Na⁺%. The Na⁺ content was significantly correlated with PCr/P_i and ΔG at the midpoint of ischemia, but, unlike Na⁺%, was not correlated with the pH.

The Na⁺% at end ischemia was inversely correlated with the values of intracellular pH, ΔG, ATP and PCr/P_i (Table 4-7). The ATP content exhibited the strongest simple correlation with Na⁺%, and was, therefore, the best independent predictor of the Na⁺% at end ischemia. Furthermore, a multiple linear regression analysis indicated that the slopes of the relationships of Na⁺% with pH and other measures of high energy phosphates became insignificant with ATP also included in the model.

The Na⁺ content at end ischemia was also most highly correlated with ATP, but was not significantly correlated with the pH (Table 4-8). Therefore, there was a detectable

Table 4-7. PREDICTORS OF Na⁺% AFTER 15 AND 30 MINUTES OF ISCHEMIA

VARIABLE	Na ⁺ % MIDPOINT ISCHEMIA			Na ⁺ % END ISCHEMIA	
	n	r	PROB > r	r	PROB > r
pH	28	-0.35	0.0350	-0.47	0.0054
ΔG (kJ/mole)	23	-0.29	NS	-0.54	0.0041
ATP (μmoles/g)	23	-0.29	NS	-0.68	0.0002
PCr/P _i	28	-0.43	0.0111	-0.60	0.0004

Notes: n is the number of observations; r is the correlation coefficient;
NS is not significant; the probabilities are one-tailed.

Table 4-8. PREDICTORS OF Na⁺ CONTENT AFTER 15 AND 30 MINUTES OF ISCHEMIA

VARIABLE	Na ⁺ MIDPOINT ISCHEMIA			Na ⁺ END ISCHEMIA	
	n	r	PROB > r	r	PROB > r
pH	25	-0.03	NS	-0.22	NS
ΔG (kJ/mole)	20	-0.48	0.0150	-0.35	NS
ATP (μmoles/g)	20	-0.12	NS	-0.62	0.0019
PCr/P _i	25	-0.37	0.0355	-0.35	0.0427

Notes: Na⁺ content is expressed as μmoles/g dry weight; n is the number of observations; r is the correlation coefficient;
NS is not significant; the probabilities are one-tailed.

relationship between $\text{Na}^+\%$ and pH, that was not apparent using Na^+ content. However, high energy phosphates were superior predictors of $\text{Na}^+\%$ compared with pH. Thus, analyses using either the $\text{Na}^+\%$ or Na^+ content were consistent in indicating that the magnitude of the increase in intracellular Na^+ was more closely related to the high energy phosphate content of the heart than to the pH at both the midpoint and end of ischemia.

Correlation coefficients of both $\text{Na}^+\%$ and Na^+ content with high energy phosphates and pH, respectively, were also determined across time for the entire 30 minute period of ischemia. The primary purpose of these analyses was to compare the relationships between changes in intracellular Na^+ , and changes in intracellular pH and high energy phosphates, respectively, between groups. The ATP, PCr/P_i , ΔG and pH were significantly correlated with the $\text{Na}^+\%$ across time for all three groups (Table 4-9).

The Na^+ content, like $\text{Na}^+\%$, was inversely correlated with both high energy phosphates and pH across the ischemic period (Table 4-10). However, the correlation of Na^+ content and pH was weaker in all three groups compared with correlations between pH and $\text{Na}^+\%$.

The relationships of $\text{Na}^+\%$ and Na^+ content, respectively, with pH and ΔG were compared between groups by evaluating differences in the slopes of the linear regressions. The linear relationship between the $\text{Na}^+\%$ and the ΔG is shown in Figure 4-24 for each of the three groups. The slope of the relationship between ΔG and $\text{Na}^+\%$ was significantly greater in the SHF compared with both the WKY ($p=0.0001$) and the SHS ($p=0.0001$). There was no difference between the WKY and the SHS hearts. Therefore, for a given decrease in ΔG there was a greater increase in $\text{Na}^+\%$ in the SHF than for either the WKY or the SHS.

Table 4-9. CORRELATIONS WITH Na⁺% DURING 30 MINUTES OF ISCHEMIA

	GROUP								
	SHF			SHS			WKY		
VARIABLE	r	p > r	n	r	p > r	n	r	p > r	n
pH	-0.76	0.0001	77	-0.48	0.0001	143	-0.62	0.0001	88
ΔG (kJ/mole)	-0.71	0.0001	44	-0.55	0.0001	132	-0.60	0.0001	77
PCr/P _i	-0.48	0.0001	76	-0.52	0.0001	143	-0.52	0.0001	88
ATP (μmole/g)	-0.74	0.0001	44	-0.42	0.0001	132	-0.53	0.0001	77

Notes: r is the correlation coefficient; p is the one-tailed probability;
n is the number of observations used in the correlation analysis.

Table 4-10. CORRELATIONS WITH Na⁺ CONTENT FOR 30 MINUTES OF ISCHEMIA

	GROUP								
	SHF			SHS			WKY		
VARIABLE	r	p > r	n	r	p > r	n	r	p > r	n
pH	-0.43	0.0001	77	-0.21	0.0130	110	-0.39	0.0001	88
ΔG (kJ/mole)	-0.80	0.0001	44	-0.27	0.0034	99	-0.47	0.0001	77
PCr/P _i	-0.33	0.0019	77	-0.18	0.0290	110	-0.30	0.0023	88
ATP (μmole/g)	-0.77	0.0001	44	-0.61	0.0001	99	-0.35	0.0008	77

Notes: Na⁺ content is expressed in μmoles/g dry weight; r is the correlation coefficient;
p is the one-tailed probability;
n is the number of observations used in the correlation analysis.

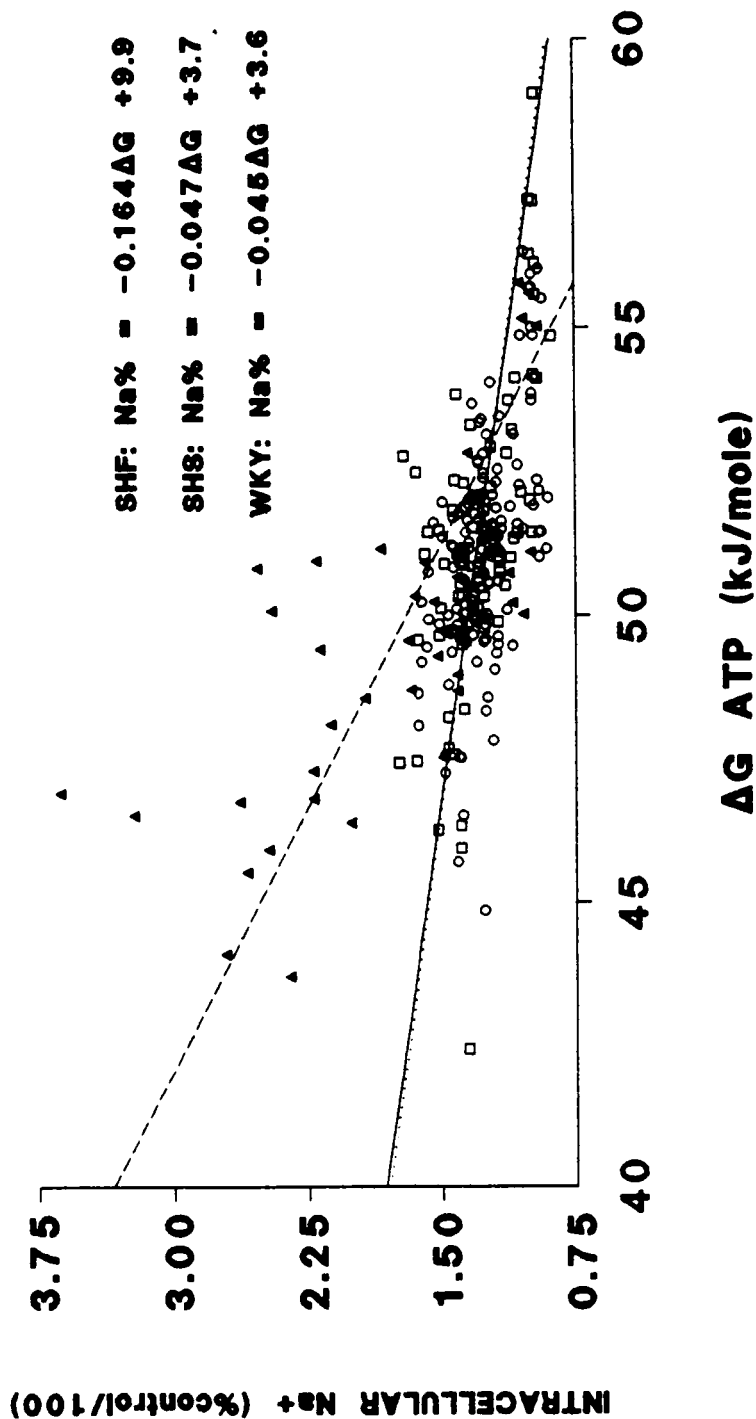


Figure 4-24. Relationship Between the Intracellular Na⁺% and the ΔG for the SHF, WKY and SHS During Ischemia. The symbols are the observed data points taken from the final 3 minutes of control and 30 minutes of ischemia. The dashed line is the regression line for the SHF ($R^2=0.49$, $p=0.0001$). The solid line is the regression line for the SHS ($R^2=0.30$, $p=0.0001$). The dotted line is the regression line for the WKY ($R^2=0.35$, $p=0.0001$). The slope of the regression was significantly greater for the SHF compared with both the WKY ($p=0.0001$) and the SHS ($p=0.0001$). There was no difference in the slopes for the WKY and the SHS.

The results of the comparison of the slopes for the regression of Na^+ content and ΔG were similar to those for $\text{Na}^+\%$ (Figure 4-25). The slope of the regression for the SHF was significantly greater compared with the WKY ($p=0.0321$) and tended to be greater compared with the SHS ($p=0.0812$). The regressions of Na^+ content and ΔG produced low R^2 values compared with the same regressions with $\text{Na}^+\%$ for the SHS and the WKY. The poorer linear fit probably reflects the greater between heart variability in the control values of Na^+ content that was reduced when Na^+ was expressed as $\text{Na}^+\%$.

The between group comparisons of the regression of $\text{Na}^+\%$ with pH are shown in Figure 4-26. For a given decrease in pH there was a significantly greater increase ($p=0.0001$) in the intracellular $\text{Na}^+\%$ in the SHF compared with both the WKY and the SHS. There was no difference in slope between the SHS and the WKY groups. The between group comparisons of the regressions of Na^+ content and pH are shown in Figure 4-27. The slope of the regression for the SHF was significantly greater compared with the slope of the regression for the WKY ($p=0.009$). The slope of the regression for the SHF was also greater compared with the SHS, but the difference did not attain statistical significance. Therefore, the results of the between group comparisons of the relationship of Na^+ content were similar to those using $\text{Na}^+\%$, but the R^2 values were superior when the intracellular Na^+ was normalized to control values.

The ΔG is dependent on the intracellular $[\text{H}^+]$, and the calculated value of ΔG is actually adjusted for this dependence (see methods). Therefore, to evaluate the importance of the collinearity between ΔG and pH, multiple linear regression was also used to examine the relationships of these variables with $\text{Na}^+\%$ and Na^+ content, respectively. The results indicated that both ΔG and pH continued to be significant predictors of the $\text{Na}^+\%$ or Na^+ content even when included in the same model.

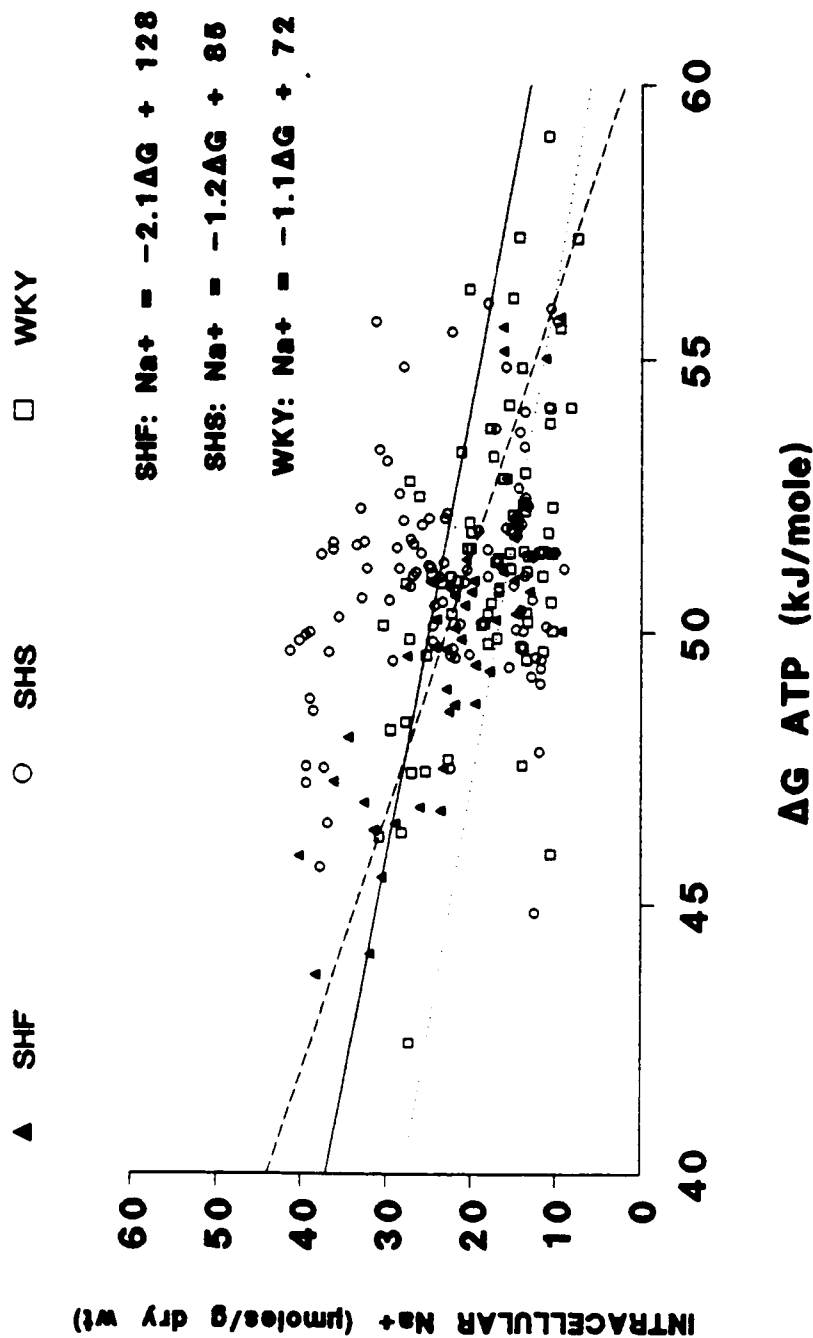


Figure 4-25. Relationship Between the Intracellular Na^+ Content and the ΔG for the SHF, SHS, and WKY. The symbols represent individual data points for the last 3 minutes of the control period and the 30 minutes of ischemia. The dashed line is the regression line for the SHF ($R^2=0.624$, $p=0.0001$). The solid line is the regression line for the SHS ($R^2=0.064$, $p=0.0068$). The dotted line is the regression line for the WKY ($R^2=0.211$, $p=0.0001$). The slope of the regression for the SHF was significantly greater compared with the WKY ($p=0.0321$), and also tended to be greater compared with the SHS ($p=0.0812$). There was no significant difference in the slope between the SHS and WKY.

