Cardiovascular response to isoproterenol in norepinephrine-pretreated and in exercised rats

by

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A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

Department: Veterinary Physiology and Pharmacology
Major: Physiology

Iowa State University
Ames, Iowa

1979

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INTRODUCTION

Cardiovascular disease kills more people in this country than all other diseases combined (Borhani 1977). This has led to a cascade of research in cardiac performance and cardiac disease. Since the early 1960's when President John F. Kennedy instituted a national program to encourage Americans to become more active and physically fit, exercise has become a vital tool to both test and treat cardiac disease. The concept that exercise might protect an individual from heart attack arose in the early 1950's when Morris et al. (1953) reported that London bus conductors had fewer heart attacks than the less active bus drivers. It was also noticed that postal carriers seemed to have fewer heart attacks than postal clerks (Morris 1959; Kahn 1963). More recently, Paffenbarger (1977) reported on a continuing study of a group of San Francisco longshoremen which began in 1951. The number of fatal heart attacks suffered by those men in inactive occupations was 1.8 times higher than those who worked in active occupations. It was also noted in this study that the number of diagnosed cardiac problems was the same in both types of occupations, but those in the active jobs had a better survival rate.

The study of the effect exercise has on heart disease was widely expanded by the work of Rona (1959a) who initiated the use of the synthetic catecholamine isoproterenol to produce an infarction in rats that closely resembled lesions caused by spontaneous infarction. The technique of isoproterenol administration to examine myocardial infarction is now widely accepted.

The purpose of this study was to examine the physiological responses to norepinephrine and isoproterenol in three groups of rats, an exercise stressed group, a norepinephrine stressed group, and a control group.

LITERATURE REVIEW

Cardiac Adaptations to Exercise

The cardiovascular response to training has been the subject of a number of review papers (Ekelund 1969, Barnard 1975, Scheuer and Tipton 1977, Segel 1977). There is general agreement that exercise training seems to protect the myocardium from infarction. The mechanism of this protection is, however, the subject of controversy, as the myocardium demonstrates a number of different adaptations to exercise training. A significant problem throughout the current literature is that there are variations in species, as well as a lack of uniformity in the type and duration of any exercise program (Segel 1977).

The ventricular mass and left ventricular volume of the heart have been shown to be increased by strenuous exercise programs in experimental animals and in the human population. Cardiac hypertrophy has been shown to occur in mice(Kiiskinen and Heikkinen 1976), rats (Oscai et al. 1971), dogs (Wyatt and Mitchell 1974), and humans (Morganroth et al. 1975). An increase in ventricular volume occurred in animals that were subjected to either swimming or running programs (Wyatt and Mitchell 1974; Morganroth et al. 1975). In humans, however, it has been reported that ventricular end-diastolic volume increases only in those individuals who participate in endurance type training such as running, swimming, and bicycling. Athletes engaged in isometric sports such as weight lifting showed ventricular hypertrophy, but no ventricular end-diastolic volume increase (Roskamm 1971). It has been demonstrated also that ventricular hypertrophy can be produced by stimulation of the beta-1 receptors on the heart (Pagano and Inchiosa

1977). These researchers also showed that stimulation of the alpha receptors seemed to oppose the effect of beta-1 stimulation.

Because there is a positive correlation between ventricular volume and maximal stroke volume, it is not surprising that exercise training also increased stroke volume in most individuals (Roskamm 1971; Saltin 1971). Some researchers believe that the increase in stroke volume achieved with exercise training is responsible for maintaining the performance of the exercised myocardium during periods of hypoxia or ischemia (Scheuer and Stezoski 1972; Bahn and Scheuer 1975). In general, the hearts from trained animals have been reported to have improved intrinsic contractility (Crews and Aldinger 1967; Scheuer and Stezoski 1972; Wyatt and Mitchell 1974). Bahn and Scheuer (1975) stated that an increase in myosin ATPase activity was probably responsible for the change in contractility. However, Tibbits et al. (1978) attribute the change in contractility to an increase in the availability of extracellular calcium to the myocardial cell rather than ATPase.

A reduction in resting heart rate is another phenomenon of strenuous exercise training (Scheuer and Tipton 1977; Barnard et al. 1976). Tipton (1965) and Tipton and Taylor (1965) demonstrated that bradycardia in swimming rats could be blocked to a certain extent by administering atropine. This effect is, however, not as easily demonstrated in animals that have been exercised on a treadmill. Therefore, some researchers have concluded that the response that these researchers blocked was more related to the diving reflex rather than a training effect (Ekblom et al. 1973). Sutton et al. (1967) showed a reduction in the intrinsic sino-atrial frequency in humans exposed to physical training. This reduction

has also been reported in trained rats (Lin and Horvath 1972). The mechanism involved in reducing the sino-atrial frequency and the resulting bradycardia is still an area of confusion (Badeer 1975). More recently, Hughson et al. (1977) have demonstrated a decrease in the sino-atrial node sensitivity to norepinephrine. These researchers noted an increase in the myocardial potassium content and concluded that the bradycardia was not a result of increased parasympathetic nervous activity, but was probably established by electrophysiological factors controlled by the membrane potentials of the cardiac cell.

An increase in the extent of coronary collateral circulation may accompany exercise training. Increases in the capillary to fiber ratio and in fiber diameter in the cardiac tissue, which are indicators of collateral vessel formation, have been reported to occur in rats exercised by both treadmill running and forced swimming programs (Ekelund 1969; Danenberg 1972, respectively). McElroy et al. (1978) used a perfusion of carbon black to demonstrate an increase in the capillary to fiber ratio in rats exercised by forced swimming. These researchers noted that this indication of collateral vessel formation occurred in the absence of ventricular hypertrophy. Marr et al. (1977) used radioactive microspheres to demonstrate that there was a positive shift in the ratio of endocardial to epicardial blood that occurred when dogs were exercise-trained. They surmised that the shift in myocardial blood distribution was helpful in preventing hypoxia in the endocardial tissue. This finding is significant as the endocardium is the portion of the myocardium most susceptible to necrosis under hypoxic conditions (Bloor 1974). Therefore, the shift in blood from the epicardium to the endocardium during exercise stress may prevent

ischemia and infarction from occurring. The development of collateral circulation is not universally accepted because current research has not proven an increase in collateral vessels in exercised human subjects (Scheuer and Tipton 1977). Sanders et al. (1978), using miniature swine with resting bradycardia and an increase in stroke volume after treadmill training, demonstrated no increase in collateral circulation using microspheres.

Physical training seems to produce an increase in the metabolic efficiency in the myocardial cell. Myocardial oxygen consumption of exercised humans at rest was less than myocardial oxygen consumption from sedentary human subjects (Heiss et al. 1976). Bersohn and Scheuer (1978) have demonstrated that hearts from exercised rats maintain their function during hypoxic conditions better than hearts from nonexercised animals. Scheuer et al. (1977) have stated that the improved physiological performance in exercised rat hearts cannot be attributed to energy stores or intermediary metabolism, but rather to improved blood flow, oxygen delivery, and changes in the contractile properties of the heart. Heiss et al. (1976) placed catheters in the coronary sinus, pulmonary artery, and abdominal aorta and monitored myocardial substrate uptake in humans during bicycle ergometer exercise. These investigators reported that hearts from exercised subjects required less energy for normal function at both resting and working conditions. The increase in mitochondrial concentration in somatic muscle exposed to exercise is well-documented (Gollnick et al. 1973; Holloszy and Booth 1976). Similar changes in cardiac mitochondrial number is less clear. Gollnick and King (1969) reported that exercised rat hearts showed an increase in mitochondria but this finding is contradicted by other researchers in the field (Oscai et al. 1971; McGill and

Stone 1977). There is a strong possibility that the confusion over cardiac mitochondrial number is the result of differences in tissue preparation, strain, and gender of animal (Segel 1977).

Plasma Catecholamine Responses to Exercise

A rise in endogenous catecholamines accompanying strenuous muscular work was first reported by Hartman et al. (1922). Unfortunately, these early investigators were unable to separate norepinephrine and epinephrine activity in their study. Gray and Beetham (1957) were among the first to distinguish between norepinephrine and epinephrine levels in humans during strenuous work. These researchers were the first to report that changes in plasma epinephrine levels were rather low and inconsistent, while norepinephrine levels rose about threefold during stressful exercise. This finding has been subsequently confirmed by several authors (Haggendahl et al. 1970; Raven et al. 1970; Kotchen et al. 1971; Hartley 1975; Cousineau et al. 1977). Elmadjian et al. (1957) reported that norepinephrine release was related to physical activity, while epinephrine release was related more to emotional anticipatory stress. Of particular interest is that exercised animals and humans demonstrate different norepinephrine responses than sedentary individuals when challenged with exercise stress. Exercised individuals have a slightly lower resting plasma level of norepinephrine and during exercise the rise in plasma norepinephrine level is only about half of that seen in an equally stressed sedentary subject (Hartley 1975; Raven et al. 1970). The reason for this difference in response to stressful stimuli is unclear at this time. However, some researchers have stated that this difference may be evidence that exercise causes a reduction in

the sympathetic nervous system activity (Hartley 1975; Paynter et al. 1977).

The role of norepinephrine in exercise-trained and untrained individuals is a broad and somewhat confused area of research. Wyatt et al. (1978) reported that training in cats may improve the catecholamine-induced improvement of myocardial contractile function and adenylate cyclase activity. Changes in the circulating levels of catecholamines may be linked to possible alterations in the number or the sensitivity of the beta-1 receptors on the myocardial cell (Scheuer and Tipton 1977; Wildenthal 1974; Banerjee and Kung 1977). Increases in the norepinephrine level may play a role in rises in plasma renin levels and be directly related to renal blood flow in exercising individuals (Kotchen et al. 1971). It also seems probable that the increase in norepinephrine may be responsible for an increase in cell membrane permeability to certain enzymes during exercise (Raven et al. 1970). Dumont and Lelorier (1977) demonstrated that rises in norepinephrine levels may actually improve the ratio of blood flow between the endocardium and epicardium of human subjects suffering from angina pectoris. These researchers believe that this redistribution of blood flow may prevent endocardial damage. In contrast to this theory are data presented by Waldenstrom et al. (1978) which demonstrate that a rapid release of endogenous stores of norepinephrine might actually initiate acute myocardial infarction in subjects with marginal coronary blood flow.