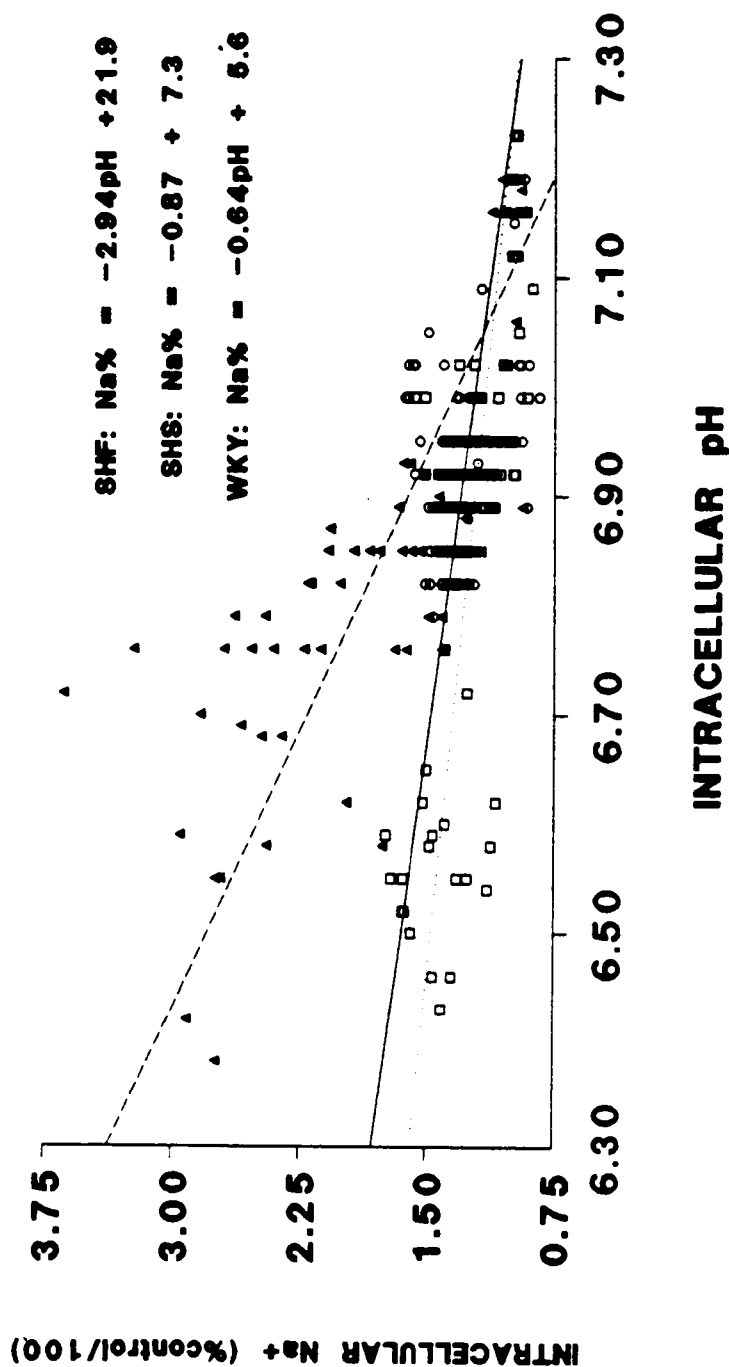


Figure 4-26. Relationship Between the Intracellular Na⁺% and the Intracellular pH for the SHF, WKY, and SHS During Ischemia. The symbols are the observed data points taken from the final three minutes of control and 30 minutes of ischemia. The dashed line is the regression line for the SHF ($R^2=0.57$, $p=0.0001$). The solid line is the regression line for the SHS ($R^2=0.22$, $p=0.0001$). The dotted line is the regression line for the WKY ($R^2=0.37$, $p=0.0001$). The slope of the regression line was significantly greater for the SHF compared with both the WKY ($p=0.0001$) and the SHS ($p=0.0001$). There was no significant difference between the slopes of the regression for the WKY and the SHS.

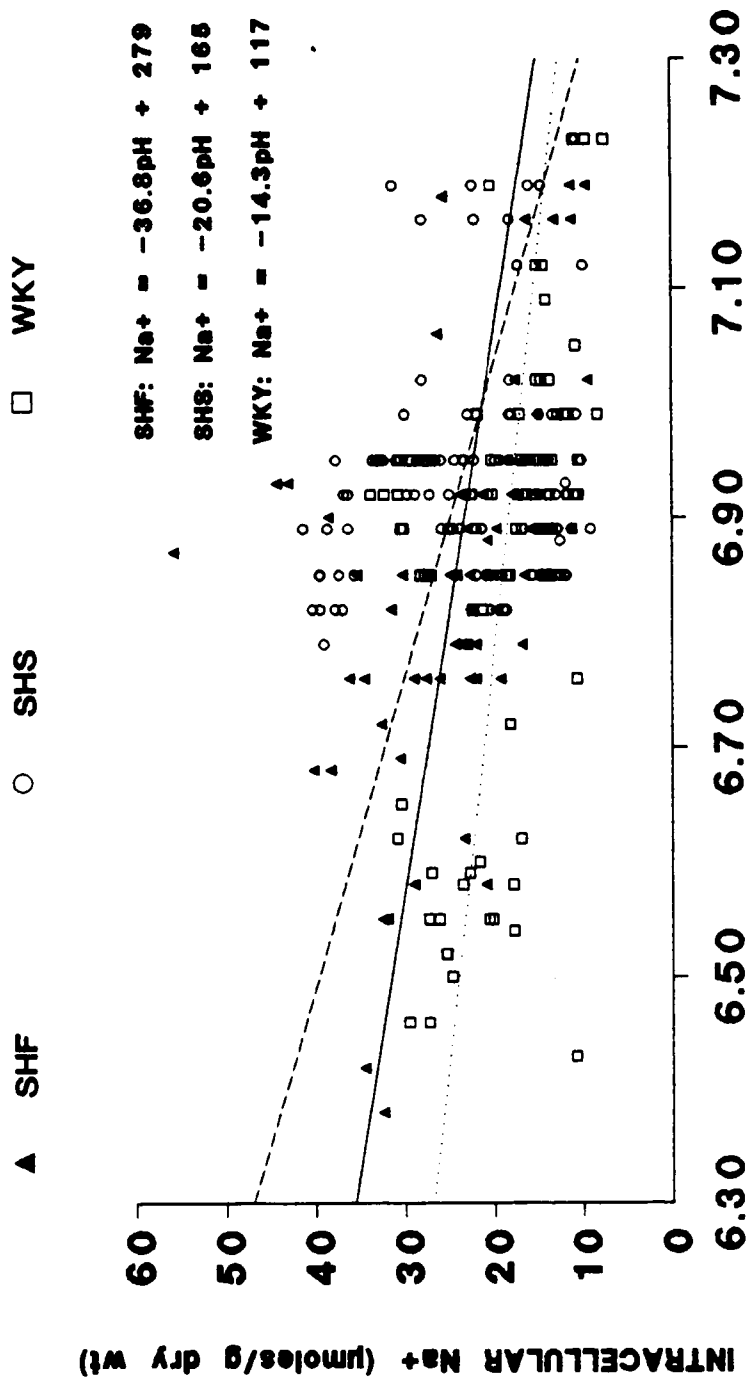


Figure 4-27. Relationship Between the Intracellular Na⁺ Content and the Intracellular pH for the SHF, SHS, and WKY. The symbols represent individual data points for the last 3 minutes of the control period and the 30 minutes of ischemia. The dashed line is the regression line for the SHF ($R^2=0.17$, $p=0.0001$). The solid line is the regression line for the SHS ($R^2=0.036$, $p=0.026$). The dotted line is the regression line for the WKY ($R^2=0.1440$, $p=0.0002$). The slope of the regression for the SHF was significantly greater compared with the WKY ($p=0.009$). There were no significant differences in the slopes of the regression lines between the SHF and SHS, or between the SHS and the WKY.

Therefore, there was a significant relationship of ΔG with $\text{Na}^+\%$ and Na^+ content, respectively, that was independent of the effect of pH on ΔG .

All the SHF hearts arrested during ischemia, while the SHS and WKY hearts continued to beat. Therefore, the apparent differences in the relationship of $\text{Na}^+\%$ with ΔG and pH, respectively, in the SHF may have been due to changes that occurred subsequent to arrest in these hearts. To evaluate the effects of contractile failure in the SHF, the comparisons between groups for the relationships of $\text{Na}^+\%$ with ΔG and pH were repeated using data from the SHF hearts only for the period of ischemia while they were still beating. The slope of the regression of $\text{Na}^+\%$ and ΔG (Figure 4-28) was significantly greater in the beating SHF hearts compared with both the SHS ($p=0.0364$) and the WKY ($p=0.0262$). Furthermore, the slope of the regression of $\text{Na}^+\%$ and ΔG following arrest in the SHF hearts (slope=0.11, $R^2=0.062$, $p=0.179$) was not significantly different from the slope of the regression prior to contractile failure in these hearts. The lower R^2 value for the regression following arrest was due in part to the smaller number of observations in this subset of data compared with the data prior to arrest.

The slope of the regression of $\text{Na}^+\%$ with pH (Figure 4-29) was also significantly greater in the SHF prior to arrest compared with both the SHS ($p=0.0013$) and the WKY ($p=0.0001$). The slope of the regression of $\text{Na}^+\%$ with pH in the SHF hearts following arrest (slope=2.07, $R^2=0.267$, $p=0.0015$) was not significantly different from the slope of the regression prior to arrest in these hearts. Since the differences in the slopes of the regression of $\text{Na}^+\%$ with both ΔG and pH in the SHF were observed prior to arrest in these hearts, the greater increase in Na^+ for a given decrease in ΔG or pH in the SHF was not due to contractile failure in these hearts.

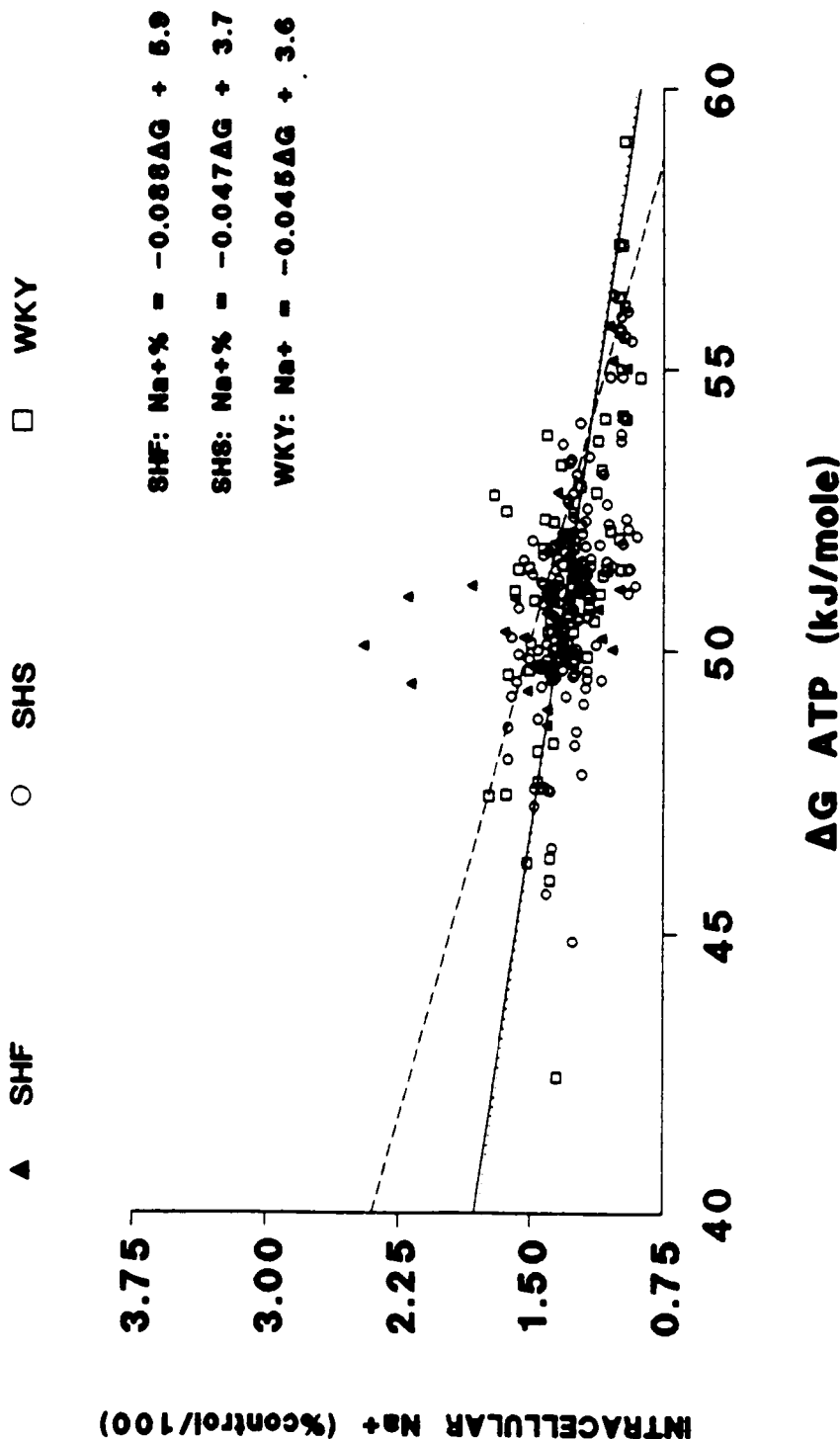


Figure 4-28. Comparison of the Linear Regression of Intracellular $\text{Na}^+\%$ and ΔG in the SHS, and WKY with the SHF Prior to Arrest. The symbols for the SHS and WKY represent the individual data points for each heart during the last 3 minutes of the control period and the entire 30 minutes of ischemia. The symbols for the SHF represent individual data points for each heart for the last 3 minutes of control through the time point prior to arrest. The dashed line is the regression line for the SHF ($R^2=0.180$, $p=0.014$). The solid line is the regression line for the SHS ($R^2=0.302$, $p=0.0001$). The dotted line is the regression line for the WKY ($R^2=0.349$, $p=0.0001$). The slope of the regression line for the SHF group was significantly greater compared with both the SHS ($p=0.0364$) and the WKY ($p=0.0262$). There was no significant difference between the slopes of the regression lines for the SHS and WKY.

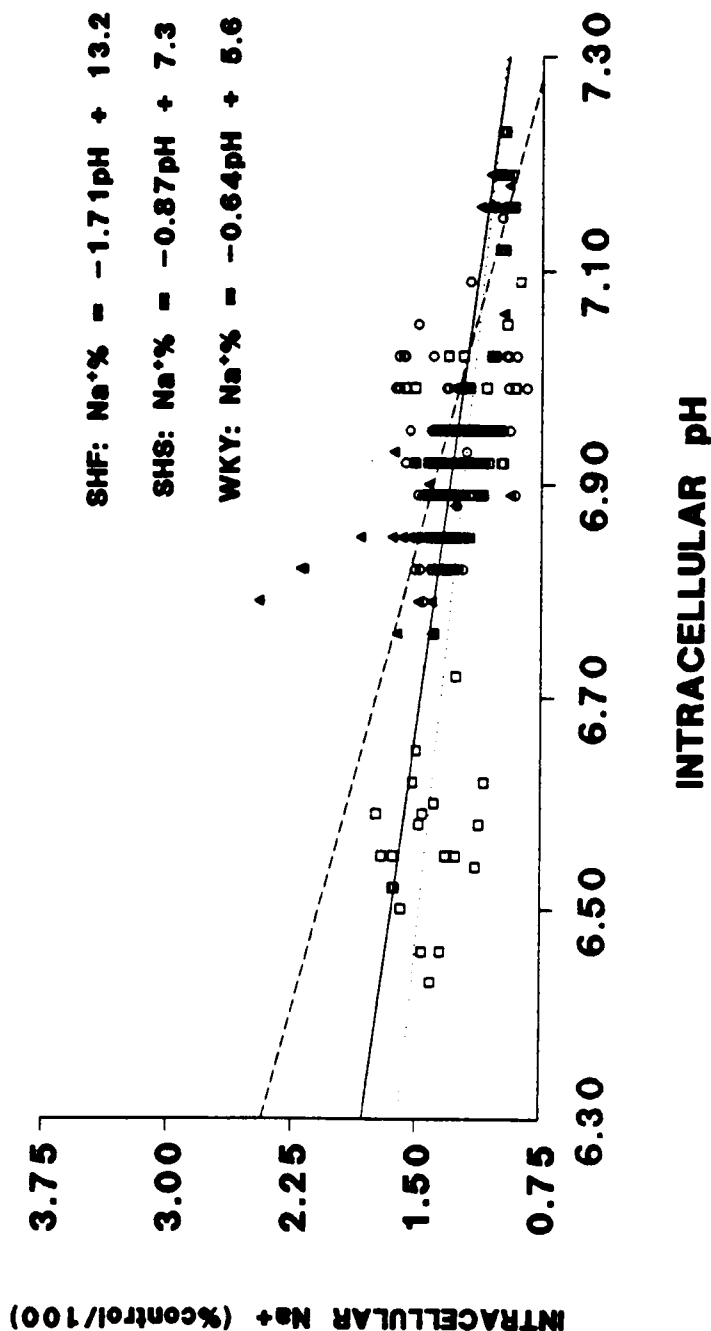


Figure 4-29. Comparison of the Linear Regression of Intracellular Na⁺% and pH in the SHS, and WKY with the SHF Prior to Arrest. The symbols for the SHS and WKY represent the individual data points for each heart during the last 3 minutes of the control period, and the entire 30 minutes of ischemia. The symbols for the SHF represent individual data points for each heart for the last 3 minutes of control through the time point prior to arrest. The dashed line is the regression line for the SHF ($R^2=0.396$, $p=0.0001$). The solid line is the regression line for the SHS ($R^2=0.222$, $p=0.0001$). The dotted line is the regression line for the WKY ($R^2=0.373$, $p=0.0001$). The slope of the regression line for the SHF was significantly greater compared with both the SHS ($p=0.0013$) and the WKY ($p=0.0001$). There was no significant difference between the slopes of the SHS and WKY regression lines.

Contractile Failure in the SHF

As noted previously, all of the hearts in the SHF group stopped contracting during ischemia, while the hearts in the other groups continued to beat. The SHF hearts arrested after a mean ($\pm$ SEM) of 18 ± 1.9 minutes of ischemia. To determine what factor(s) might have contributed to contractile failure in these hearts the mean values of Na^+ , pH, and high energy phosphates at the time of arrest in the SHF were compared with values at end ischemia for the SHS and WKY. End ischemic values were used for the WKY and SHS because maximal changes in all variables were observed at this time point. The results are presented in Table 4-11. The $\text{Na}^+\%$ was significantly higher at the time of arrest in the SHF compared with the values at end ischemia in the other groups ($p < 0.0048$). In contrast, the pH and contents of high energy phosphate were not significantly different at the time of arrest. Therefore, contractile failure during ischemia in the SHF was apparently associated with greater increases in Na^+ , but not with significantly lower contents of high energy phosphates than those observed in the SHS and WKY hearts.

The increase in LVEDP during ischemia was measured as an index of ischemic dysfunction in this study. The maximal increase in LVEDP generally occurred at end ischemia. Therefore, the correlations between the increase in LVEDP at end ischemia, with respect in control, and the values of other key variables at end ischemia were determined. The purpose of these analyses was to determine what factors may have contributed to the ischemic dysfunction in these hearts.

The maximal increase in LVEDP was significantly correlated with $\text{Na}^+\%$, Na^+ content, ΔG , ATP, PCR/P_i , and pH (Table 4-12). The $\text{Na}^+\%$ at end ischemia was the best independent predictor of the maximal increase in LVEDP during ischemia. Multiple linear regression analysis confirmed that Na^+ was the best predictor of the increase in LVEDP.

Table 4-11. CONDITIONS AT TIME OF ARREST IN THE SHF

VARIABLE	SHF AT TIME OF ARREST (n)	SHS END ISCHEMIA (n)	WKY END ISCHEMIA (n)
Na ⁺ (% control)	1.8 ± 0.13 ^{ab} (7)	1.3 ± 0.03 (13)	1.4 ± 0.05 (8)
Na ⁺ (μmoles/g)	26.3 ± 3.00 (7)	23.1 ± 2.80 (10)	21.2 ± 2.70 (8)
pH	6.8 ± 0.04 (7)	6.9 ± 0.01 (13)	6.7 ± 0.08 (8)
ΔG (kJ/mole)	49.2 ± 0.70 (4)	49.6 ± 0.60 (12)	50.2 ± 1.00 (7)
PCr (μmoles/g)	4.2 ± 0.70 (4)	4.9 ± 0.80 (12)	5.2 ± 1.40 (7)
P _i (μmoles/g)	36.5 ± 3.50 (4)	25.4 ± 2.10 (12)	35.3 ± 3.90 (7)
ATP (μmoles/g)	11.5 ± 2.20 (4)	11.3 ± 0.90 (7)	8.9 ± 0.90 (7)
PCr/P _i	0.11 ± 0.02 (7)	0.21 ± 0.03 (13)	0.17 ± 0.05 (8)

^a p = 0.0001 vs SHS^b p = 0.0048 vs WKY

Table 4-12. CORRELATIONS WITH THE INCREASE IN LVEDP AT END ISCHEMIA

VARIABLE	n	r	PROB > r
Na ⁺ % (%control/100)	28	0.75	0.0001
ΔG (kJ/mole)	23	-0.66	0.0003
ATP (μmoles/g)	23	-0.57	0.0024
PCR/P _i	28	-0.56	0.0001
pH	28	-0.60	0.0004
LV DRY/BW (g/kg)	28	0.26	NS
RV DRY/BW (g/kg)	28	0.33	0.0400
Na ⁺ (μmoles/g)	25	0.65	0.0005

Notes: n is the number of observations; r is the Correlation Coefficient; PROB is the one-tailed probability; NS is not significant.

The increase in LVEDP is plotted against the $\text{Na}^+\%$ at end ischemia in Figure 4-30. When all of the data were used in the regression analysis the relationship was linear as shown. However, it is clear that the heart that exhibited the greatest increase in $\text{Na}^+\%$, but only a moderate increase in LVEDP, had a lot of influence in forcing the data to the linear fit. When the data were evaluated without this heart, the relationship between the increase in LVEDP and $\text{Na}^+\%$ was fit best by a curvilinear function that was approximated by a second degree polynomial (Figure 4-30). The curvilinear function indicates that there was little tendency for the LVEDP to increase until the $\text{Na}^+\%$ increased to more than twice the control value. Clearly, there is uncertainty in fitting either model to predict the relationship between the change in LVEDP and $\text{Na}^+\%$ with only a small number of hearts. However, both the linear and polynomial models confirm that there is a significant relationship between the increase in LVEDP and the increase in the $\text{Na}^+\%$.

Recovery of Left Ventricular Function

It was shown above that SHF hearts required significantly longer to recover systolic function. Given that these hearts had significantly greater changes in pH, high energy phosphates and Na^+ compared with both the SHS and WKY, additional analyses were done to determine how these variables were related to the rates of recovery of LVDEVP. More specifically, the analyses were used to determine whether the rate of recovery of LVDEVP was related to the increase in Na^+ even given the interdependence or collinearity between Na^+ and other variables.

Correlations of the recovery time of LVDEVP with the pH, Na^+ and high energy phosphates at end ischemia, and with indices of left and right ventricular hypertrophy are shown in Table 4-13. The recovery time was directly correlated with the Na^+ , and

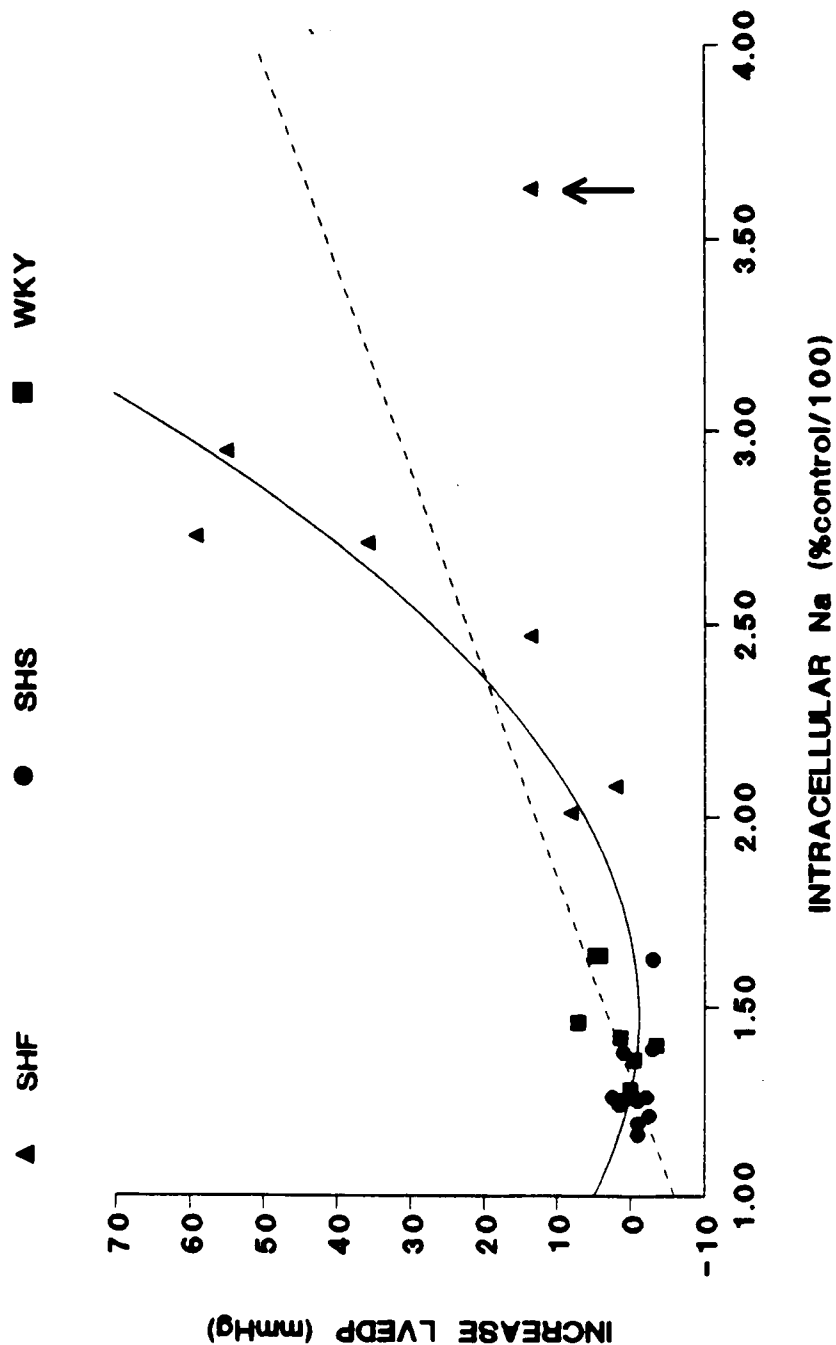


Table 4-13. CORRELATIONS WITH RECOVERIES OF VENTRICULAR PRESSURES

	TIME TO RECOVERY OF LVDEVP ^a			RECOVERY OF LVEDP ^b	
	n	r	PROB > r	r	PROB > r
LV DRY/BW (g/kg)	28	0.42	0.0128	0.51	0.0030
RV DRY/BW (g/kg)	28	0.53	0.0020	0.41	0.0140
Na ⁺ % (%control)	28	0.76	0.0001	0.32	0.0450
pH	28	-0.40	0.0182	-0.07	NS
Δ G (kJ/mole)	23	-0.60	0.0014	-0.26	NS
ATP (μ moles/g)	23	-0.53	0.0050	-0.17	NS
Δ CPP ^d (mmHg)	27	-	-	0.45	0.0084
Na ⁺ (μ moles/g)	25	0.59	0.0010	0.58	0.0011
PCr/P _i	27	-0.64	0.0002	- 0.23	NS

Notes: n is the number of observations used in the correlation analysis; r is the correlation coefficient. PROB is the one-tailed probability.

^a The time in minutes required for the LVDEVP to recover to 80% of control.

^b Recovery of LVEDP was calculated as the increase in LVEDP at end reperfusion with respect to control in mmHg.

^c Δ CPP was calculated as the increase in CPP at end reperfusion with respect to control.

inversely related to the pH, ΔG , ATP, and PCr/ P_i at end ischemia. The time to recovery of LVDEVP was also correlated directly with the LV dry/BW and RV dry/BW indicating that hearts with more severe hypertrophy required longer to recover systolic function following ischemia.

Of the variables measured, the best independent predictor of the time to recovery of LVDEVP was the Na^+ %. Furthermore, the results of a multiple linear regression analysis indicated that no other variable, or combination of variables contributed significantly to the prediction of the recovery time of LVDEVP when Na^+ % was also included in the model. Thus, of the variables measured, the end ischemic Na^+ % was the best predictor of the rate of recovery of systolic function even after considering the influence of other variables on Na^+ %.

Inspection of the observed data and the residuals for the linear model relating the recovery time of LVDEVP and Na^+ % indicated that the relationship was described more accurately by an asymptotic model. The relationship between the time to recovery of LVDEVP and Na^+ is shown in Figure 4-31. The asymptotic regression, like the linear model, indicated that hearts which had accumulated more Na^+ at end ischemia generally required longer to recover LVDEVP. Unlike the linear model, the asymptotic model accounted for the apparent plateau in the recovery time of LVDEVP at values of Na^+ % above 2. That is, hearts with Na^+ % of greater than 2.5 had recovery times that were similar to hearts with Na^+ % of 2.