Induction of Myocardial Infarction by Isoproterenol

The induction of myocardial necrosis by catecholamines for the purpose of producing and studying myocardial infarction was first reported

by Rona et al. (1959a). Prior to this time, the surgical technique of coronary occlusion was the only method of producing infarction. Coronary occlusion is still used in larger animals to study regional blood flow changes (Bloor 1974; Sanders et al. 1978). Because of the size of the small laboratory animal, surgical coronary occlusion was not practical and, therefore, the induction of myocardial damage by catecholamines was an alternate solution to this problem (Rona et al. 1959a). The synthetic catecholamine isoproterenol (DL 3'4'-dihydroxyphenol-2-isopropylaminoethanol) hydrochloride was first used by Rona et al. (1959a) and others to achieve reproducible myocardial infarction in rats (Wexler and Kittinger 1963; Handforth 1962).

The L form of isoproterenol was soon reported to be more potent than the racemic mixture in producing myocardial infarction (Rosenblume et al. 1966). Since then, it has been well-documented that the L form of isoproterenol, like the L isomer of most adrenergic catecholamines, is more potent than either the D or DL mixture of the compound (Alexander et al. 1975; Harden et al. 1976; Mukherjee and Lefkowitz 1976).

Isoproterenol is a pure beta agonist (Innes and Nickerson 1975). The effect on the heart is both inotropic and chronotropic (Wexler 1973; Petery and Meirop 1977). It is assumed by most investigators that stimulation of a beta receptor causes an activation of adenyl cyclase and a subsequent rise in intracellular cyclic AMP (Mukherjee and Lefkowitz 1976). It is the cyclic AMP concentration that actually determines the activity of the cardiac cell (Haber and Wrenn 1976).

The physiological responses of administering high doses of isoproterenol to rats are tachycardia and hypotension (Rona et al. 1959a; Beznak

and Hacker 1963; Wexler et al. 1968; Buckberg and Ross 1973; Laughlin et al. 1978). An 18-25 percent increase in heart rate in rats given isoproterenol has been reported by several authors (Beznak and Hacker 1963). Accompanying the tachycardia is an increase in energy demand (Milei et al. 1976) and oxygen consumption (Rona et al. 1959b; Zbinden and Nutley 1960). The systemic vascular effect of isoproterenol is to decrease the peripheral resistance in the major muscle beds by causing vasodilatation. The resulting systemic hypotension is characterized as classic of high doses of isoproterenol (Innes and Nickerson 1975; Petery and Meirop 1977).

The hypotension is thought to be responsible for the reduction in perfusion of the myocardium (Kirk et al. 1977). Since the cardiac tissue is supplied primarily during diastole, the diastolic pressure is most critical in retaining cardiac perfusion (Bloor 1974). Investigators Beznak and Hacker (1963) indicated that the endocardium is the area of the heart most seriously affected by isoproterenol injection. Handforth (1962) reported that the blood flow to the endocardium appeared to actually stop in rats treated with isoproterenol. Buckberg and Ross (1973) demonstrated that a low dose of isoproterenol in the dog produced a heart rate of 161 beats per minute and an 83 percent increase in coronary blood flow. However, when the dose of isoproterenol was increased to produce a heart rate of 197 beats per minute, the drug lost its positive inotropic effect and blood flow in the subendocardium fell 35 percent while systemic diastolic pressure dropped 50 percent from control values. Johannsen et al. (1978) reported that the ratio of endocardial to epicardial blood flow was greatly reduced by high doses of isoproterenol. Kirk et al. (1977) suggested that the hypoperfusion of the myocardium in general and

particularly the endocardium is the primary mechanism by which isoproterenol caused myocardial ischemia and infarction. Most investigators, however, maintain that the cardiac damage results from an imbalance between the increased oxygen demand and the decrease in perfusion pressure caused by isoproterenol injection (Zbinden and Nutley 1960; Handforth 1962; Ferrans et al. 1964; Wexler 1973; Laughlin et al. 1978). These investigators believe that isoproterenol-induced myocardial necrosis is produced by an increased heart rate and increased cardiac contractility which causes an oxygen demand that cannot be met by the hypofunctioning coronary circulation. Myocardial hypoxia soon develops which then leads to ischemia and eventually irreversible infarction. This mechanism is supported by the fact that the endocardium is the first and most likely area to become necrotic when circulatory insufficiency develops quickly, and this is also the most common area affected by isoproterenol (Handforth 1962; Ferrans et al. 1964).

Rona et al. (1959b) noted that there was a direct relationship between body weight and the severity of the infarction produced by isoproterenol. This observation has been corroborated by other investigators who have reported that heavier rats are more susceptible to isoproterenol infarction (Balazs et al. 1962; Wexler et al. 1968). Although Balazs et al. (1972) have recently suggested that the amount of body fat and not a change in either the activity of the cardiac beta receptors or the breakdown rate of isoproterenol was responsible for the differences in heavier rats, it is at present still uncertain as to the specific effect weight has on the cardiotoxic effects of isoproterenol administration.

Use of Plasma Enzymes as Indicators of Cardiac Damage

The use of plasma or serum enzymes for diagnostic purposes of cardiac damage is widely accepted today, however, the first report of such use for indication of myocardial infarction was not made until 1954 by LaDue et al. They were the first to report that serum glutamic oxaloacetic transaminase (SGOT) was elevated in patients that had suffered heart attacks. Karmen et al. (1955) stated that the increase in serum transaminase was from the damaged cardiac cells. In 1961, Highman and Altland demonstrated that hypoxia increased the cell membrane permeability to intracellular enzymes. This work then suggested that ischemia as well as necrosis was responsible for the rise of cardiac enzymes in the systemic circulation. In an attempt to correlate the amount of cardiac damage with the enzyme concentration in the blood, Kjekshus and Sobel (1970) demonstrated a linear relationship between the blood enzyme concentration and the cardiac damage in the rabbit. Maclean et al. (1976) went a step further and reported that they could predict the percentage of damaged myocardium from the plasma enzyme levels.

Wroblewski and LaDue (1955) reported that another enzyme, lactic dehydrogenase (LDH), also rose in patients suffering from myocardial infarction. These researchers concluded that LDH, like SGOT, was liberated from damaged cardiac cells. Sobel and Shell (1972) reported that LDH levels reached a peak at 36-38 hours after infarction and declined slowly over the next seven days. Although these studies were done on human subjects, other investigators have shown that cardiac damage in rats, dogs, and rabbits produces elevated levels of LDH in the systemic circulation (Schenk et al. 1966; Wexler 1970; Shell et al. 1971; Maclean

et al. 1976).

The first report of multiple forms of LDH came from Vessel and Bearn (1957). Using electrophoresis, these researchers isolated three forms of LDH. Soon others demonstrated the existence of five distinct forms of LDH (Wroblewski et al. 1960; Weiland and Pfleiderer 1961). Markert (1963) and Dawson et al. (1964) reported that these LDH isoenzymes were of similar molecular weight and formed by tetrameric association of two distinct subunits which they designated M or H. Each isoenzyme was composed of four of these subunits. It was shown that the LDH from somatic muscle was composed of four M subunits (M_4), while the LDH isolated from cardiac muscle was composed of four H subunits (H_4). The other three isoenzymes are composed of the remaining combinations of M and H subunits, or H_1M_3, H_2M_2, and H_3M_1 (Weiland and Pfleiderer 1961). The isoenzymes are designated in the literature by their relative mobility in an electric field, so that LDH-1 is the most mobile isoenzyme which is the H_4 species and LDH-5 is the rather immobile M_4 species (Dawson et al. 1964). Dawson et al. (1964) reported that the LDH-5 found in skeletal muscle has a relatively low Km for pyruvate and is normally used for anaerobic glycolysis. The LDH-1 found in cardiac muscle, however, has a very high Km for pyruvate and can actually be inhibited by pyruvate. This finding corresponds to the idea that cardiac muscle is aerobic in nature.

The distribution of the various isoenzymes in humans and animals has shown that LDH-1 and LDH-2 are found primarily in the heart, although small amounts are found in the kidney and red blood cells also. LDH-3 is found primarily in red blood cells, while LDH-4 and LDH-5 are found only in liver and somatic muscle (Hess and Walter 1961; Weiland and Pfeiderer 1961).

Because of these findings, some researchers began to question if LDH was a good diagnostic tool since more than one organ system was often represented by a single isoenzyme (Cohen et al. 1964; Wexler 1970; Sobel and Shell 1972). In spite of these problems, it has been shown that LDH-1 is a much better indicator of cardiac damage than total LDH or SGOT (Wroblewski et al. 1960; Sobel and Shell 1972; Berstein et al. 1974). Sobel and Shell (1972) have shown that LDH-1 elevation can indicate myocardial damage long before total LDH levels elevate significantly. It has been stated that LDH-1 determinations are a better diagnostic tool than serial electrocardiograms and have been shown to be more than 95 percent reliable in detecting the onset of myocardial infarction in humans (Cohen et al. 1964; Sobel and Shell 1972).

MATERIALS AND METHODS

Male virgin Sprague Dawley rats (Sprague Dawley Farms, Madison, Wisconsin) were maintained in group cages in an animal room with a constant light cycle (7:00 AM-10:00 PM) and temperature (26 $\pm$ 2^{o} C). Animals were randomly divided into three groups, a sedentary control group (S), a drug pretreated group (N), and an exercise-trained group (Ex). Ex animals were given tap water and Teklad rat chow Ad. libitum while the S and N animals were given a restricted diet of Teklad rat chow in order to maintain weekly body weights in the same range as the Ex animals. The general health of all the animals was closely monitored and antibiotics were administered to all animals when necessary. No antibiotics were administered three weeks prior to the termination of the study.

Exercise Program

Ex animals were exercised on a motor driven treadmill (Jette et al. 1969) 30 minutes per day, 5 days per week for the entire 20 week program. The treadmill speed was 17 meters per minute and the animals ran on an 8^{o} incline. Electrical stimulation was used to encourage the rats to run. This level of exercise has been reported to be a sufficient work load to produce training effects and is a work load of about 70 percent of maximal oxygen uptake in the rat (Severson et al. 1978; Scheurer and Tipton 1977). Animals received no exercise 48 hours prior to being killed.

Drug Pretreatment

Animals assigned to the N group were treated with norepinephrine 5 days per week. Two intraperitoneal injections of 2 μg/kg were given

15 minutes apart during the time the Ex animals were running on the treadmill. The dosage of norepinephrine selected produced heart rate and blood pressure increases in test animals without causing ectopic beats. The C animals were injected with equal volumes of physiological saline on the same schedule.

Recording of Data

At the end of the 20 week exercising period, all animals were removed from exercise and drug treatment for 48 hours. Each animal was anesthetized with sodium pentobarbital (25 mg/kg). The right common carotid artery was isolated and cannulated with a polyethylene cannula tipped with a 23 gage needle. A Beckman R611 Dynograph was used to record the physiological events. Pressure measurements were made using a Statham P23Db pressure transducer. Standard electrocardiogram (ECG) electrodes were placed subcutaneously and each animal was positioned in dorsal recumbency. After control data were obtained, each animal was given an intravenous injection of norepinephrine (2 μg/kg), and the response was recorded. After the physiological events returned to control values, a second dose of norepinephrine (4 μg/kg) was given to each animal, and the parameters were again recorded. After the physiological events returned to control the third time, the animals were given at random either physiological saline or L-isoproterenol (80 mg/kg) subcutaneously. Following either the isoproterenol or saline response, the animal was allowed to recover, removing the cannula and suturing the incision. After a recovery period of twenty-four hours, each animal was again anesthetized with sodium pentobarbital (25 mg/kg). Blood was drawn from the post vena cava into

plastic syringes and the animal was killed by exsanguination. The hearts were removed and wet ventricular weights were obtained. The blood was placed in heparinized centrifuge tubes and centrifuged for 15 minutes at 1400 x g. The plasma was harvested and placed in sealed plastic tubes kept at room temperature until the enzymatic assay could be carried out. All enzymatic assays were completed within 6 hours after plasma collection.

Interpretation of Data

Mean blood pressure was calculated using the formula:

$$\text{Mean blood pressure} = \text{diastolic pressure} + 1/3(\text{pulse pressure}).$$

Systolic and diastolic pressures were obtained from the calibrated pressure recordings. The mean blood pressure was determined at maximum pressure response for norepinephrine and at the minimum pressure response for isoproterenol. Heart rates were determined by counting the number of QRS peaks on the ECG tracing for a period of not less than four seconds. The heart rate response for a given dose of norepinephrine was determined before the blood pressure had peaked, in an attempt to avoid baroreceptor regulation. The isoproterenol heart rate response was obtained during the time of maximum drug effect. The duration of the norepinephrine blood pressure response was determined as the time from the initial rise in systolic blood pressure until the blood pressure returned to control values. Because of the extremely long blood pressure duration and the cardiotoxic effects of the isoproterenol injection, the response time for isoproterenol was determined as the time from the onset of drug action to the point where the systolic blood pressure began to return toward the

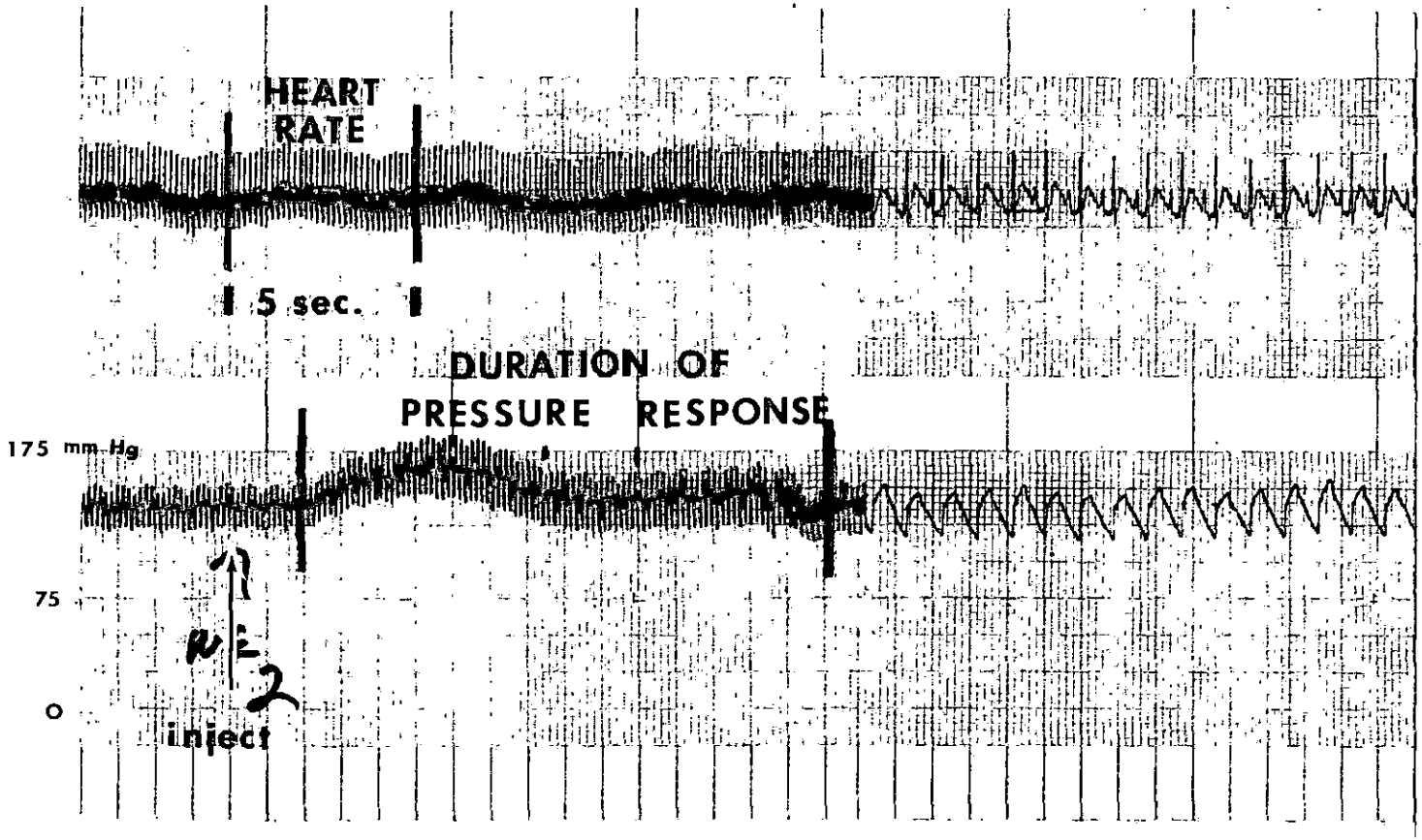


Figure 1. Norepinephrine blood pressure and heart rate response

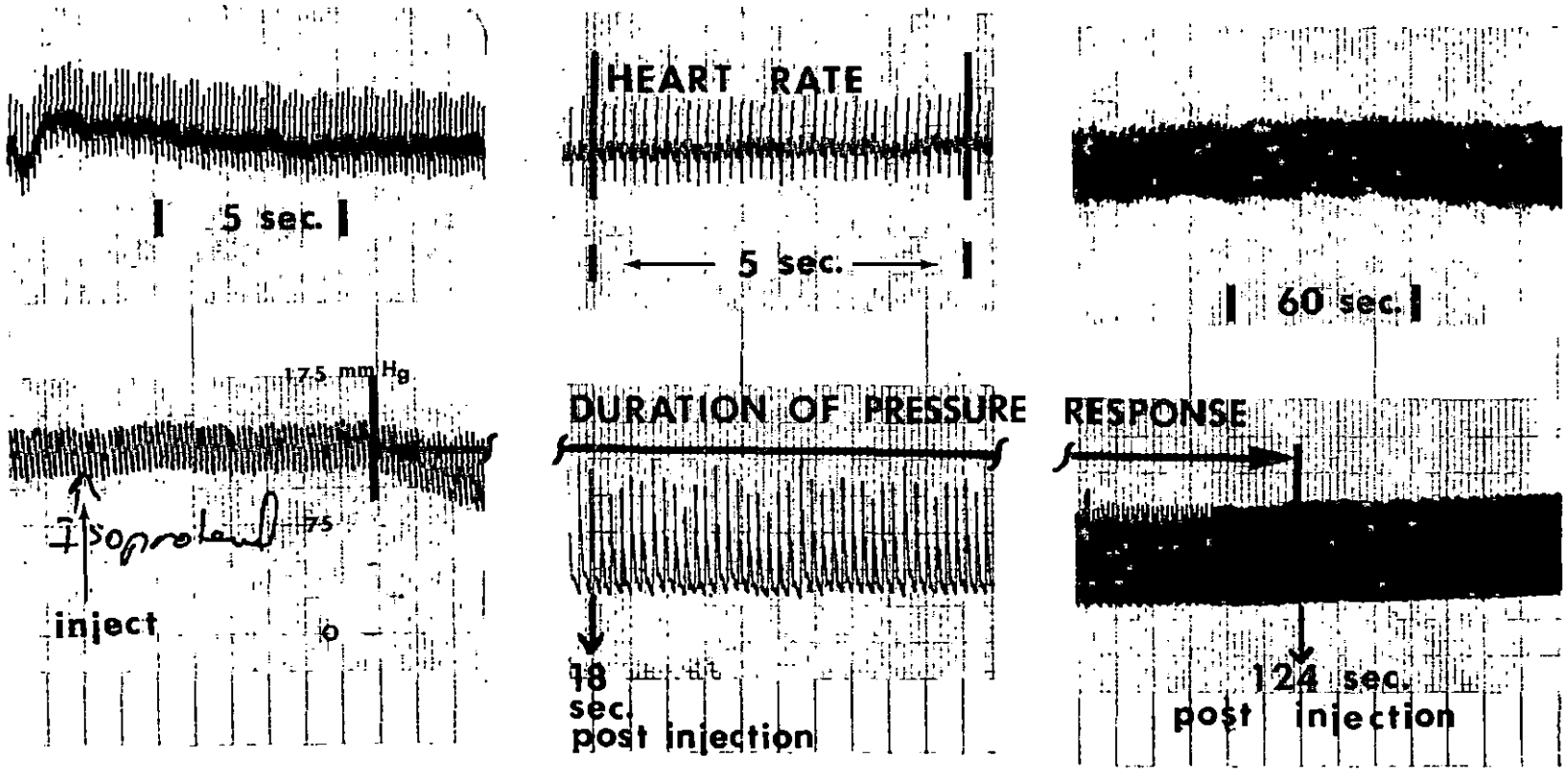


Figure 2. Isoproterenol blood pressure and heart rate response

preinjection level. Examples of recordings are shown with the duration and heart rates indicated.