There was also a significant relationship between the recovery of LVDEVP and the recovery of Na^+ during reperfusion. The time required for the LVDEVP to recover by 80% was significantly correlated with the recovery time for Na^+ ($r=0.39, p=0.016$). In 71% of the hearts studied, the intracellular Na^+ recovered completely to control values prior to

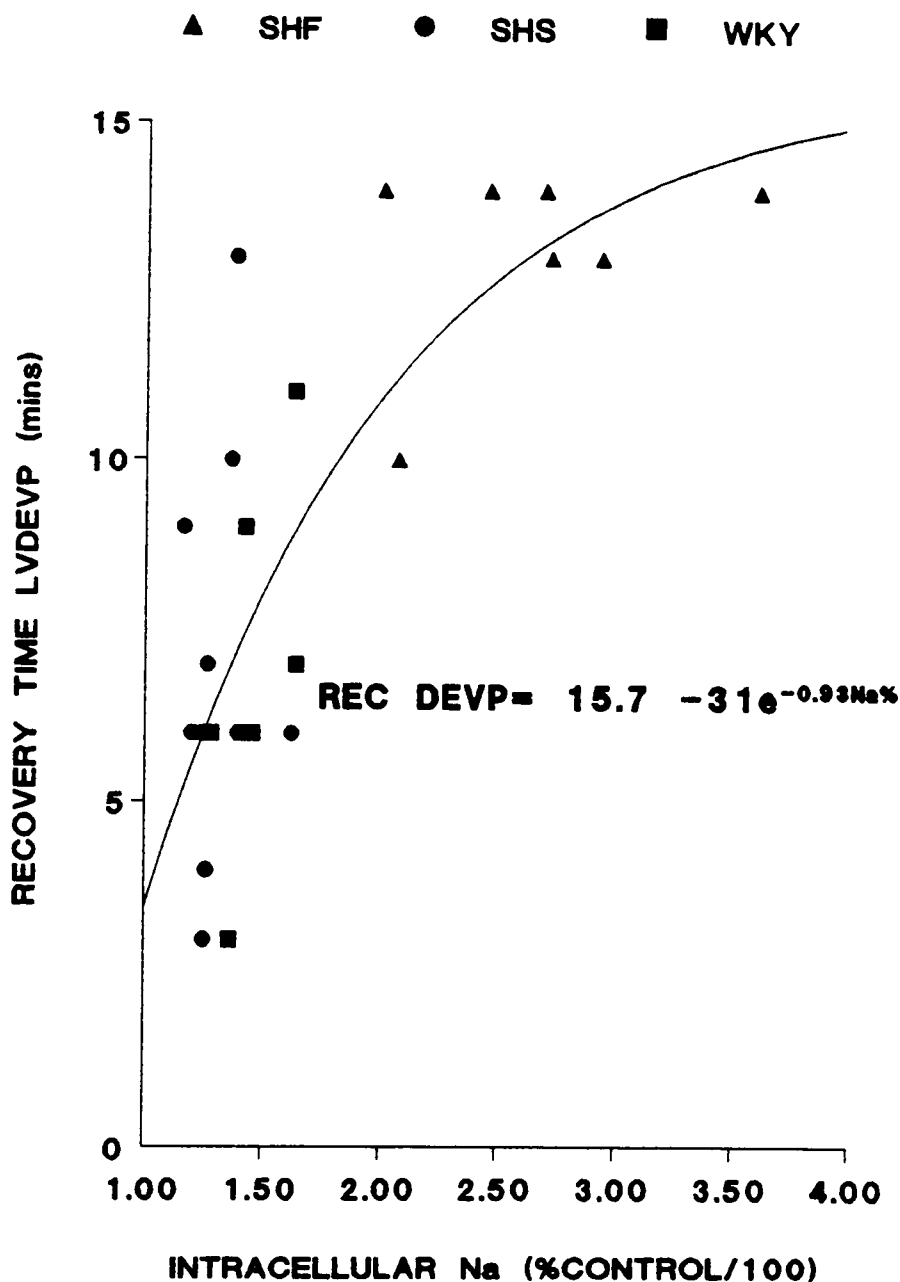


Figure 4-31. Relationship of the Rate of Recovery of Developed Pressure During Reperfusion and the Intracellular Na^+ At End Ischemia. Symbols represent observed data, and the curve represents the asymptotic model as shown. Although the linear relationship between REC LVDEVP and $\text{Na}^+\%$ was also significant, the asymptotic model provided a superior fit. The asymptotic model predicted that hearts which accumulated more Na^+ during ischemia generally required longer to recover LVDEVP, but the relationship plateaued such that hearts with Na^+ values 3-fold higher than the control value had similar recovery times as hearts in which the Na^+ was only twice the control value.

the time when the LVDEVP had recovered to 80% of the control value.

The recovery of LVEDP was calculated as the increase in LVEDP with respect to control following 30 minutes of reperfusion. Correlations between LVEDP and other key variables are shown in Table 4-13. A sustained increase in LVEDP at end reperfusion was correlated directly with the LV dry/BW, the RV dry/BW, the Na⁺%, and the Na⁺ in $\mu\text{moles/g dry wt}$ at end ischemia. There was also a significant correlation between the recovery of LVEDP and the increase in coronary perfusion pressure at end reperfusion with respect to control ($p=0.0084$). In contrast, a sustained increase in LVEDP was not associated with the pH or contents of high energy phosphates at end ischemia.

D. DISCUSSION

Characteristics of SHR Groups

Previous studies have shown that hearts with hypertrophy associated with pressure overload have an increased susceptibility to ischemic dysfunction and damage, but the causes of this predisposition have not been identified (26,27,34-36,41-43). The primary objective of this study was to determine whether hypertrophied SHR hearts exhibited greater accumulation of intracellular Na⁺ during ischemia, and whether the greater increase in Na⁺ was associated with greater ischemic dysfunction and/or damage following reperfusion compared with nonhypertrophied WKY. Additionally, the association of the increase in intracellular Na⁺ with changes in high energy phosphates and pH, respectively, during ischemia were evaluated.

The answers to these questions are somewhat complicated because the ischemic protocol distinguished two different groups of hypertrophied SHR. The SHS group, which

represented the majority of the SHR studied, did not exhibit either greater accumulation of intracellular Na^+ during ischemia, or greater ischemic dysfunction or damage compared with the WKY controls.

In contrast, another subset of SHR hearts, the SHF, exhibited a significantly greater increase in Na^+ during ischemia compared with the WKY. Furthermore, the greater accumulation of Na^+ in the SHF hearts was associated with greater systolic and diastolic dysfunction during ischemia, and poorer recovery following reperfusion. The observation that only a subset of SHR exhibited a greater increase in Na^+ during ischemia indicated that the presence of hypertrophy per se was not a sufficient cause for impaired Na^+ handling in this model of hypertension. However, in the subgroup of hypertrophied SHR hearts that did exhibit impaired Na^+ handling, altered Na^+ homeostasis was associated with greater ischemic dysfunction and poorer functional recovery following reperfusion.

The SHR is a well established model of hypertensive hypertrophy (2,22,61) in which the severity of hypertrophy is relatively mild compared with models such as the aortic banded rat (26), or the DOCA-salt rat (27,41). Earlier studies have indicated that 6-12 month old SHR exhibit a stage of significant hypertrophy in which the hearts are functionally compensated, and capable of generating equal or even greater isometric tension compared with WKY (2,80,157). Previous studies have also indicated that SHR hearts do not typically exhibit clinical signs of failure until the rats are 14-18 months of age (46,158).

The reasons for the different responses to ischemia in the two SHR groups is unclear. There was no apparent evidence of pulmonary edema or dyspnea in the SHF rats that would indicate that these animals were in congestive heart failure. In addition,

both groups of SHR developed equal left ventricular isovolumic wall stress at lower end diastolic wall stress compared with the WKY, indicating that contractile function was not compromised in either the SHS or the SHF. The perfusion conditions, including coronary flow and end diastolic wall stress, were similar for the SHF and SHS. There were also no differences in the duration of preischemic perfusion times, or evidence of deterioration of function in the SHF that would indicate that these hearts were compromised by the isolation procedure.

The LV dry/BW ratios were increased by 56% and 67% in the SHS and SHF, respectively, with respect to the WKY. The severity of LV hypertrophy was significantly greater in the SHF compared with the SHS. Additionally, the RV dry weight to body weight ratio was significantly higher in the SHF, but not in the SHS, compared with the WKY controls. The SHF, therefore, exhibited greater left ventricular hypertrophy compared with the SHS, and evidence of right ventricular hypertrophy that was not evident in the SHS. It should be emphasized that differences in the severity of hypertrophy were based on mean differences for the two groups of SHR, and that there was some overlap in the ranges of LV and RV weight to body weight ratios between groups. Nevertheless, these data do indicate that the SHF exhibited a relatively more severe stage of hypertrophy compared with the SHS. Thus, it is also possible that the SHF hearts may have also had alterations in Na^+ homeostasis or metabolism, on the cellular level, that were not yet present in the SHS.

Estimation of Intracellular Na^+

The NMR visibility of intracellular Na^+ in the myocardium remains controversial. Many investigators have found that the concentrations of Na^+ measured in complex tissues

such as cardiac muscle with NMR are systematically lower than concentrations measured with other methods such as atomic absorption or flame photometry (159). The Na^+ nucleus is quadrupolar with a nuclear spin of $3/2$. The fact that a portion of the tissue Na^+ is not detectable with NMR has been attributed to static and/or dynamic quadrupolar effects produced by local electric field gradients in the tissue (159). These effects may produce shifting and/or broadening of the $3/2$ to $1/2$ transitions of the Na^+ nucleus which account for 60% of the ^{23}Na NMR signal. In contrast, the $1/2$ to $-1/2$ transition is not believed to be affected by electric field gradients, and is therefore NMR visible. The latter transition produces 40% of the NMR signal, and therefore, it has been argued that the NMR visibility of intracellular Na^+ should be 40%. This theoretical argument has been supported by studies that have produced empirical estimates of approximately 40% for the NMR visibility of intracellular sodium (159).

The NMR visibility of Na^+ was not estimated directly in the current study. However, the mean Na^+ contents ($\mu\text{moles/gram dry wt}$) measured in this study were actually within the ranges reported previously for measurements of Na^+ using flame photometry and ion selective electrodes in combination with extracellular markers (1,2,110). Therefore, comparisons of the estimated control Na^+ content with values from studies using other techniques did not provide evidence of systematic underestimation, or invisibility, of intracellular Na^+ in the current study.

The between heart variability of the intracellular Na^+ content measured in the present study was quite high. The between heart coefficient of variation for estimation of the control intracellular content was 35-38% for the three groups of rats, and the observed range of intracellular Na^+ was 8 to 43 $\mu\text{moles/gram}$ at control. The range of values of Na^+ at end ischemia overlapped with the control range in all three groups including the SHF

group that exhibited a 2-3 fold increase in Na^+ during ischemia. The large variability in Na^+ content may have originated from several sources including real between heart variability, measurement of content rather than concentration, variability in the resolution of the intracellular resonance, and radiofrequency field inhomogeneity.

The between heart coefficient of variation of 35-38% observed in the present study for estimation of the control intracellular Na^+ content is comparable to the coefficients of variation for the estimation of Na^+ in homogenates in previous studies using flame photometry (110) and ion selective electrodes (1-2) in combination with extracellular markers. Additionally, the coefficient of variation for the measurement of intracellular Na^+ activity with microelectrodes (112,122) was also 30-40%. Therefore, although the coefficient of variation for the estimation of the intracellular Na^+ content with NMR was high, the variability was similar to that reported for other techniques.

Incomplete resolution of the intracellular and extracellular Na^+ resonances in the current study (Figure 4-7), resulted in a failure to integrate a small portion of the intracellular resonance. The resolution of the intracellular Na^+ peak was variable between hearts, and the poorer resolution of the intracellular resonance for some hearts may have produced erroneously low estimates of the intracellular Na^+ content. These low estimates of intracellular Na^+ could have contributed to the between heart variability in the intracellular Na^+ measurement. The incomplete resolution would also have contributed error to the estimation of $\text{Na}^+\%$ if the resolution changed during ischemia or reperfusion.

Another potential source for the lack of precision in the estimation of the intracellular Na^+ content between hearts was the inhomogeneity of the radiofrequency field for the home-built probe. The nonuniformity in the applied magnetic field made positioning of the heart within the NMR tube critical. The radiofrequency field was uniform

at the center of the probe, but the magnitude of the field decreased so that the pulse power was attenuated by 30% at the edges of the 20mm NMR sample tube containing the heart. The signal from parts of the heart positioned in areas of reduced power near the edge of the tube would have been attenuated. Therefore, differences between experiments in the positioning of the heart within the tube could have produced differences in the estimated Na^+ content between hearts. The effects of the radiofrequency field inhomogeneity were minimized by positioning each heart as closely as possible to the center of the tube. In addition, the Na^+ standard was contained in the balloon within the left ventricle so that the standard was positioned in the center of the tube with the heart, and should have served as an appropriate reference for the heart.

The large between heart variability of the estimates of intracellular Na^+ content would make it difficult to detect small changes in intracellular Na^+ that may have been physiologically important in the heart. Therefore the intracellular Na^+ content was normalized to control values within each heart and expressed as a fraction of the control content for most analyses. The observation that the basal Na^+ content in $\mu\text{moles/g}$ dry weight was similar in all three groups suggests that no information was lost as a result of the expression of Na^+ as a fraction of control. In addition, it should be noted that the difference in the accumulation of intracellular Na^+ at end ischemia in the SHF group was great enough to be significant using either $\text{Na}^+\%$, or the Na^+ content in $\mu\text{moles/g}$. However, analyses used to relate Na^+ with functional variables were sensitive to the variability in the Na^+ content, and correlations of high energy phosphates, pH, and functional variables were generally stronger with $\text{Na}^+\%$ than with Na^+ content in $\mu\text{moles/g}$ dry weight.

Changes in Intracellular Na⁺

Increased intracellular Na⁺ has been observed during ischemia and early reperfusion in normal hearts from several species including the rat (101-108). The magnitude of this increase in Na⁺ has been shown to increase with the duration and severity of ischemia (101-108). The intracellular Na⁺ increased significantly in both groups of SHR and in the nonhypertrophied WKY hearts in the present study. Similar maximal increases in intracellular Na⁺ of 40-50% were observed during 30 minutes of low flow ischemia for both the WKY and SHS. These changes are in the same range as those reported previously for brief 10-15 minute periods of zero flow ischemia in normal rat hearts (101,107). In addition, similar increases in Na⁺ during 30 minutes of low flow ischemia were previously observed in nonhypertrophied Sprague Dawley rat hearts (106). Tani and Neely (102) were unable to measure a significant increase in intracellular Na⁺ during 40 minutes of low flow anoxia in rat hearts. However, the intracellular Na⁺ was estimated in the latter study using ion selective electrodes in homogenates from freeze clamped hearts with ¹⁴C-D-sorbitol as an extracellular marker. Since the intracellular Na⁺ content is relatively low, small errors in estimating the intracellular volume in this last study would have produced large errors in the estimation of the intracellular Na⁺ concentration. Therefore, errors in estimating the intracellular volume may have resulted in an underestimation of changes in intracellular Na⁺ during ischemia in the study utilizing ion selective electrodes.

The more than 2.5 fold increase in Na⁺ observed for the SHF hearts was substantially higher than that observed for the WKY and the SHS in this study. The increase in Na⁺ in the SHF was comparable to that observed previously during more severe ischemic protocols including partial coronary occlusion in dogs (103), and 20

minutes of zero flow ischemia in rat hearts (108).

Few studies have measured the change in intracellular Na^+ during ischemia or hypoxia in hypertrophied hearts. In a single preliminary report, Stewart and others (160) measured increases in intracellular Na^+ of 56% in both hypertrophied SHR and control WKY hearts during 16 minutes of zero flow ischemia. The increase in Na^+ in this study is similar to that observed in the SHS and WKY in the present study, but less than the increase in Na^+ observed in the SHF.

Association Between Na^+ and Functional Impairment

A number of studies have shown an association between the increase in intracellular Na^+ during ischemia and the development of ischemic dysfunction and damage in nonhypertrophied hearts (101,102,108). The present study tested the hypothesis that hypertrophied hearts would exhibit greater ischemic damage and dysfunction secondary to a greater accumulation of intracellular Na^+ during ischemia.

The hypertrophied SHF hearts exhibited both greater accumulation of intracellular Na^+ during ischemia, and greater functional impairment during ischemia and reperfusion compared with both the WKY controls and the hypertrophied SHS hearts. These observations suggest an association between the accumulation of Na^+ and the development of ischemic dysfunction and damage. However, the SHF also exhibited greater changes in ΔG and ATP compared with the WKY and SHS. Therefore, additional analyses were done to determine the relative importance of Na^+ , pH and high energy phosphates in promoting ischemic dysfunction and damage.

Contractile failure occurred in all SHF hearts during low flow ischemia. In contrast, the SHS and WKY hearts were still contracting at end ischemia despite decreases in pH

and high energy phosphates to levels similar to those present in the SHF hearts at the time of failure. Therefore, contractile failure in the SHF hearts was apparently not due to either depletion of energy stores, or the effects of low pH on the myofibrils. In contrast, the increase in Na^+ at the time of failure in the SHF hearts was significantly greater than the maximal increase in Na^+ observed in the SHS and WKY hearts. This indicates that these hearts may have stopped beating due to the effects of the increase in intracellular Na^+ content. Contractile failure in the SHF hearts was preceded by periods of arrhythmias during which the hearts became refractory to pacing. These arrhythmias may have been due to the effects of the elevated intracellular Na^+ on the membrane potential and excitability.

Contractile failure in the SHF hearts was followed by the development of contracture. The increase in resting tension was concurrent with a continuous increase in intracellular Na^+ , and the maximal increase in LVEDP was significantly correlated with the maximal accumulation of intracellular Na^+ . This suggests the possibility that the rise in tension was provoked by an increased influx of Ca^{2+} via Na^+ - Ca^{2+} exchange. However, the increase in LVEDP was also correlated with the ΔG at end ischemia. Therefore, the possibility that low ΔG contributed to the development of contracture via a decrease in the energy available for Ca^{2+} uptake by the sarcoplasmic reticulum (51), or by promoting the formation of rigor bonds between myofilaments (36,49) cannot be excluded.

Greater diastolic dysfunction has been reported during ischemia or hypoxia in several models of pressure overload hypertrophy (27,33,41,43). Wexler and others (33) measured a significantly greater increase in diastolic tension during hypoxia in hypertrophied hearts from DOCA-salt rats compared with controls despite similar rates

of depletion of high energy phosphates. These results indicated that diastolic dysfunction in this model was not related to differences in high energy phosphate metabolism. The investigators proposed that diastolic dysfunction in the DOCA-salt hearts may have been promoted by elevated intracellular Ca^{2+} due to impaired Ca^{2+} handling (33).

Cunningham and others (27) found that diastolic dysfunction in hypertrophied DOCA-salt rat hearts during hypoxia could be mitigated by increasing the perfusate glucose concentration from 5.5 to 27.5mM and providing insulin. The improved function at higher substrate concentrations was associated with normalization of lactate production which was reduced in the hypertrophied DOCA-salt hearts perfused with 5.5 mM glucose. These investigators concluded that the hypertrophied hearts had an decreased ability to utilize anaerobic glycolysis (27). Decreased anaerobic glycolysis was not the cause of the increase in LVEDP in the current study since the hypertrophied SHF hearts actually produced more lactate during ischemia than the WKY controls. Greater lactate production during ischemia and hypoxia was observed previously in hypertrophied hearts from aortic banded rats (26).

The LVEDP did not increase significantly during ischemia in either the control WKY or hypertrophied SHS. Therefore, there is no indication that the 40-50% increase in intracellular Na^+ in these hearts promoted an influx of Ca^{2+} via $\text{Na}^+ - \text{Ca}^{2+}$ exchange during ischemia. However, the LVEDP did increase transiently in the WKY and SHS at the beginning of reperfusion. During these first few minutes of reperfusion the increase in intracellular Na^+ was sustained at the level attained at end ischemia, and the recovery of LVEDP followed a similar time course as the recovery of Na^+ . Therefore, this transient increase in diastolic tension may have been due to an enhanced influx or decreased removal of Ca^{2+} via $\text{Na}^+ - \text{Ca}^{2+}$ exchange (101,102,108) during this phase of reperfusion.

Additionally, the increase in pH back towards control levels may have facilitated the transient increase in diastolic tension by increasing the sensitivity of the myofibrils to Ca^{2+} (149,150) that had entered the myocytes either during ischemia or with the initiation of reperfusion.

The time required for the hypertrophied SHF hearts to recover 80% of the preischemic LVDEVP hearts was longer than that observed for the control WKY. The recovery time for the other hypertrophied group, the SHS, was similar to that of the WKY. Previous studies have also demonstrated slower recovery of systolic function in hypertrophied hearts (44). The time required for the recovery of LVDEVP was correlated directly with the Na^+ % and inversely correlated with pH and high energy phosphates at end ischemia. Multiple linear regression analysis, however, indicated that the time required for recovery of LVDEVP was more closely associated with Na^+ % than with the pH, concentrations of high energy phosphates, or a combination of these variables. Thus, these data suggest that the prolonged time for recovery may have been directly associated with greater increases in intracellular Na^+ . Normal excitation contraction coupling is dependent on the gradients for Ca^{2+} and Na^+ ; therefore, the prolonged time for functional recovery may have reflected the greater time required for the restoration of normal cation gradients in the SHF hearts. This concept is supported by the observation that Na^+ recovered completely to control levels in most hearts prior to the time when LVDEVP recovered to 80% of the control level.

The increase in LVEDP at the end of reperfusion with respect to control was indicative of a sustained increase in diastolic stiffness. This increased diastolic stiffness was greater in the SHF compared with the WKY. There was, however, no difference in the recovery of diastolic function in the SHS compared with the WKY. The severity of left

ventricular edema was not different in the SHF hearts compared with the other groups, and therefore edema was not the cause of the greater diastolic stiffness in the SHF hearts. Increased diastolic stiffness was also not correlated with high energy phosphate concentrations at end ischemia. The sustained increase in LVEDP was directly correlated with the severity of hypertrophy, the CPP, and the Na^+ at end ischemia. Therefore, the development of both systolic and diastolic dysfunction during ischemia, slower recovery of LVDEVP during reperfusion, and incomplete recovery of LVEDP could be related to a greater accumulation of intracellular Na^+ during ischemia.

Functional recovery was complete in the WKY and SHS hearts in this study despite a 40-50% increase in intracellular Na^+ . Complete functional recovery was also observed in an earlier study in which a similar increase in Na^+ of approximately 50% was produced by 10-15 minutes of zero flow ischemia in normal rat hearts (101). The substantial increase in intracellular Na^+ in the SHF was associated with significantly greater dysfunction during ischemia and poorer recovery of function and ATP following reperfusion compared with the control WKY and the hypertrophied SHS. However, the ischemic injury in this group was still rather mild compared with earlier studies which have employed longer periods of zero flow ischemia (38,101,108) or hypoxia (104), even though some of these studies measured similar increases in intracellular Na^+ (108). The relatively mild ischemic dysfunction and damage in the current experiment may be attributed to the relative mildness of the ischemic protocol, and the use of a relatively low perfusate free Ca^{2+} concentration.

The mean ($\pm$ SEM) perfusate free Ca^{2+} concentration used in this study was in the low physiologic range for rats ($1.01 \pm 0.02\text{mM}$). In contrast, many previous studies have perfused hearts with higher concentrations of 1.2 to 2.0 mM free Ca^{2+}

(38,101,104,108). We have observed that a decrease in free Ca^{2+} from 1.5 to 1.0 mM significantly decreases the severity of ischemic damage during both low flow and zero flow ischemia (unpublished observations). This is consistent with the results of studies which have indicated that lowering the extracellular Ca^{2+} has a protective effect on the myocardium during ischemia (23,148). This dependence of ischemic damage and dysfunction on the extracellular Ca^{2+} concentration supports the hypothesis that an influx of Ca^{2+} , presumably via Na^{+} - Ca^{2+} exchange, is important in the development of ischemic dysfunction and damage (148).

Low flow ischemia is intermediate in severity between zero-flow ischemia and hypoxia. The delivery of oxygen is compromised, but delivery of substrates and removal of the metabolites is maintained at a somewhat reduced rate (17). Therefore, energy production by anaerobic glycolysis is supported by the provision of substrates, and the accumulation of metabolites such as lactate and H^{+} is less than during zero flow ischemia, but probably greater than during hypoxia. This type of ischemia has physiologic relevance in that it mimics the conditions that may exist in the peripheral zone of a myocardial infarct (17).

The low flow model of ischemia was used in this study as an alternative to zero flow ischemia which has been shown to produce larger areas of no-reflow in hypertrophied hearts compared with controls (38,47,62). Although the possibility of heterogeneity of reperfusion cannot be excluded in the low flow model of ischemia, there was no evidence of splitting of the P_i peak or incomplete recoveries of PCr or P_i that have been associated with no-reflow in previous studies (26,160). Therefore, although the amount of ischemic dysfunction and damage is rather mild in this model of ischemia, the ischemic protocol was adequate to facilitate the demonstration of a relationship between

the accumulation of intracellular Na^+ and differences in the severity of ischemic dysfunction and damage.

Possible Mechanisms for the Increase in Intracellular Na^+

The increase in intracellular Na^+ during ischemia could occur as a result of an increased influx of Na^+ via mechanisms such as $\text{Na}^+ - \text{H}^+$ exchange and/or decreased removal via the $\text{Na}^+ - \text{K}^+$ ATPase. The decrease in oxygen supply during ischemia promotes an increase in lactate and H^+ production via anaerobic glycolysis. Lazdunski and others (109) demonstrated that $\text{Na}^+ - \text{H}^+$ exchange was an important mechanism for pH regulation in the heart, and were the first to propose that elimination of H^+ by $\text{Na}^+ - \text{H}^+$ exchange might play a key role in the increase in intracellular Na^+ during ischemia.

A number of investigators have provided direct evidence that Na^+ increases during ischemia and reperfusion in normal hearts via $\text{Na}^+ - \text{H}^+$ exchange. Tani and Neely (101) demonstrated that treatment of rat hearts with the $\text{Na}^+ - \text{H}^+$ exchange inhibitor amiloride mitigated the accumulation of Na^+ during ischemia and early reperfusion in a dose dependent manner. Similar attenuation of the increase of intracellular Na^+ with amiloride was reported by Murphy and others (108). Additionally, ethylisopropylamiloride (EIPA), a more specific inhibitor of $\text{Na}^+ - \text{H}^+$ exchange, also reduced the increase in Na^+ during both zero flow and low flow ischemia in nonhypertrophied rat hearts (106).

The influx of Na^+ via $\text{Na}^+ - \text{H}^+$ exchange during ischemia may be even more important in hypertrophied hearts if these hearts do indeed exhibit a greater capacity to utilize anaerobic glycolysis as proposed previously (26,32). In the current study, hypertrophied SHF and SHS hearts produced more lactate during ischemia than the WKY. These results are consistent with those of Anderson and others (26) who measured

greater lactate production during ischemia and hypoxia in hearts from aortic banded rats compared with nonhypertrophied hearts. Presumably, the greater lactate and H^+ production during ischemia in the SHS and SHF hearts provided an increased stimulus for the influx of Na^+ via Na^+-H^+ exchange, and thereby promoted a greater influx of Na^+ in these hearts compared with WKY controls.

The intracellular pH was measured in this study to determine whether differences in Na^+ accumulation could be related to differences in the accumulation of H^+ . There was a significant correlation between Na^+ and pH across the ischemic period in all three groups. However, the relationship was not a simple one. That is, although the WKY hearts exhibited small increases in Na^+ , similar to those observed in the SHS, these hearts exhibited large changes in pH similar to those in the SHF hearts. In addition, the SHF exhibited greater changes in Na^+ for a given change in pH compared with both the WKY and the SHS. Therefore, there was no a simple direct relationship between the decrease in pH and the increase in Na^+ during ischemia in this study.

The dissociation between pH and the increase in Na^+ does not eliminate the possibility that Na^+-H^+ exchange is an important mechanism for the increase in intracellular Na^+ . The pH actually corresponds to the free H^+ that accumulates in the cardiocyte, and may not accurately reflect the total H^+ load, or the H^+ that is removed via Na^+-H^+ exchange. Murphy and others (108) also found a dissociation of pH and Na^+ during ischemia when Na^+-H^+ exchange was inhibited with amiloride. Amiloride attenuated the increase in Na^+ during ischemia, but did not affect the pH. Similar results were observed in experiments in which the ischemic increase in Na^+ was blocked with EIPA (106). These observations indicate that other mechanisms in addition to Na^+-H^+ exchange may be important for the regulation of pH in the heart.

The measured increase in intracellular Na^+ represents the difference between the influx of Na^+ via Na^+/H^+ exchange and the removal of Na^+ via the Na^+/K^+ ATPase. Thus, the relationship between the increase in intracellular Na^+ and the decrease in pH may also have been complicated by differences in the rate of Na^+ removal via the Na^+/K^+ ATPase in the different groups. The intracellular Na^+ concentration is an important regulator of the Na^+/K^+ ATPase activity (82). Therefore, an increased influx of Na^+ would be expected to stimulate increased removal of Na^+ via the Na^+/K^+ ATPase. However, the capacity of the Na^+/K^+ ATPase to respond to a Na^+ load may be limited by decreased energy availability during ischemia (102,110).

Previous studies have provided evidence that the Na^+/K^+ pump may be controlled directly by energy availability during hypoxia and ischemia (110). Therefore, high energy phosphates were monitored in the present study, and related to the changes in intracellular Na^+ in order to determine whether there was evidence of reduced energy availability in the hypertrophied hearts that might have compromised the capacity of the Na^+/K^+ ATPase to respond to an influx of Na^+ .