Determination of Total LDH Activity

Total plasma LDH activity was determined spectrophotometrically by the method of Wacker et al. (1956) with the modifications by Calbiochem (Calbiochem, La Jolla, California; Appendix A).

Isolation of Plasma LDH Isoenzyme Activity

Plasma LDH isoenzymes were separated by electrophoresis on cellulose polyacetate strips using the method of Preston et al. (1965) (Appendix B). The activity of a specific isoenzyme was obtained by multiplying the total activity by the relative percentage of the specific isoenzyme (Vander Tuig 1976).

Data Analysis

Statistical analyses of data were performed using the Statistical Analysis System (Barr and Goodnight 1972). The correlation procedure was used to determine correlation coefficients between data. The means procedure was used to calculate the means and standard errors. The means were compared using the Student's T test. Means were considered significantly different if $p < 0.05$.

RESULTS AND DISCUSSION

Body Weight and Heart Weight

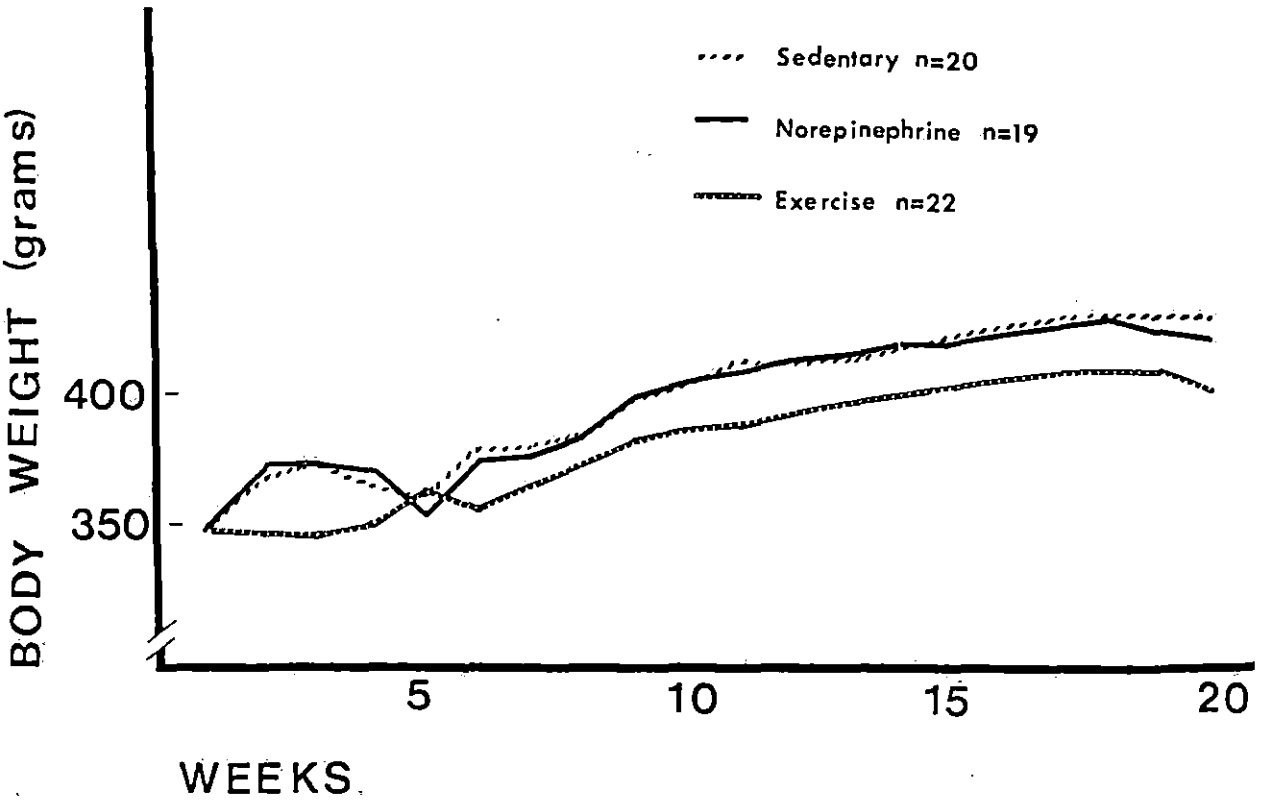
Weekly mean body weights for the three groups of rats are shown in Figure 3. No significant difference was observed among any of the three groups during the 20-week experiment. At the conclusion of the experiment, there was no significant difference observed among the wet ventricular weights of any of the animals. Therefore, there was no difference in the heart weight to body weight ratios as shown in Table 1.

Table 1. Heart weight to body weight ratios

Group name	Mean body weight at sacrifice (grams)		Heart weight to body weight ratio (grams/kilogram)
Sedentary control	431 ± 10[a]	n = 22	3.14 ± 0.47[a]
Norepinephrine pretreated	418 ± 12	n = 17	2.87 ± 0.43
Exercised	409 ± 10	n = 21	2.88 ± 0.49

[a]All numbers are expressed as mean ± standard error of the mean.

It has been known for some time that male rats do not increase their food consumption adequately to compensate for the energy expended during strenuous activity (Ocsai et al. 1971; Dohm et al. 1977). It has also been demonstrated that heavier rats are more susceptible to isoproterenol cardiac damage than rats of the same age and lighter body weight (Rona et al. 1959b; Balazs et al. 1962; Riggs et al. 1977). Riggs et al. (1977)



Sedentary--rats not exercised on the treadmill
Norepinephrine--rats pretreated with norepinephrine 5 days per week
Exercise--rats exercised on the treadmill 5 days per week

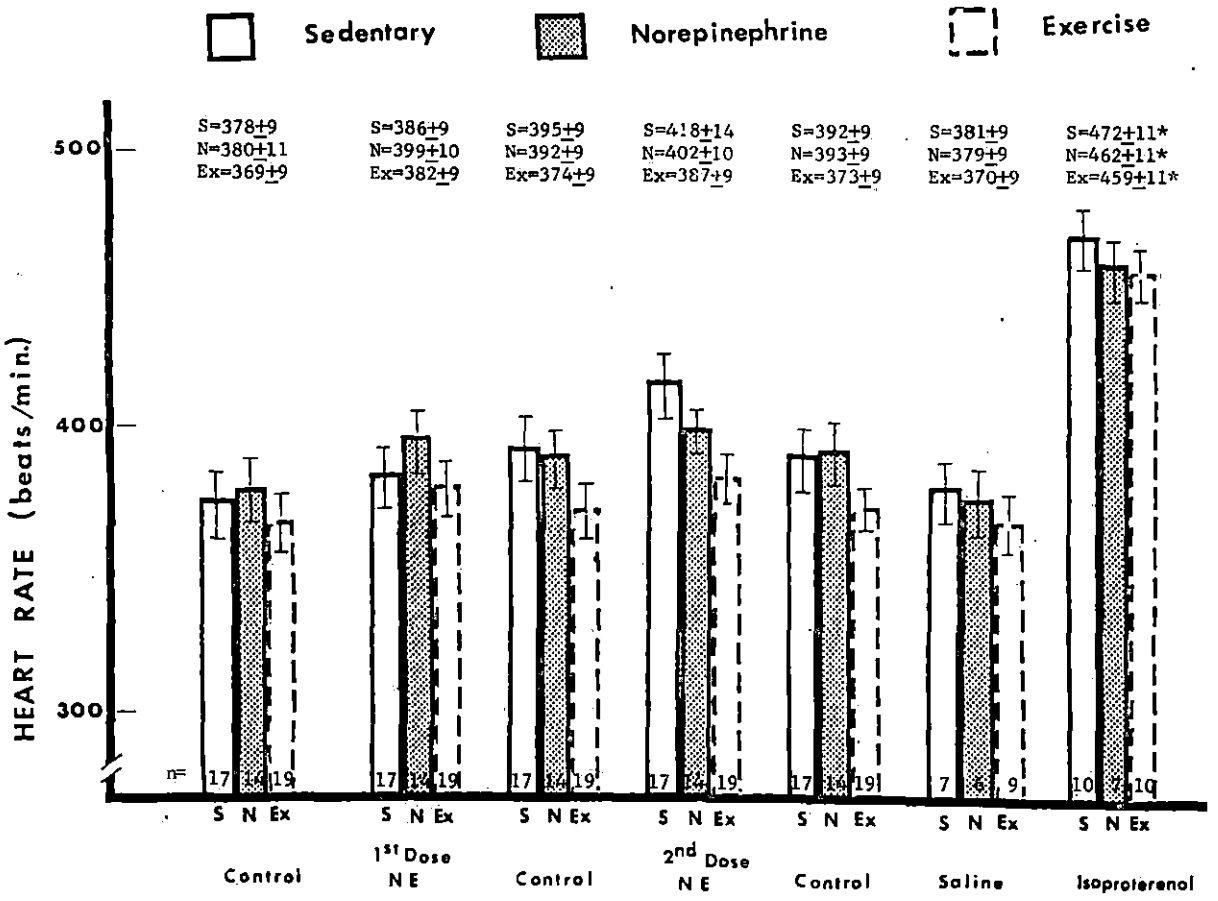
Figure 3. Mean weekly body weights

hypothesized that the protective effect they observed in exercised animals treated with isoproterenol was derived from the difference in the body weights and the resulting difference they observed in heart weight to body weight ratio. These researchers postulated that, since exercised animals demonstrated lower body weights and larger ventricular mass than sedentary animals, the hearts of the exercised rats would have a reduced afterload. From these data, Riggs et al. (1977) stated that the protection from isoproterenol infarction observed in the exercised rats was derived from lower body weight and higher heart weight in exercised animals.