The decrease in ATP was significantly greater, and the ΔG of ATP hydrolysis at end ischemia was significantly lower in the SHF compared with the control WKY. There were no significant differences in the decrease in high energy phosphates between the WKY and the SHS. Therefore, the increase in intracellular Na^+ was substantially greater in the SHF hearts which also exhibited greater changes in high energy phosphates. Furthermore, the SHS and WKY groups which had similar changes in high energy phosphates also exhibited a similar accumulation of Na^+ during ischemia. These observations suggest that the magnitude of increase in intracellular Na^+ may have been related to the severity of the decline in high energy phosphates. This concept is

supported by the significant correlations between the maximal increase in Na^+ and the ATP content and ΔG , respectively, both at end ischemia and across the ischemic period.

The concept that the level of high energy phosphates is important in determining the magnitude of the increase in intracellular Na^+ during ischemia is consistent with the results of a study by Tani and Neely (102). They found that the increase in intracellular Na^+ during low flow anoxia without substrate was eliminated by the provision of glucose and subsequent preservation of ATP content during anoxia. The attenuation of the increase in Na^+ was blocked by ouabain, and therefore the beneficial effect of glucose was attributed to the maintenance of $\text{Na}^+ - \text{K}^+$ ATPase by energy derived from anaerobic glycolysis (102). Although greater anaerobic glycolysis may have also promoted a greater influx of Na^+ via $\text{Na}^+ - \text{H}^+$ exchange, the energy availability was apparently adequate to support the removal of Na^+ by the $\text{Na}^+ - \text{K}^+$ ATPase so that no net increase in intracellular Na^+ was observed.

The intracellular Na^+ plateaued following an initial increase in both the SHS and the WKY, but not in the SHF. The plateau represents a balance of Na^+ influx, presumably via $\text{Na}^+ - \text{H}^+$ exchange, and removal via the $\text{Na}^+ - \text{K}^+$ ATPase. The change in ΔG in the SHS and WKY followed a similar pattern in which the ΔG decreased immediately at the beginning of ischemia and then plateaued at a lower value for the duration of the ischemic period.

Fiolet and others (110) observed a similar plateau in both the intracellular Na^+ and the ΔG observed during ischemia and anoxia in normal rat hearts. They proposed that the plateau in ΔG represented the new equilibrium of energy supply and demand established by anaerobic energy production. In addition, as the ΔG decreased dramatically towards the end of ischemia or anoxia in this study, the intracellular Na^+ and

K^+ also changed proportionately. These investigators were able to demonstrate close agreement between measured membrane gradients for Na^+ and K^+ , and gradients predicted by assuming that the Na^+-K^+ ATPase was in thermodynamic equilibrium during anoxia and ischemia. They concluded that these results were consistent with the concept that the Na^+-K^+ ATPase is subject to direct thermodynamic control by the ΔG during ischemia and hypoxia in the heart.

Therefore, using the theory that the Na^+-K^+ ATPase is controlled by the ΔG during ischemia, one could speculate that the plateau in ΔG observed in the WKY and SHS in the current study represented an equilibrium of ischemic energy production and demand in these hearts. The new energy equilibrium, in turn, established a new level of intracellular Na^+ at which the Na^+-K^+ ATPase was able to keep pace with the influx of Na^+ via Na^+-H^+ exchange. The initial rise in Na^+ in these hearts would therefore reflect a mismatch between the increased influx of Na^+ and a reduced maximal capacity for Na^+ removal by the Na^+-K^+ ATPase at reduced energy levels.

The Na^+ did not plateau in the SHF hearts during ischemia; therefore, the Na^+-K^+ ATPase activity in these hearts was not sufficient to prevent a continuous rise in Na^+ . There was, however, a plateau in the ΔG in the SHF hearts at a level similar to that observed for the WKY and SHS during approximately the first half of the ischemic period. This plateau was followed by an abrupt decrease in ΔG during the later half of ischemia that resulted in a significantly greater decrease in ΔG at end ischemia in the SHF compared with the SHS. The observation that the initial plateau in ΔG during early ischemia in the SHF did not result in a similar plateau in Na^+ suggests that the coupling between energy supply and the removal of Na^+ via the Na^+-K^+ ATPase was somehow less efficient in these hearts. This concept is supported by the observation that the

increase in intracellular Na^+ for a given decrease in ΔG was greater in the SHF hearts compared with the other groups. Therefore, even at similar levels of ΔG the capacity of the SHF hearts to prevent an increase in Na^+ was apparently less in the SHF compared with the WKY and SHS.

Discussion of Differences Between Groups

Although the SHS produced more lactate, and therefore H^+ ions, during ischemia than the WKY, the pH actually decreased less in the SHS than in the WKY. This indicates that the SHS had either a greater buffer capacity or more efficient mechanisms for H^+ removal compared with the WKY. One of the most important mechanisms for pH regulation in the myocardium is $\text{Na}^+ - \text{H}^+$ exchange (109,116,117). The results of a recent study suggest that the SHR may have an enhanced ability to utilize $\text{Na}^+ - \text{H}^+$ exchange compared with WKY (161). These investigators found that both the rate of increase and the maximal increase in the H^+ concentration during repetitive stimulation of SHR skeletal muscle was lower compared with WKY. Since the reduced accumulation of H^+ in the SHR was eliminated by the $\text{Na}^+ - \text{H}^+$ exchange antagonist amiloride, it was concluded that the SHR had increased efficiency for the removal of H^+ via $\text{Na}^+ - \text{H}^+$ exchange (161). A similar enhancement of the $\text{Na}^+ - \text{H}^+$ exchange mechanism in SHR cardiac muscle would account for the superior pH regulation observed in the SHS hearts in the present study.

Assuming that the improved pH regulation in the SHS compared with the WKY was indeed due to increased $\text{Na}^+ - \text{H}^+$ exchange, these hearts were also exposed to a greater influx of Na^+ via this mechanism. However, Na^+ plateaued at the same level as that observed for the WKY. This similar accumulation of intracellular Na^+ in the SHS and

WKY suggests that the SHS may also have removed more Na^+ than the WKY during ischemia to prevent greater Na^+ accumulation.

The greater energy production via anaerobic glycolysis in the SHS hearts may have been utilized to maintain the activity of the Na^+/K^+ ATPase at a level that was adequate to compensate for the greater influx of Na^+ . This hypothesis is supported by the observation that there were no significant differences in the decreases in PCr, ATP, or ΔG between the SHS and the WKY despite greater anaerobic energy production in the SHS. Energy demand was similar in the SHS and WKY during ischemia since these groups developed similar left ventricular pressures at the same heart rate. Since low flow ischemia certainly provided a limited amount of oxygen delivery during ischemia, it is possible that the WKY hearts were able to maintain energy levels by generating more ATP via oxidative phosphorylation rather than by anaerobic glycolysis. Since oxygen consumption was not measured in this study this possibility cannot be excluded.

In contrast to the SHS, the SHF hearts exhibited a greater increase in Na^+ , a greater decrease in ATP, and an equivalent decrease in pH compared with the WKY. The greater increase in Na^+ in the SHF hearts may have been due to increased influx, and/or decreased removal via the Na^+/K^+ ATPase compared with the SHS.

The lactate production in the two SHF hearts for which lactate measurements were obtained was in the upper range of the lactate production observed for the SHS. Therefore, anaerobic energy production, and the subsequent H^+ load were apparently at least as great in the SHF as in the SHS. Although the decrease in pH was greater in the SHF compared with the SHS, the pH did not decrease significantly more in the SHF hearts compared with the WKY. Furthermore, the rate of pH recovery during the initial three minutes of reperfusion was greater in the SHF compared with the WKY. These

observations are consistent with the hypothesis that the SHF, like the SHS, may have had enhanced $\text{Na}^+ - \text{H}^+$ exchange compared with the WKY. Greater $\text{Na}^+ - \text{H}^+$ exchange would have contributed to the greater increase in Na^+ .

In contrast to the SHS hearts, the SHF hearts apparently had inadequate $\text{Na}^+ - \text{K}^+$ ATPase activity to compensate for Na^+ influx, and subsequently the intracellular Na^+ increased to very high levels. Decreased removal of Na^+ via the $\text{Na}^+ - \text{K}^+$ ATPase could have resulted from decreased $\text{Na}^+ - \text{K}^+$ ATPase activity due to decreased energy availability, or a deficiency in the number of $\text{Na}^+ - \text{K}^+$ pumps. The possibility that the SHF had insufficient energy available to maintain the $\text{Na}^+ - \text{K}^+$ ATPase activity at a level adequate to keep pace with the influx of Na^+ is supported by observations that the decrease in ΔG and ATP were significantly greater in the SHF, and that the increase in Na^+ was significantly correlated with both ATP and ΔG .

The energy availability is determined by a balance of energy production and demand. The lactate production in the SHF hearts indicated that the production of ATP via anaerobic glycolysis may have been comparable to that observed for the SHS, and therefore greater than that observed for the WKY. Assuming that there were no differences in the limited aerobic energy production in the SHF compared with the WKY, then net energy production would have been greater in the SHF. Therefore, there was no evidence of impaired anaerobic energy production in the SHF hearts.

There was, however, evidence of greater energy consumption by the myofibrils in the SHF during ischemia which may have accounted for the greater decrease in ΔG in these hearts. Developed pressures were less in the SHF during the last minutes of ischemia, but the diastolic tension was higher. If this increased diastolic tension was due to persistent cross-bridge cycling resulting from a Ca^{2+} overload (162), then the energy

consumption by the SHF was greater than that in the WKY and SHS. Calcium overload may have also contributed to energy depletion by stimulating other ATPases in the heart (23,162). Therefore, greater energy consumption in the SHF hearts may have depleted energy stores during the last half of ischemia, and thereby contributed to the greater increase in Na^+ in these hearts. However, greater energy depletion apparently does not explain the greater increase in Na^+ during the initial minutes of ischemia in the SHF when the energy levels were similar between groups.

The hypothesis that the greater increase in intracellular Na^+ observed in the SHF was not due exclusively to insufficient energy availability is supported by the observation that the increase in Na^+ was greater for a given change in ΔG in the SHF compared with the WKY and SHS. Another possible explanation for the reduced capacity of the $\text{Na}^+ - \text{K}^+$ ATPase to respond to the influx of Na^+ in the SHF hearts is that the SHF had an intrinsic defect in the $\text{Na}^+ - \text{K}^+$ ATPase such as a decreased number of pumps.

Several studies have reported that the $\text{Na}^+ - \text{K}^+$ ATPase activity was reduced in sarcolemmal microsomes from hypertrophied SHR hearts (83-86). The reduction in $\text{Na}^+ - \text{K}^+$ ATPase activity has been attributed to a decrease in the $V_{\max}$ of the enzyme due to a decreased number of pumps (83). A deficiency in the number of $\text{Na}^+ - \text{K}^+$ pumps would reduce the maximal capacity for Na^+ removal by the $\text{Na}^+ - \text{K}^+$ ATPase at any given energy level. The greater increase in intracellular Na^+ at similar levels of ΔG observed in this study, therefore, is consistent with the possibility that the SHF had a deficiency in the number of $\text{Na}^+ - \text{K}^+$ pumps compared with the other groups.

In summary, the pH data support the hypothesis that the influx of Na^+ via $\text{Na}^+ - \text{H}^+$ exchange was greater in both the SHF and SHS. The greater intracellular Na^+ accumulation in the SHF indicates that the removal of Na^+ via the $\text{Na}^+ - \text{K}^+$ ATPase was

less effective in the SHF compared with the other groups. This decreased capacity for removal of Na^+ via the Na^+-K^+ ATPase may have resulted from a combination of decreased energy availability, and an intrinsic deficiency in the number of Na^+-K^+ ATPase pumps in the SHF. It is unclear why the number of Na^+-K^+ pumps would be reduced in only a subset of the SHR at a given age. In vitro studies have reported decreased Na^+-K^+ ATPase activity in the SHR at ages younger than the SHR in the present study (83-86). It is conceivable, however, that the decrease in Na^+-K^+ ATPase activity may represent one of the initial cellular and molecular changes which ultimately results in the transition to failure in hypertrophied hearts.

Given that the SHR are genetically hypertensive, it is possible that the defect in Na^+ handling is also a genetic defect that was present in only a subset of the rats in this strain. However, all of the animals were acquired from the same vendor, and therefore were derived from the same genetic stock. In addition, defects in Na^+ handling have also been detected in vitro in other models of pressure overload hypertrophy (87). Furthermore, in a preliminary report, Atkinson and others (163) described a similar disparity in Na^+ handling during ischemia in hypertrophied thyrotoxic hearts. Approximately half of the hypertrophied hearts exhibited contractile failure during ischemia which was associated with a significantly greater increase in intracellular Na^+ compared with both the controls and a second subset of thyrotoxic hearts. Additionally, in preliminary experiments in our lab, we have found a similar divergence in Na^+ homeostasis during ischemia in Sprague Dawley rat hearts with hypertrophy induced by aortic constriction.

In a study in canine hearts hypertrophied by aortic banding, Gaasch and others (81) recently found that hypertrophied hearts did not demonstrate greater ischemic

dysfunction and damage until the hearts exhibited signs of myocardial failure. The results of the present study indicate that more subtle changes may occur in the hypertrophied heart that determine the response to ischemia. These changes were not detectable either as impaired basal systolic function or other overt signs of myocardial failure.

In conclusion, a subset of hypertrophied SHR hearts exhibited impaired Na^+ handling during ischemia that was detected as a greater accumulation of intracellular Na^+ compared with the WKY controls and a second subgroup of SHR. The impaired Na^+ handling was not associated with hypertrophy per se since not all hypertrophied hearts were affected. We propose that the development of impaired Na^+ handling may be indicative of a subtle transition towards a less compensated state that precedes the development of congestive heart failure, and is manifest as an increased sensitivity to ischemia. Finally, the results of the present study indicate that impaired Na^+ handling may indeed contribute to the increased sensitivity of hypertrophied hearts to ischemic dysfunction and damage.