In an effort to remove the confusion involving body weight, the rats in this study were pair fed to maintain body weights that were not significantly different from each other. Therefore, any physiological differences between exercised and sedentary animals reported in this study cannot be attributed to the body-weight differences described by Riggs et al. (1977).

Heart Rate

The heart rate responses to the two doses of norepinephrine and to the dose of isoproterenol are shown in Figure 4. The exercise group showed a slight but nonsignificant bradycardia during the control recordings. There were no significant differences observed among the three groups for any drug treatment. The failure to demonstrate a resting bradycardia in the exercised animals indicated that the exercise regime was not strenuous enough. However, the trend shown for resting bradycardia demonstrates that the exercise load used was approaching a sufficient work stress to produce bradycardia. Using light exercise loads is advantageous in that



Control--baseline recording before and after drug treatments

1st Dose NE--first intravenous injection of norepinephrine (2 µg/kg)

2nd Dose NE--second intravenous injection of norepinephrine (4 µg/kg)

Saline--subcutaneous injection of physiological saline

Isoproterenol--subcutaneous injection of L-isoproterenol (80 mg/kg)

* indicates a significant rise in heart rate in all three groups treated with l-isoproterenol ($p < .05$)

Figure 4. Mean heart rates ± standard error of the mean

increases in cardiac stroke volume and increased cardiac contractility have been well-documented in exercise programs that fail to demonstrate cardiac hypertrophy or resting bradycardia (Bersohn and Scheuer 1978; Scheuer et al. 1977). The lighter exercise load allows the study of the effects of exercise without the physical changes in cardiac and skeletal muscle that occur at heavier work loads (Shepherd and Gollnick 1976; Holloszy and Booth 1976). These lighter work loads are probably more representative of the human exercise program.

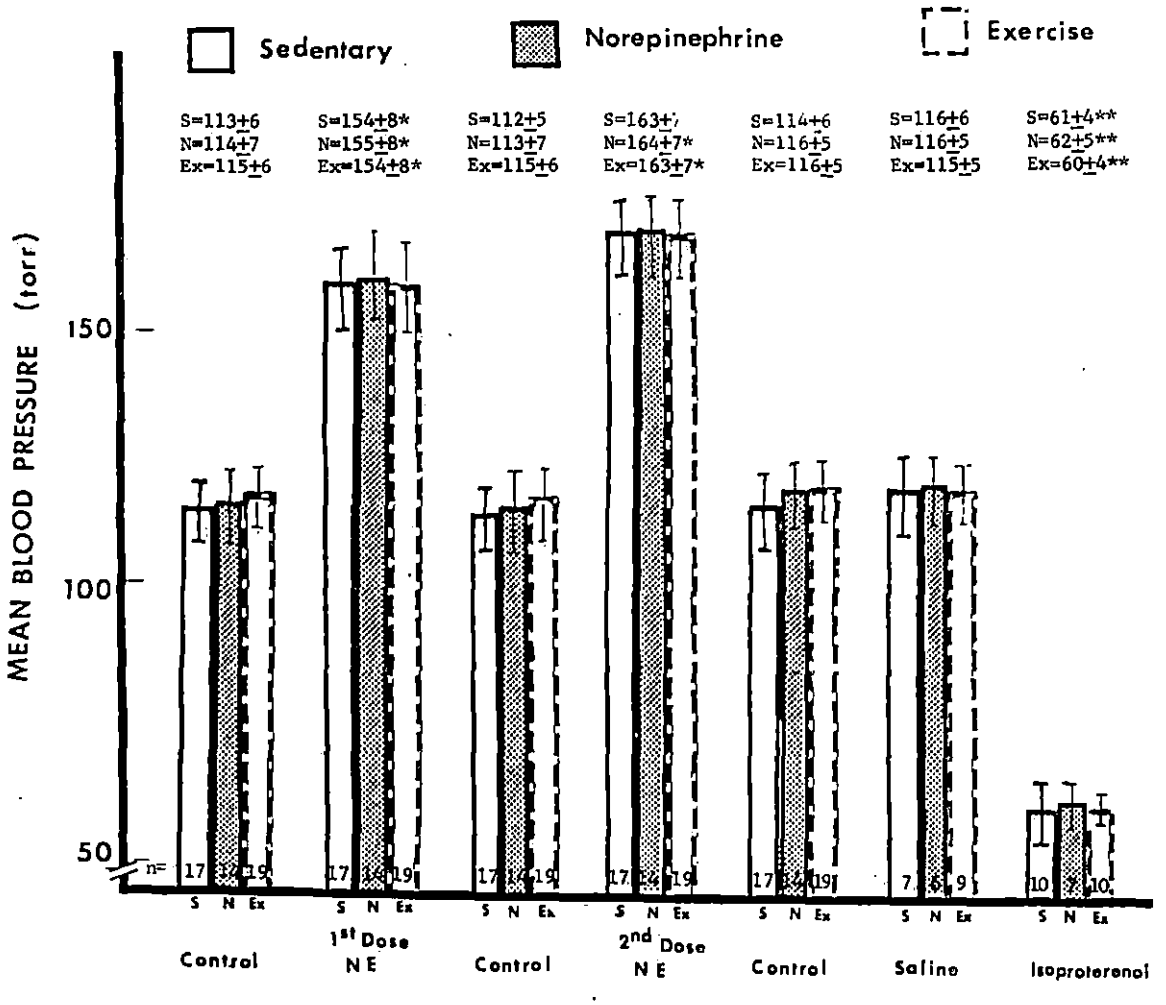
It has been reported that the number or the affinity of the beta receptors on the heart might be altered by exercise training (Scheuer and Tipton 1977; Mickey et al. 1976). The real possibility that changes in the number of beta receptors can occur was demonstrated by Banerjee and Kung (1977). These researchers used a radioactive labeling technique to quantify the number of beta receptor binding sites on rat cardiac cell membranes. Their findings indicate that animals without thyroxin had fewer receptor binding sites than normal animals or animals receiving thyroid hormone replacement. From these data, they concluded that endogenous norepinephrine may be important in determining the number of beta receptor sites on the cardiac cell. This conclusion was based on the assumption that thyroid hormones have an integral part in the regulatory mechanism associated with sympathetic discharge. If exercise training reduced the number of active beta receptor sites on the rat myocardium, isoproterenol would be tolerated better by the exercised animal than the nonexercised animals with the normal number of beta receptors. If the exercise training of the rats in this study had reduced the number or the affinity of the beta receptors, the exercise animals would have demonstrated a reduced heart rate response

to norepinephrine and isoproterenol compared to the sedentary rats. No such differences were observed in this study. Therefore, at the level of exercise used in this study, there is no evidence to indicate that the beta receptors of the exercised rats were reduced in either number or affinity for catecholamines. The very high heart rates produced by isoproterenol injection were similar to heart rate responses reported by other researchers (Riggs et al. 1977; Rona et al. 1959a; Beznak and Hacker 1963).

Blood Pressure

The blood pressure responses to norepinephrine and isoproterenol are shown in Figure 5. There are no significant differences among any of the three groups for any drug treatment. There is a significant increase in blood pressure ($p < .05$) during each of the two doses of norepinephrine and a significant drop in blood pressure after isoproterenol injection ($p < .01$).

The rise in systemic blood pressure was due to the norepinephrine action on the alpha receptors of the peripheral vascular system (Innes and Nickerson 1975). There is a possibility that the rapid rise in systemic blood pressure may have masked changes in the heart rate by initiating the baroreceptor bradycardia reflex (Ahlquist 1965). There is also some evidence to suggest that the administration of norepinephrine into the systemic circulation causes a suppression of the cardioaccelerator center in the brain (Kaneko et al. 1960). The baroreceptor bradycardia reflex was not seen during the isoproterenol response, as the peripheral resistance was decreased so rapidly that the mean pressure fell to below 65 torr. Under



Control--baseline recording before and after drug treatments

1st Dose NE--first injection of norepinephrine (2 μg/kg)

2nd Dose NE--second injection of norepinephrine (4 μg/kg)

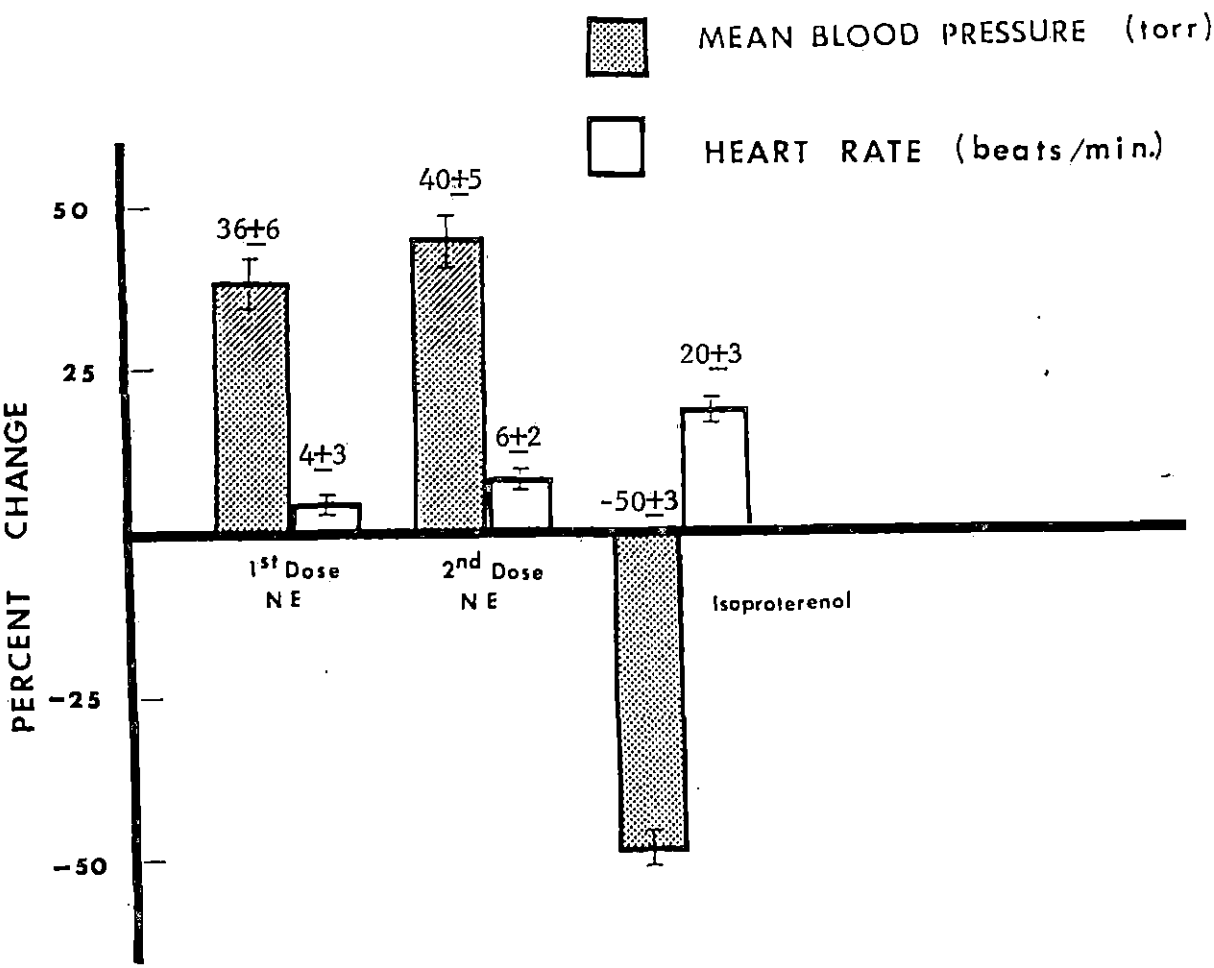
Saline--subcutaneous injection of physiological saline

Isoproterenol--subcutaneous injection of L-isoproterenol (80 mg/kg)

* indicates a significant rise in mean blood pressure in all three groups at 1st Dose NE and at 2nd Dose NE ($p < .05$)

** indicates a significant drop in mean blood pressure in all three groups treated with isoproterenol ($p < .01$)

Figure 5. Mean blood pressure ± standard error of the mean



1^{st} Dose NE--first intravenous injection of norepinephrine, n = 50
2^{nd} Dose NE--second intravenous injection of norepinephrine, n = 50
Isoproterenol--subcutaneous injection of L-isoproterenol, n = 27

Figure 6. Percent change in mean blood pressure for all animals expressed as percent ± standard error of the mean

these conditions the heart rate is not depressed by vagal baroreceptor control (Ginsburg and Cobbold 1960). Figure 6 expresses the blood pressure and heart rate responses as percent change from control values for all animals in this study. The combined effect of a 50% drop in blood pressure accompanied by a 20-25% increase in heart rate is a classic isoproterenol response. This heart rate response increases the substrate and oxygen requirements for the myocardial cell. This combined effect is believed to be the major cause of the myocardial necrosis associated with isoproterenol injection (Kirk et al. 1977; Scheuer et al. 1977; Bersohn and Scheuer 1978; Wexler 1970).

Plasma Enzyme Levels

Determination of specific serum or plasma enzymes has been shown to be an effective indicator of cellular damage (LaDue et al. 1954; Sobel and Shell 1972). In this study, total plasma LDH levels were evaluated (Figure 7). All three groups show a significant rise in total LDH activity when given isoproterenol ($p < .05$). The exercise group demonstrated a slightly lower total LDH activity, however, this difference is not significant. These data are similar to those presented by Wexler and Greenberg (1974) who reported no difference in total LDH levels between sedentary rats and swimming rats injected with isoproterenol. The duration of the exercise program used by Wexler and Greenberg (1974) was only two weeks and may account for the lack of difference between exercised and sedentary animals. Tajuddin et al. (1975) reported that total serum LDH and creatine phosphokinase (CPK) levels following isoproterenol injection were lower in swimming rats exercised for eight weeks. Because of the reduced responses of

LDH and CPK, these researchers concluded that the exercised animals had suffered less myocardial damage from isoproterenol injection than the sedentary rats. The problem in using only total LDH activity is that damage of muscle, kidney, pulmonary, and red blood cells will elevate total LDH activity. It is, therefore, unreliable to base conclusions on total LDH levels alone (Sobel and Shell 1972). A better way to look for cardiac damage is by using the isoenzyme LDH-1 which is found almost exclusively in the heart muscle (Hess and Walter 1961; Weiland and Pfleiderer 1961). The use of LDH-1 in signaling the occurrence of myocardial infarction is better than serial ECG recordings and is more than 95% reliable (Sobel and Shell 1972). The data for LDH-1 activity are presented in Figure 8. The plasma levels of LDH-1 were significantly lower in the exercised animals given isoproterenol than in the sedentary control animals subjected to isoproterenol ($p < .05$). The norepinephrine pretreated group showed a reduction in LDH-1 activity, indicating less cardiac damage than the sedentary control group, however, this difference was not significantly different. In all three groups, the LDH-1 activity was higher in those animals receiving isoproterenol than in saline injected animals ($p < .05$). This would indicate that there may have been some cardiac cell damage in all isoproterenol treated animals. This finding conflicts with data reported by Feirer (1978) who showed no difference between exercised rats injected with saline and those injected with isoproterenol. Feirer (1978) reported LDH-1 activities in saline injected animals of 20-26 mU/ml of plasma which is more than two times higher than the highest LDH-1 activity reported for saline animals in this study. The LDH-1 activities of animals treated with isoproterenol are almost identical

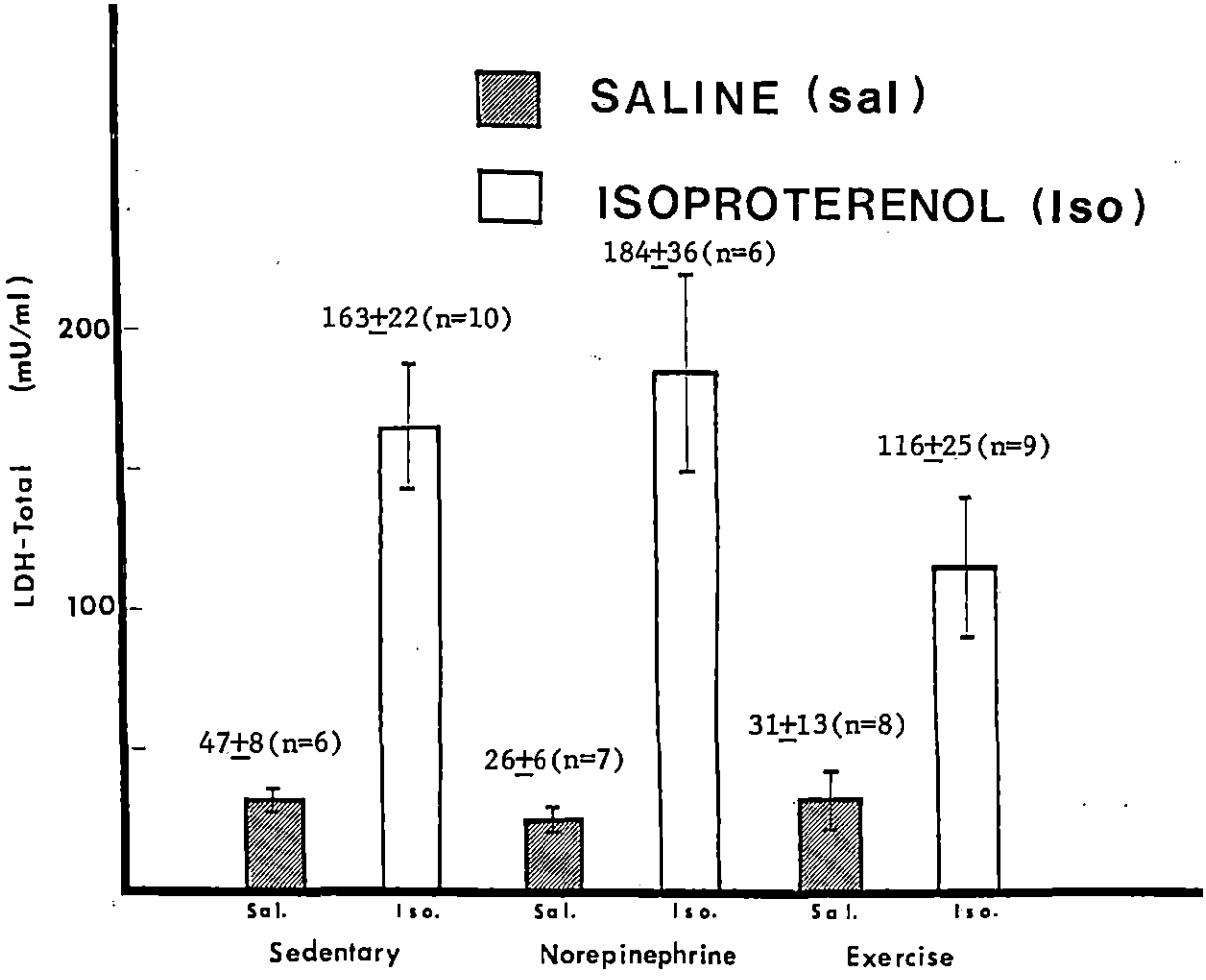


Figure 7. Total LDH activity expressed as mean activity ± standard error. All animals receiving isoproterenol show a significant rise in LDH activity ($p < .05$)