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APPENDIX

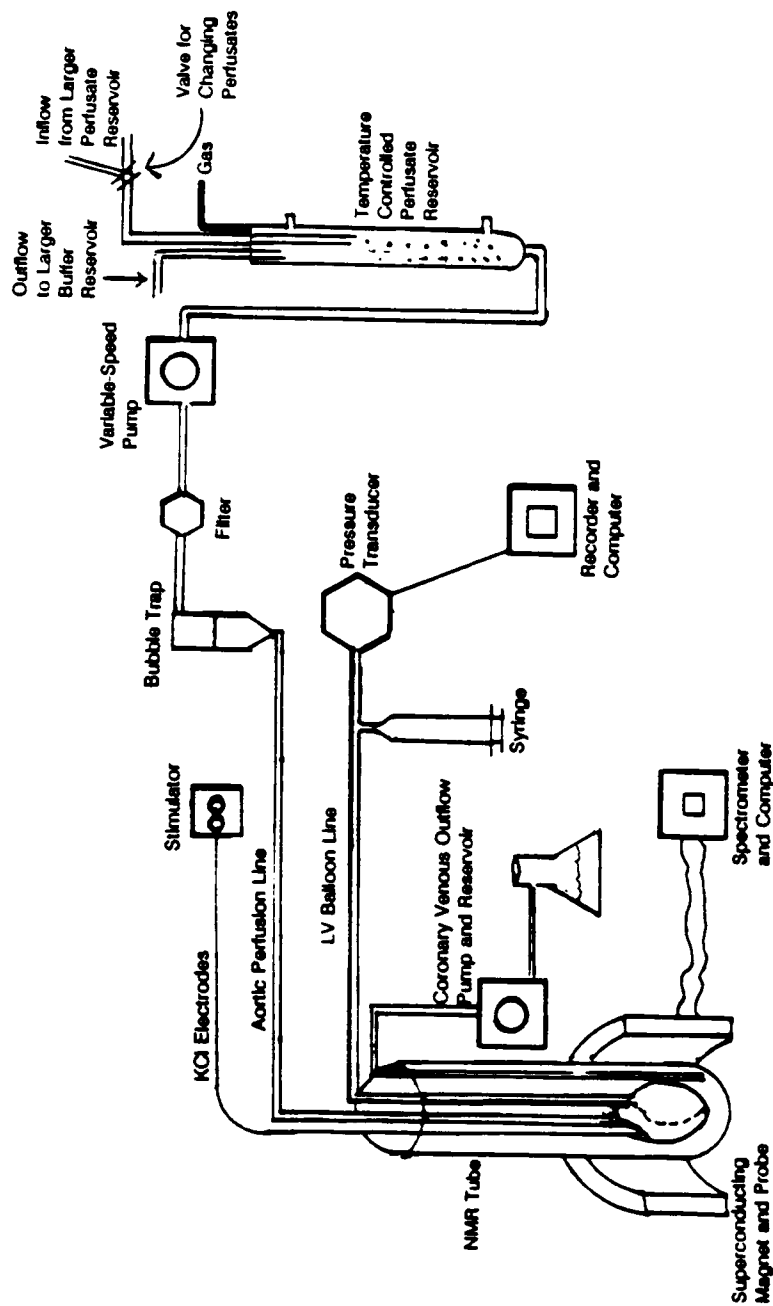


Figure A-1. Experimental Apparatus Used for Constant Flow Perfusion of Isolated Hearts During Collection of NMR and Functional Data. The temperature of the NMR probe and the aortic perfusion line were controlled to maintain the temperature of the heart and perfusate at 37°C. The perfusate was switched from normal Krebs Henseleit buffer to perfusate containing the shift reagent Tm(DOTP)^{5-} via the valve shown in the inflow to the perfusate reservoir. The buffer containing Tm(DOTP)^{5-} was maintained at 4°C in a separate flask not shown in the diagram. The shift reagent buffer was also circulated through a 25ft coil of silastic tubing that was bathed in fluid aerated with 95% O_2 and 5% CO_2 at 37°C prior to entering the reservoir shown above. See the methods sections in chapters 3 and 4 for additional details.

Table A-1 COMPLETE SODIUM DATA FOR EXPERIMENT IN CHAPTER 4

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHF	11	-9	27.56	1.02
SHF	11	-6	26.28	0.98
SHF	11	-3	26.92	1.00
SHF	11	0	25.64	0.95
SHF	11	3	26.28	0.98
SHF	11	6	38.46	1.43
SHF	11	9	44.23	1.64
SHF	11	12	42.95	1.60
SHF	11	15	44.23	1.64
SHF	11	18	55.77	2.07
SHF	11	21	64.74	2.40
SHF	11	24	72.44	2.69
SHF	11	27	76.28	2.83
SHF	11	30	79.49	2.95
SHF	11	33	72.44	2.69
SHF	11	36	46.15	1.71
SHF	11	39	32.05	1.19
SHF	11	42	29.49	1.10
SHF	11	45	31.41	1.17
SHF	11	48	32.69	1.21
SHF	11	51	33.33	1.24
SHF	11	54	32.05	1.19
SHF	11	57	32.05	1.19
SHF	11	60	31.41	1.17
SHF	13	-9	17.51	1.03
SHF	13	-6	16.84	0.99
SHF	13	-3	15.42	0.91
SHF	13	0	17.48	1.03
SHF	13	3	18.37	1.08
SHF	13	6	20.70	1.22
SHF	13	9	21.33	1.26
SHF	13	12	23.79	1.40
SHF	13	15	23.75	1.40
SHF	13	18	22.59	1.33
SHF	13	21	24.65	1.45
SHF	13	24	27.01	1.59
SHF	13	27	30.20	1.78
SHF	13	30	35.22	2.08
SHF	13	33	37.54	2.22
SHF	13	36	21.26	1.25
SHF	13	39	17.28	1.02
SHF	13	42	17.28	1.02

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHF	13	45	16.28	0.96
SHF	13	48	16.28	0.96
SHF	13	51	16.94	1.00
SHF	13	54	18.27	1.08
SHF	13	57	17.94	1.06
SHF	13	60	17.61	1.04
SHF	14	-9	8.79	0.98
SHF	14	-6	8.97	1.00
SHF	14	-3	8.79	0.98
SHF	14	0	9.52	1.06
SHF	14	3	9.34	1.04
SHF	14	6	14.83	1.65
SHF	14	9	16.47	1.84
SHF	14	12	19.77	2.20
SHF	14	15	19.59	2.18
SHF	14	18	21.97	2.45
SHF	14	21	22.70	2.53
SHF	14	24	23.61	2.63
SHF	14	27	28.92	3.22
SHF	14	30	32.58	3.63
SHF	14	33	30.39	3.39
SHF	14	36	15.19	1.69
SHF	14	39	10.43	1.16
SHF	14	42	8.79	0.98
SHF	14	45	9.88	1.10
SHF	14	48	8.79	0.98
SHF	14	51	8.79	0.98
SHF	14	54	8.79	0.98
SHF	14	57	9.15	1.02
SHF	14	60	9.15	1.02
SHF	15	-9	16.45	1.01
SHF	15	-6	16.84	1.04
SHF	15	-3	15.50	0.95
SHF	15	0	16.26	1.00
SHF	15	3	16.26	1.00
SHF	15	6	20.66	1.27
SHF	15	9	21.24	1.31
SHF	15	12	22.96	1.41
SHF	15	15	24.30	1.49
SHF	15	18	27.55	1.69
SHF	15	21	34.44	2.12
SHF	15	24	36.16	2.22

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHF	15	27	38.27	2.35
SHF	15	30	40.18	2.47
SHF	15	33	38.84	2.39
SHF	15	36	18.18	1.12
SHF	15	39	15.50	0.95
SHF	15	42	16.07	0.99
SHF	15	45	14.73	0.91
SHF	15	48	14.73	0.91
SHF	15	51	13.58	0.84
SHF	15	54	13.01	0.80
SHF	15	57	12.05	0.74
SHF	15	60	13.39	0.82
SHF	36	-9	11.86	1.00
SHF	36	-6	11.51	0.97
SHF	36	-3	13.25	1.12
SHF	36	0	11.16	0.94
SHF	36	3	11.16	0.94
SHF	36	6	13.95	1.18
SHF	36	9	16.74	1.41
SHF	36	12	19.18	1.62
SHF	36	15	20.92	1.76
SHF	36	18	23.36	1.97
SHF	36	21	28.94	2.44
SHF	36	24	32.43	2.74
SHF	36	27	34.52	2.91
SHF	36	30	32.43	2.74
SHF	36	33	35.91	3.03
SHF	36	36	23.01	1.94
SHF	36	39	16.74	1.41
SHF	36	42	15.34	1.29
SHF	36	45	14.99	1.26
SHF	36	48	14.99	1.26
SHF	36	51	16.39	1.38
SHF	36	54	15.69	1.32
SHF	36	57	16.39	1.38
SHF	36	60	15.69	1.32
SHF	54	-9	12.61	1.07
SHF	54	-6	11.76	1.00
SHF	54	-3	11.59	0.99
SHF	54	0	11.25	0.96
SHF	54	3	13.13	1.12
SHF	54	6	15.00	1.28

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHF	54	9	15.85	1.35
SHF	54	12	15.00	1.28
SHF	54	15	17.90	1.52
SHF	54	18	19.60	1.67
SHF	54	21	22.67	1.93
SHF	54	24	26.08	2.22
SHF	54	27	30.51	2.59
SHF	54	30	31.88	2.71
SHF	54	33	29.49	2.51
SHF	54	36	11.42	0.97
SHF	54	39	11.25	0.96
SHF	54	42	10.74	0.91
SHF	54	45	12.10	1.03
SHF	54	48	11.59	0.99
SHF	54	51	12.27	1.04
SHF	54	54	12.10	1.03
SHF	54	57	12.44	1.06
SHF	54	60	12.78	1.09
SHF	55	-9	16.39	1.05
SHF	55	-6	15.04	0.96
SHF	55	-3	14.85	0.95
SHF	55	0	16.20	1.04
SHF	55	3	17.16	1.10
SHF	55	6	20.06	1.28
SHF	55	9	20.83	1.33
SHF	55	12	22.95	1.47
SHF	55	15	24.11	1.54
SHF	55	18	21.99	1.41
SHF	55	21	24.88	1.59
SHF	55	24	21.99	1.41
SHF	55	27	23.34	1.49
SHF	55	30	31.44	2.01
SHF	55	33	32.59	2.09
SHF	55	36	17.74	1.14
SHF	55	39	14.08	0.90
SHF	55	42	14.85	0.95
SHF	55	45	13.11	0.84
SHF	55	48	13.69	0.88
SHF	55	51	13.89	0.89
SHF	55	54	12.15	0.78
SHF	55	57	13.31	0.85
SHF	55	60	12.15	0.78

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	16	-9	25.83	1.07
SHS	16	-6	24.36	1.01
SHS	16	-3	23.48	0.98
SHS	16	0	22.30	0.93
SHS	16	3	22.89	0.95
SHS	16	6	28.47	1.18
SHS	16	9	29.93	1.24
SHS	16	12	30.82	1.28
SHS	16	15	32.58	1.35
SHS	16	18	32.87	1.37
SHS	16	21	32.28	1.34
SHS	16	24	28.47	1.18
SHS	16	27	29.64	1.23
SHS	16	30	28.76	1.20
SHS	16	33	25.83	1.07
SHS	16	36	18.49	0.77
SHS	16	39	17.32	0.72
SHS	16	42	18.49	0.77
SHS	16	45	20.84	0.87
SHS	16	48	19.96	0.83
SHS	16	51	18.49	0.77
SHS	16	54	15.85	0.66
SHS	16	57	17.61	0.73
SHS	16	60	16.43	0.68
SHS	19	-9	32.58	1.04
SHS	19	-6	28.91	0.93
SHS	19	-3	31.21	1.00
SHS	19	0	31.21	1.00
SHS	19	3	27.99	0.90
SHS	19	6	33.50	1.07
SHS	19	9	33.04	1.06
SHS	19	12	37.63	1.21
SHS	19	15	36.71	1.18
SHS	19	18	39.47	1.26
SHS	19	21	38.55	1.24
SHS	19	24	41.30	1.32
SHS	19	27	36.25	1.16
SHS	19	30	36.25	1.16
SHS	19	33	37.63	1.21
SHS	19	36	29.83	0.96
SHS	19	39	30.75	0.99
SHS	19	42	29.37	0.94

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	19	45	30.75	0.99
SHS	19	48	30.29	0.97
SHS	19	51	27.99	0.90
SHS	19	54	28.91	0.93
SHS	19	57	27.99	0.90
SHS	19	60	30.29	0.97
SHS	30	-9	.	1.04
SHS	30	-6	.	0.96
SHS	30	-3	.	1.01
SHS	30	0	.	0.99
SHS	30	3	.	0.96
SHS	30	6	.	1.09
SHS	30	9	.	1.17
SHS	30	12	.	1.17
SHS	30	15	.	1.29
SHS	30	18	.	1.23
SHS	30	21	.	1.26
SHS	30	24	.	1.27
SHS	30	27	.	1.21
SHS	30	30	.	1.21
SHS	30	33	.	1.18
SHS	30	36	.	0.90
SHS	30	39	.	0.94
SHS	30	42	.	0.94
SHS	30	45	.	0.95
SHS	30	48	.	0.94
SHS	30	51	.	0.87
SHS	30	54	.	0.82
SHS	30	57	.	0.82
SHS	30	60	.	0.82
SHS	31	-9	21.24	1.11
SHS	31	-6	16.86	0.89
SHS	31	-3	19.36	1.02
SHS	31	0	18.11	0.95
SHS	31	3	18.11	0.95
SHS	31	6	23.11	1.21
SHS	31	9	24.98	1.31
SHS	31	12	23.42	1.23
SHS	31	15	25.92	1.36
SHS	31	18	27.17	1.43
SHS	31	21	26.86	1.41
SHS	31	24	24.36	1.28

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	31	27	27.17	1.43
SHS	31	30	26.54	1.39
SHS	31	33	22.80	1.20
SHS	31	36	17.49	0.92
SHS	31	39	17.49	0.92
SHS	31	42	16.55	0.87
SHS	31	45	17.49	0.92
SHS	31	48	19.67	1.03
SHS	31	51	18.42	0.97
SHS	31	54	18.42	0.97
SHS	31	57	17.49	0.92
SHS	31	60	17.49	0.92
SHS	33	-9	.	1.06
SHS	33	-6	.	0.97
SHS	33	-3	.	0.97
SHS	33	0	.	1.03
SHS	33	3	.	1.18
SHS	33	6	.	1.40
SHS	33	9	.	1.49
SHS	33	12	.	1.57
SHS	33	15	.	1.61
SHS	33	18	.	1.58
SHS	33	21	.	1.57
SHS	33	24	.	1.61
SHS	33	27	.	1.63
SHS	33	30	.	1.63
SHS	33	33	.	1.32
SHS	33	36	.	1.04
SHS	33	39	.	1.13
SHS	33	42	.	1.08
SHS	33	45	.	0.96
SHS	33	48	.	1.07
SHS	33	51	.	1.04
SHS	33	54	.	1.04
SHS	33	57	.	1.04
SHS	33	60	.	1.04
SHS	34	-9	14.03	0.93
SHS	34	-6	16.11	1.07
SHS	34	-3	15.85	1.05
SHS	34	0	14.55	0.97
SHS	34	3	12.73	0.84
SHS	34	6	16.37	1.09

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	34	9	17.93	1.19
SHS	34	12	18.19	1.21
SHS	34	15	18.19	1.21
SHS	34	18	19.75	1.31
SHS	34	21	20.27	1.34
SHS	34	24	18.45	1.22
SHS	34	27	21.31	1.41
SHS	34	30	18.97	1.26
SHS	34	33	16.89	1.12
SHS	34	36	11.69	0.78
SHS	34	39	10.91	0.72
SHS	34	42	9.62	0.64
SHS	34	45	9.87	0.66
SHS	34	48	9.62	0.64
SHS	34	51	9.87	0.66
SHS	34	54	8.06	0.53
SHS	34	57	9.10	0.60
SHS	34	60	9.10	0.60
SHS	37	-9	.	1.07
SHS	37	-6	.	0.93
SHS	37	-3	.	1.01
SHS	37	0	.	0.99
SHS	37	3	.	1.07
SHS	37	6	.	1.26
SHS	37	9	.	1.29
SHS	37	12	.	1.40
SHS	37	15	.	1.31
SHS	37	18	.	1.11
SHS	37	21	.	1.29
SHS	37	24	.	1.25
SHS	37	27	.	1.25
SHS	37	30	.	1.25
SHS	37	33	.	1.26
SHS	37	36	.	1.14
SHS	37	39	.	0.81
SHS	37	42	.	0.75
SHS	37	45	.	0.83
SHS	37	48	.	0.88
SHS	37	51	.	0.82
SHS	37	54	.	0.78
SHS	37	57	.	0.78
SHS	37	60	.	0.74