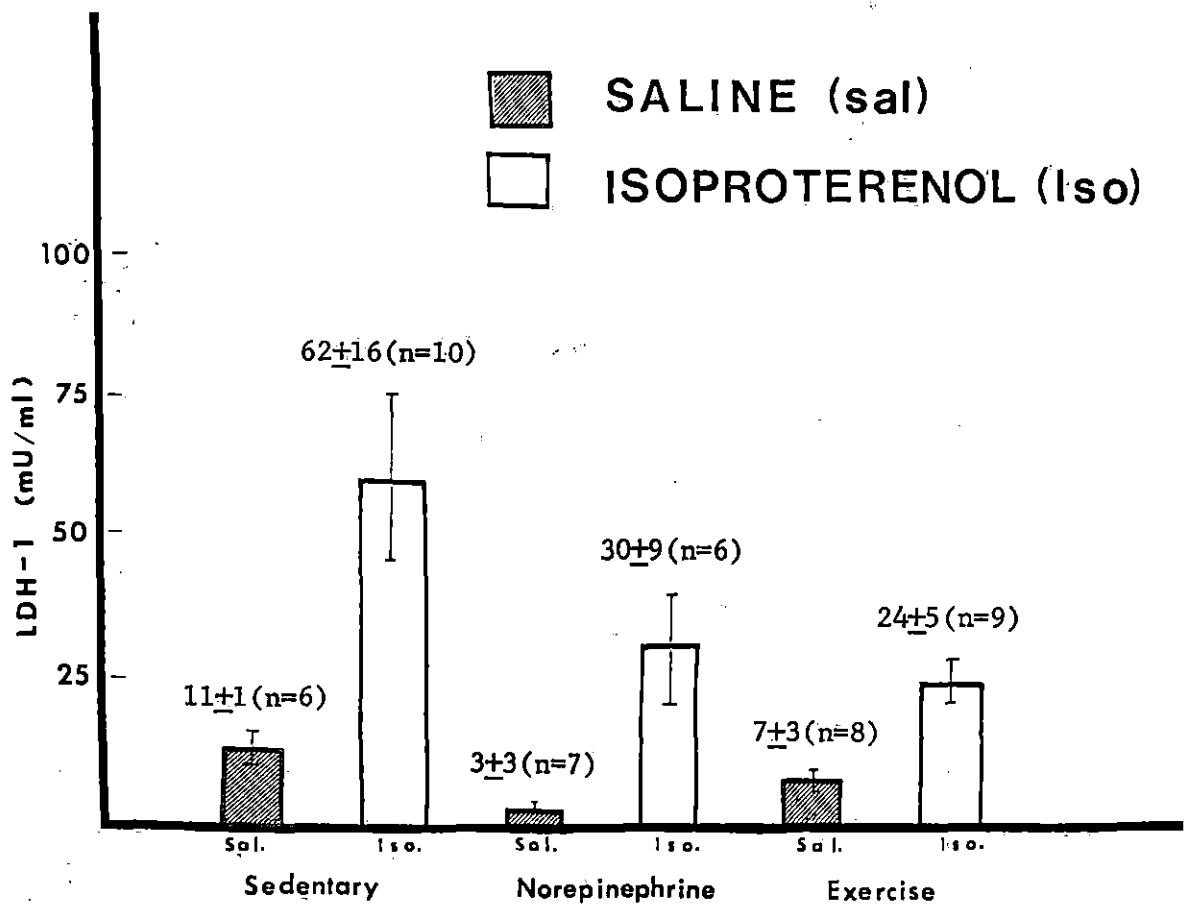


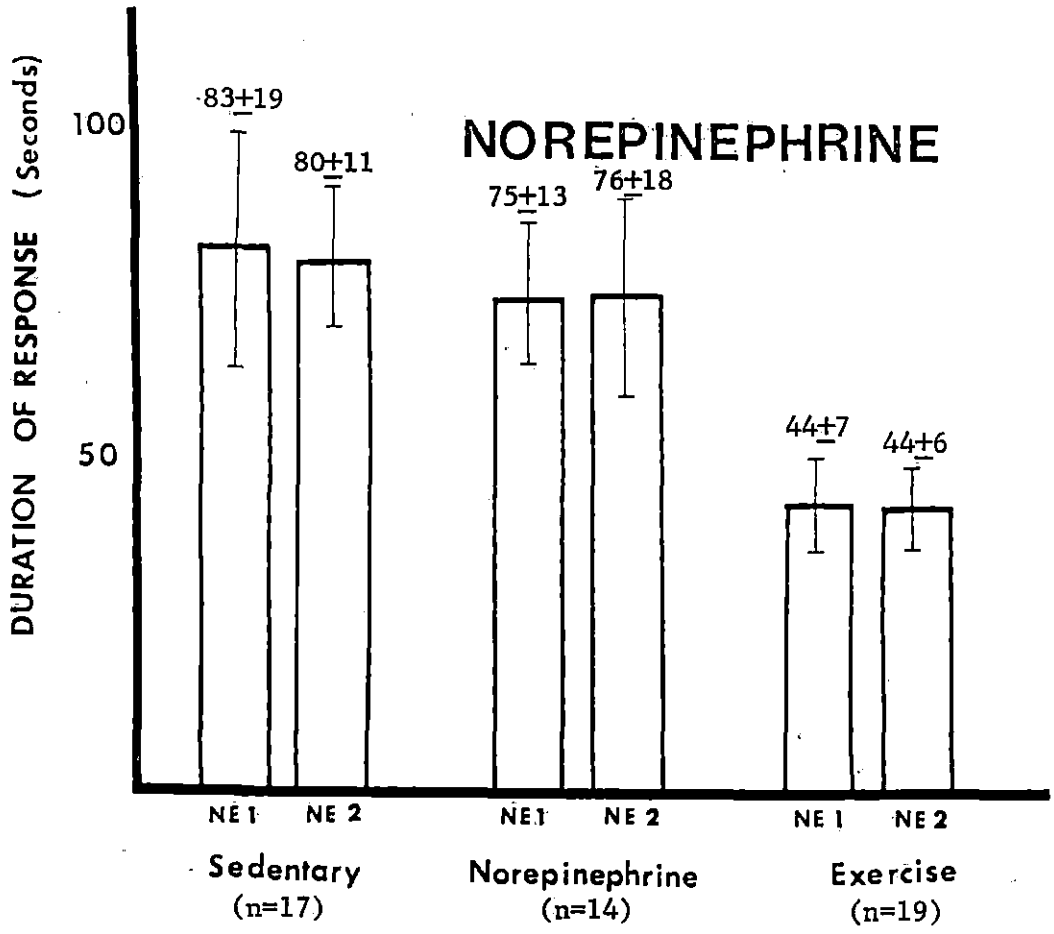
Figure 8. LDH-1 activity expressed as mean activity ± standard error. Sal. animals have a significantly lower LDH-1 activity than the Iso. animals ($p < .05$). The Exercise-Iso. animals have a significantly lower LDH-1 activity than the Sedentary-Iso. animals ($p < .05$)

with the values reported by Feirer (1978). The LDH-1 activities in saline injected animals reported in this study correspond very closely to those reported by Vander Tuig (1976). Even though this study indicates the possibility of cardiac damage in the exercised animals, the sedentary animals clearly experienced much more cardiac damage from the isoproterenol injection as indicated by the LDH-1 activity found in the plasma. The data further suggest that the animals treated with norepinephrine are afforded some manner of protection from isoproterenol induced infarction. Sufficient data were not obtained to substantiate beta receptor alteration and no differences in heart weight to body weight ratio, it is concluded that factors other than these are responsible for the apparent protective effect seen in this study.

Because of the apparent reduction in cardiac damage indicated by LDH-1 levels in the animals pretreated with norepinephrine, it is believed that the protective effect might be a result of repeated fluctuation in plasma catecholamine level. The fluctuation in norepinephrine level was produced artificially in the norepinephrine pretreated group by daily injections. In the exercise group, this fluctuation is presumed to occur naturally during exercise training (Kotchen et al. 1971). There are a number of physiological adaptations that may occur as a result of this fluctuation and these will be discussed later.

Duration of Blood Pressure Response

The data indicate no significant difference in the duration of blood pressure response (Figure 9) between the first and second doses of norepinephrine. A significant decrease in the duration of the blood pressure



NE 1--first intravenous dose of norepinephrine (2 μg/kg)

NE 2--second intravenous dose of norepinephrine (4 μg/kg)

Figure 9. Duration of the blood pressure response to norepinephrine injection. Values shown are means ± standard errors. The exercise animals have a significantly shorter duration than either the sedentary or the norepinephrine pretreated animals ($p < .05$)

response in the exercised animals is evident when compared with either the sedentary or the norepinephrine pretreated animals ($p < .05$). The data indicate approximately a 40% reduction in the duration of the blood pressure response.

The blood pressure response duration to isoproterenol is shown in Figure 10. The exercised animals show a much shorter duration, approximately 1/5, than the sedentary animals. The rats given norepinephrine pretreatments exhibited a blood pressure duration in between the exercised and sedentary rats. There is a very positive correlation (0.97) between the duration of the blood pressure response and the amount of LDH-1 activity found in the plasma of isoproterenol injected animals. These data, therefore, indicate that the apparent protective effect as indicated by the reduction in LDH-1 activity may be in some way related to the duration of the blood pressure response produced by isoproterenol injection.

The uptake and removal of catecholamines from the plasma occurs in many tissues. Alabaster (1977) states that the primary organ believed to be involved with norepinephrine removal is the pulmonary vasculature, while the removal of isoproterenol is believed to occur primarily in the vasculature of the somatic muscle beds. The uptake of all catecholamines from the circulatory system is believed to occur by active transport while the breakdown of the catecholamines is accomplished intracellularly by mono amine oxidase and catechol-o-methyl transferase (Iwasawa and Gillis 1974). It may be that the catecholamine uptake system, the enzyme system, or both could be influenced by exercise. If the uptake or degradation of norepinephrine and isoproterenol were enhanced by exercise or induced by the chronic administration of norepinephrine, this would explain why