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	38	-9	15.89	0.98
SHS	38	-6	16.44	1.02
SHS	38	-3	16.16	1.00
SHS	38	0	15.89	0.98
SHS	38	3	15.89	0.98
SHS	38	6	19.18	1.19
SHS	38	9	21.37	1.32
SHS	38	12	20.55	1.27
SHS	38	15	22.19	1.37
SHS	38	18	22.47	1.39
SHS	38	21	22.47	1.39
SHS	38	24	20.82	1.29
SHS	38	27	21.92	1.36
SHS	38	30	20.27	1.25
SHS	38	33	19.45	1.20
SHS	38	36	15.89	0.98
SHS	38	39	15.62	0.97
SHS	38	42	16.99	1.05
SHS	38	45	16.44	1.02
SHS	38	48	17.53	1.08
SHS	38	51	16.99	1.05
SHS	38	54	16.71	1.03
SHS	38	57	16.44	1.02
SHS	38	60	16.71	1.03
SHS	39	-9	10.78	1.00
SHS	39	-6	10.78	1.00
SHS	39	-3	10.92	1.01
SHS	39	0	10.64	0.99
SHS	39	3	10.08	0.94
SHS	39	6	12.74	1.18
SHS	39	9	13.58	1.26
SHS	39	12	13.72	1.27
SHS	39	15	14.28	1.32
SHS	39	18	13.58	1.26
SHS	39	21	15.54	1.44
SHS	39	24	14.14	1.31
SHS	39	27	14.42	1.34
SHS	39	30	14.70	1.36
SHS	39	33	12.88	1.19
SHS	39	36	11.06	1.03
SHS	39	39	10.22	0.95
SHS	39	42	10.64	0.99

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	39	45	10.78	1.00
SHS	39	48	10.50	0.97
SHS	39	51	11.76	1.09
SHS	39	54	10.78	1.00
SHS	39	57	10.64	0.99
SHS	39	60	10.36	0.96
SHS	51	-9	9.78	0.94
SHS	51	-6	10.38	1.05
SHS	51	-3	9.72	0.98
SHS	51	0	9.84	0.99
SHS	51	3	9.05	0.91
SHS	51	6	11.16	1.13
SHS	51	9	11.71	1.18
SHS	51	12	11.83	1.20
SHS	51	15	12.91	1.30
SHS	51	18	12.79	1.29
SHS	51	21	12.43	1.26
SHS	51	24	11.77	1.19
SHS	51	27	11.95	1.21
SHS	51	30	12.49	1.26
SHS	51	33	11.65	1.18
SHS	51	36	8.27	0.84
SHS	51	39	9.05	0.91
SHS	51	42	8.81	0.89
SHS	51	45	9.23	0.93
SHS	51	48	8.57	0.87
SHS	51	51	8.09	0.82
SHS	51	54	9.05	0.91
SHS	51	57	8.81	0.89
SHS	51	60	8.03	0.81
SHS	57	-9	11.78	1.05
SHS	57	-6	10.45	0.93
SHS	57	-3	11.78	1.05
SHS	57	0	10.83	0.97
SHS	57	3	10.64	0.95
SHS	57	6	13.29	1.19
SHS	57	9	14.81	1.32
SHS	57	12	13.67	1.22
SHS	57	15	14.05	1.25
SHS	57	18	14.62	1.31
SHS	57	21	15.00	1.34
SHS	57	24	14.43	1.29

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	57	27	13.67	1.22
SHS	57	30	13.86	1.24
SHS	57	33	12.16	1.08
SHS	57	36	9.69	0.86
SHS	57	39	10.83	0.97
SHS	57	42	10.07	0.90
SHS	57	45	10.64	0.95
SHS	57	48	10.07	0.90
SHS	57	51	10.07	0.90
SHS	57	54	10.07	0.90
SHS	57	57	7.98	0.71
SHS	57	60	8.93	0.80
SHS	58	-9	19.17	1.10
SHS	58	-6	16.36	0.94
SHS	58	-3	17.13	0.99
SHS	58	0	17.13	0.99
SHS	58	3	18.15	1.04
SHS	58	6	23.26	1.34
SHS	58	9	26.84	1.54
SHS	58	12	24.80	1.43
SHS	58	15	25.82	1.49
SHS	58	18	23.77	1.37
SHS	58	21	24.54	1.41
SHS	58	24	25.05	1.44
SHS	58	27	24.54	1.41
SHS	58	30	21.98	1.26
SHS	58	33	24.03	1.38
SHS	58	36	16.87	0.97
SHS	58	39	16.11	0.93
SHS	58	42	17.89	1.03
SHS	58	45	15.85	0.91
SHS	58	48	18.41	1.06
SHS	58	51	17.89	1.03
SHS	58	54	17.89	1.03
SHS	58	57	16.62	0.96
SHS	58	60	15.59	0.90
SHS	59	-9	25.83	0.97
SHS	59	-6	27.10	1.02
SHS	59	-3	25.41	0.95
SHS	59	0	27.95	1.05
SHS	59	3	29.22	1.10
SHS	59	6	35.57	1.33

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
SHS	59	9	37.27	1.40
SHS	59	12	37.69	1.41
SHS	59	15	39.38	1.48
SHS	59	18	40.23	1.51
SHS	59	21	39.38	1.48
SHS	59	24	38.96	1.46
SHS	59	27	38.96	1.46
SHS	59	30	36.84	1.38
SHS	59	33	36.42	1.37
SHS	59	36	25.41	0.95
SHS	59	39	23.29	0.87
SHS	59	42	24.56	0.92
SHS	59	45	23.29	0.87
SHS	59	48	23.29	0.87
SHS	59	51	24.99	0.94
SHS	59	54	27.53	1.03
SHS	59	57	22.87	0.86
SHS	59	60	22.44	0.84
WKY	4	-9	26.36	1.10
WKY	4	-6	23.30	0.97
WKY	4	-3	23.61	0.99
WKY	4	0	22.07	0.92
WKY	4	3	21.77	0.91
WKY	4	6	27.28	1.14
WKY	4	9	30.66	1.28
WKY	4	12	29.12	1.22
WKY	4	15	30.04	1.26
WKY	4	18	32.19	1.35
WKY	4	21	33.72	1.41
WKY	4	24	30.35	1.27
WKY	4	27	30.35	1.27
WKY	4	30	30.66	1.28
WKY	4	33	29.74	1.24
WKY	4	36	22.38	0.94
WKY	4	39	23.61	0.99
WKY	4	42	24.22	1.01
WKY	4	45	23.91	1.00
WKY	4	48	24.83	1.04
WKY	4	51	25.14	1.05
WKY	4	54	24.22	1.01
WKY	4	57	23.61	0.99
WKY	4	60	25.75	1.08

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
WKY	9	-9	7.79	1.02
WKY	9	-6	7.94	1.04
WKY	9	-3	7.32	0.96
WKY	9	0	7.47	0.98
WKY	9	3	8.25	1.08
WKY	9	6	10.43	1.37
WKY	9	9	11.52	1.51
WKY	9	12	11.99	1.57
WKY	9	15	10.90	1.43
WKY	9	18	11.52	1.51
WKY	9	21	10.43	1.37
WKY	9	24	10.59	1.39
WKY	9	27	10.59	1.39
WKY	9	30	10.74	1.41
WKY	9	33	10.74	1.41
WKY	9	36	8.25	1.08
WKY	9	39	7.16	0.94
WKY	9	42	6.85	0.90
WKY	9	45	6.54	0.86
WKY	9	48	6.85	0.90
WKY	9	51	7.79	1.02
WKY	9	54	5.61	0.73
WKY	9	57	6.23	0.82
WKY	9	60	5.76	0.76
WKY	20	-9	16.67	1.05
WKY	20	-6	16.67	1.05
WKY	20	-3	16.67	1.05
WKY	20	0	14.02	0.88
WKY	20	3	15.54	0.98
WKY	20	6	17.43	1.10
WKY	20	9	20.08	1.26
WKY	20	12	21.22	1.33
WKY	20	15	22.36	1.40
WKY	20	18	17.81	1.12
WKY	20	21	20.08	1.26
WKY	20	24	23.49	1.48
WKY	20	27	27.28	1.71
WKY	20	30	26.14	1.64
WKY	20	33	23.11	1.45
WKY	20	36	19.70	1.24
WKY	20	39	17.81	1.12

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
WKY	20	42	16.67	1.05
WKY	20	45	15.91	1.00
WKY	20	48	12.50	0.79
WKY	20	51	13.64	0.86
WKY	20	54	16.29	1.02
WKY	20	57	14.40	0.90
WKY	20	60	14.40	0.90
WKY	21	-9	18.48	0.91
WKY	21	-6	22.00	1.09
WKY	21	-3	20.24	1.00
WKY	21	0	20.24	1.00
WKY	21	3	20.24	1.00
WKY	21	6	22.44	1.11
WKY	21	9	27.72	1.37
WKY	21	12	27.28	1.35
WKY	21	15	28.16	1.39
WKY	21	18	27.72	1.37
WKY	21	21	30.36	1.50
WKY	21	24	30.80	1.52
WKY	21	27	27.28	1.35
WKY	21	30	29.48	1.46
WKY	21	33	33.00	1.63
WKY	21	36	24.64	1.22
WKY	21	39	18.04	0.89
WKY	21	42	17.16	0.85
WKY	21	45	16.28	0.80
WKY	21	48	16.72	0.83
WKY	21	51	19.80	0.98
WKY	21	54	15.84	0.78
WKY	21	57	18.92	0.93
WKY	21	60	17.60	0.87
WKY	22	-9	10.54	0.94
WKY	22	-6	10.85	0.97
WKY	22	-3	12.25	1.10
WKY	22	0	10.85	0.97
WKY	22	3	10.70	0.96
WKY	22	6	13.64	1.22
WKY	22	9	14.57	1.31
WKY	22	12	14.73	1.32
WKY	22	15	13.64	1.22
WKY	22	18	13.33	1.19
WKY	22	21	13.80	1.24

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
WKY	22	24	15.35	1.38
WKY	22	27	14.73	1.32
WKY	22	30	15.19	1.36
WKY	22	33	13.33	1.19
WKY	22	36	10.70	0.96
WKY	22	39	11.47	1.03
WKY	22	42	10.54	0.94
WKY	22	45	10.54	0.94
WKY	22	48	10.54	0.94
WKY	22	51	8.68	0.78
WKY	22	54	10.54	0.94
WKY	22	57	10.85	0.97
WKY	22	60	10.08	0.90
WKY	24	-9	14.34	1.00
WKY	24	-6	14.34	1.00
WKY	24	-3	14.53	1.01
WKY	24	0	14.34	1.00
WKY	24	3	15.09	1.05
WKY	24	6	16.23	1.13
WKY	24	9	16.79	1.17
WKY	24	12	16.98	1.18
WKY	24	15	17.17	1.20
WKY	24	18	18.11	1.26
WKY	24	21	16.79	1.17
WKY	24	24	18.49	1.29
WKY	24	27	18.87	1.32
WKY	24	30	18.11	1.26
WKY	24	33	17.55	1.22
WKY	24	36	12.64	0.88
WKY	24	39	11.51	0.80
WKY	24	42	12.26	0.86
WKY	24	45	10.94	0.76
WKY	24	48	10.75	0.75
WKY	24	51	9.25	0.64
WKY	24	54	11.51	0.80
WKY	24	57	10.75	0.75
WKY	24	60	10.94	0.76
WKY	28	-9	16.52	1.06
WKY	28	-6	14.69	0.95
WKY	28	-3	15.60	1.01
WKY	28	0	15.06	0.97
WKY	28	3	15.42	0.99

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
WKY	28	6	16.88	1.09
WKY	28	9	17.70	1.14
WKY	28	12	20.53	1.32
WKY	28	15	21.63	1.39
WKY	28	18	22.72	1.46
WKY	28	21	27.01	1.74
WKY	28	24	24.73	1.59
WKY	28	27	25.28	1.63
WKY	28	30	25.37	1.64
WKY	28	33	24.37	1.57
WKY	28	36	21.44	1.38
WKY	28	39	20.53	1.32
WKY	28	42	20.90	1.35
WKY	28	45	18.98	1.22
WKY	28	48	20.26	1.31
WKY	28	51	18.52	1.19
WKY	28	54	18.52	1.19
WKY	28	57	19.53	1.26
WKY	28	60	18.07	1.16
WKY	29	-9	10.24	1.04
WKY	29	-6	9.50	0.97
WKY	29	-3	9.99	1.02
WKY	29	0	9.50	0.97
WKY	29	3	10.48	1.07
WKY	29	6	11.58	1.18
WKY	29	9	13.91	1.42
WKY	29	12	13.42	1.37
WKY	29	15	13.48	1.38
WKY	29	18	13.36	1.36
WKY	29	21	13.48	1.38
WKY	29	24	13.85	1.41
WKY	29	27	14.16	1.44
WKY	29	30	13.97	1.43
WKY	29	33	15.51	1.58
WKY	29	36	11.83	1.21
WKY	29	39	9.44	0.96
WKY	29	42	10.48	1.07
WKY	29	45	11.03	1.13
WKY	29	48	11.09	1.13
WKY	29	51	11.03	1.13
WKY	29	54	10.85	1.11
WKY	29	57	11.83	1.21

Table A-1 (continued)

GROUP	RAT (#)	TIME (min)	Na ⁺ CONTENT (μmoles/g)	Na ⁺ % (%control/100)
WKY	29	60	10.54	1.08

Note: Decimal points (.) represent missing data.

VITA

A. Lynelle Golden was born in Thomasville Georgia on November 18, 1958 where she lived until graduating from high school in 1977. She received an Associate of Arts degree from Oxford College of Emory University in 1979 and continued her education at Emory University in Atlanta Georgia. She studied philosophy and biology and received a B.S. in biology from Emory in 1981.

Following graduation Lynelle worked as a research technician in the departments of Poultry Science and Zoology, respectively, at the University of Georgia in Athens until beginning graduate study in the Life Sciences Program at the University of Tennessee, Knoxville in September 1984. She was a teaching assistant in the department of Zoology, and also assisted with clinical research in the College of Veterinary Medicine while working on her Master's degree. She received an M.S. in Life Sciences, with concentration in Physiology, in December 1987.

Lynelle began working on her Ph.D. in the Life Sciences program in 1988. She began her Ph.D. research and training at the University of Tennessee College of Veterinary Medicine. As part of a collaborative research project, she completed the last 2 years of her dissertation research as a visiting graduate student in the Division of Cardiovascular Disease at the University of Alabama, Birmingham. She received a Ph.D. in Life Sciences, with concentration in physiology, in May 1992.

Lynelle is a member of the American Physiological Society, and the American Heart Association. She began working as a postdoctoral associate in the Division of Cardiovascular Disease at the University of Alabama at Birmingham in April 1992.