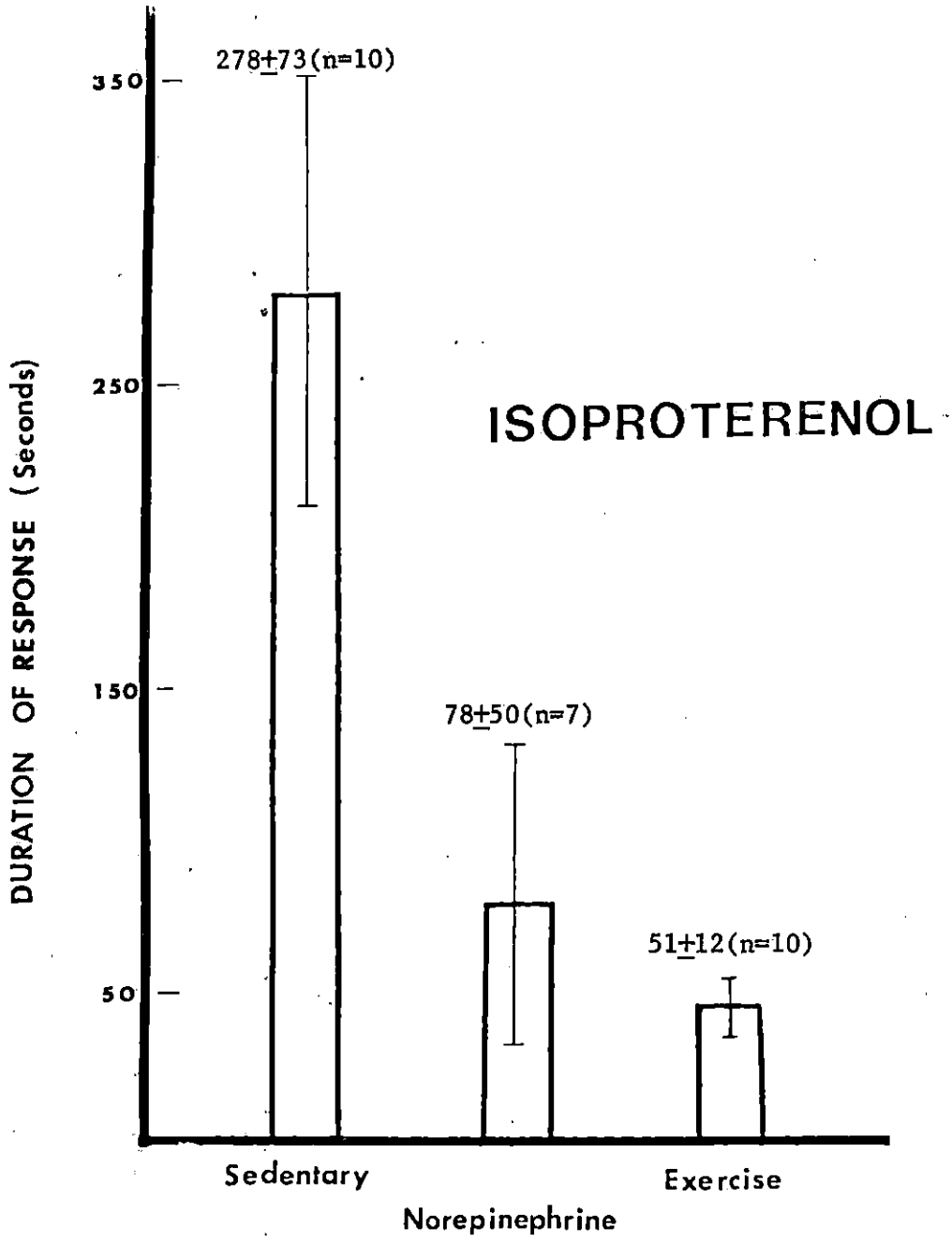


Figure 10. Duration of blood pressure response to isoproterenol injection. Values are expressed as means ± standard errors. The exercise animals have a significantly shorter duration than the sedentary animals ($p < .05$)

heart rate and blood pressure responses were not different among the three groups. Yet, the exercised animals and the rats pretreated with norepinephrine demonstrated lower LDH-1 levels indicating that these groups suffered less cardiac damage than the sedentary animals. An alteration of the uptake or degradation of catecholamines by exercise stress would also help explain the pattern of norepinephrine fluctuation seen in trained animals and humans. Initially upon exercise stress, there is a rise in plasma norepinephrine levels, but, after conditioning occurs, the resting norepinephrine levels are slightly lower in exercised individuals than in control subjects, and, during exercise stress, the norepinephrine levels in conditioned individuals do not rise as they do in untrained subjects (Kotchen et al. 1971; Galbo et al. 1976). Alabaster (1977) reports that, while the work remains to be done, there is the possibility that the process of catecholamine removal may be influenced by changes in the concentration of circulating endogenous amines. It seems most likely that the greatest effect on removal of circulating catecholamines would be an alteration of the uptake system. In the lung, the uptake system has been shown to be the rate limiting step in the removal of norepinephrine (Iwasawa and Gillis 1974). Since the uptake system is an energy requiring system, it may be possible that this system could be induced by the physiological changes that accompany exercise training. Clearly there needs to be more work done in the area of inducement of catecholamine removal before any clear conclusions concerning exercise can be made.

SUMMARY

Mean blood pressures and heart rates were used to measure physiological sensitivity to two doses of norepinephrine and to one dose of L-isoproterenol. Plasma LDH-1 was used to determine the extent of myocardial damage caused by the isoproterenol injection. Virgin male rats were aged to 100 days and randomly divided into three groups--an exercise group, a norepinephrine pretreated group, and a sedentary control group. The exercise animals ran on a motor driven treadmill 30 minutes per day, 5 days per week for 20 weeks. The speed was 17 meters per minute at a grade of 8^{o}. The norepinephrine pretreated animals were given intraperitoneal injections of norepinephrine (2 ug/kg) in a split dose 5 days per week during the 30 minute period when the exercise animals were running. The sedentary control rats received saline injections and were not exercised. Sedentary and norepinephrine pretreated rats were pair fed with exercise animals in an attempt to maintain all animals at the same body weights. After 20 weeks, each rat was anesthetized with sodium pentobarbital (25 mg/kg). Each animal was challenged with two doses of norepinephrine, the first dose of 2 µg/kg, and the second dose of 4 µg/kg. Carotid blood pressure recordings were made before and after each dose. Finally, each rat was given a subcutaneous dose of either saline of L-isoproterenol (80 mg/kg). Twenty-four hours later, blood plasma was obtained for LDH determination. Hearts were trimmed and wet ventricular weights were recorded. LDH assays were carried out within 6 hours after blood collection.

The results of this study are as follows:

1. Exercise had no effect on ventricular mass.
2. There were no significant differences in heart weight to body weight ratios demonstrated for any of the three groups of rats.
3. Exercise had no effect in reducing resting heart rate.
4. Neither exercise nor norepinephrine pretreatment had any effect on the animal's sensitivity to norepinephrine or isoproterenol.
5. Administration of isoproterenol caused a significant rise in heart rate and a significant drop in blood pressure compared to the animals receiving saline injections.
6. Exercise protected the animals from myocardial infarction as indicated by the significantly lower LDH-1 levels.
7. Animals pretreated with norepinephrine also demonstrated a trend towards a reduction in cardiac damage as indicated by the LDH-1 levels.
8. Exercise caused a reduction in the duration of the blood pressure response to norepinephrine and to isoproterenol.
9. There was a trend in the isoproterenol response time that indicated norepinephrine pretreatment caused a reduction in the blood pressure duration.
10. There was a positive correlation (0.97) between the degree of cardiac damage indicated by the LDH-1 level and the duration of the isoproterenol pressure response.

The indications of this study are that exercise protects the rat from isoproterenol infarction and that this protective effect is not due to differences in body weight nor to differences in heart weight to body

weight ratios, nor is the protection derived from changes in the peripheral vascular response or cardiac response to isoproterenol. It seems more likely that the protective effect demonstrated in this study results from a physiological mechanism not evaluated in this study.

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ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Richard Engen, for his help and constructive critisism during the preparation of this thesis. Many thanks go also to Dr. Donald Dyer and Dr. Yola Forbes for their interest in this work.

My first major professor was Dr. David Griffith. His untimely death left a void in my life, for he was more than a source of ideas, he was a friend. His good nature and easy-going manner made him a joy to work with. Were it not for his help and encouragement, this manuscript would never have come to pass. To say he is missed is a gross understatement. I hope that I can retain all that he taught me and can continue in a manner that will do justice to the reputation he had with his graduate students.

I would like to express my appreciation to my fellow colleagues--Dr. David Crandall, Denis Meerdink, Machell Trankina, Kevin Custer, Russ Feirer, Ann Nielsen, Mike Engwall, and Mark Darrah for their help and encouragement. Thanks go also to Jean Bodensteiner for her efforts and patience in transposing my illegible handwriting into typewritten copy.

Finally, my sincere thanks to to my parents and to Jan, a loving and extremely patient wife, for all their support during this project.

APPENDIX A. DETERMINATION OF TOTAL LDH ACTIVITY

Test Principle

The test principle used for the determination of total plasma LDH activity was as follows:

$$\text{L-lactate} + NAD^{+} \dashrightarrow NADH + \text{pyruvate.}$$

NADH production per unit of time was measured as the increase in absorbance of light at 340 nm using a Bausch and Lomb spectronic 600 spectrophotometer.

Procedure

1. Reagents were obtained from Calbiochem and Sigma (Sigma Chemical Company, St. Louis, Missouri). Reagents were dissolved in double distilled water to yield a substrated solution containing:

   0.05 M glycine buffer

   0.07 M lactate

   0.035 M NAD^{+}

   pH adjusted to 8.8 with NaOH

2. The solution was checked for accuracy by using a prepared LDH kit (Calbiochem) as a standard.

3. Plasma and substrate solutions were placed in a constant temperature water bath of 30° C.

4. Suitable pipets were used to pipet 200 ul of plasma into 3 ml of substrate solution in a clean quartz cuvet. The cuvet was covered with parafilm and inverted 4-5 times to insure complete mixing.

5. The cuvet was placed in a constant temperature cuvet holder (30° C) within the spectrophotometer. Initial absorbance (Ao) was read 30 seconds after the cuvet was placed into the spectrophotometer.

6. Exactly 2 minutes after the initial reading, the final absorbance was read.

Calculations

Total plasma LDH activity was calculated using the following formula:

LDH activity in mU/ml = change in absorbance over 2 minutes

(A2-Ao) X 1286 = LDH activity in mU/ml (Calbiochem 1976)

The conversion constant of 1286 was determined as follows:

$$\frac{\text{A/2 min X T.V. X 1000}}{\text{mM abosrptivity X S.V. X P X min}} = \text{mU LDH/ml of sample}$$

mM absorptivity = 6.22 @340 nm for NADH

p = length of the light path in cm

S.V. = sample volume in milliliters

T.V. = total assay volume in milliliters

A = change in absorption

Therefore, $\frac{\text{A/2 min X 3.2 X 1000}}{\text{6.22 X 0.2 X 1 X 2}} = \text{A/2 min X 1286}$ (Calbiochem 1976). One milliunit of LDH equals one micromole of NADH produced per minute per milliliter of plasma (Wacker et al. 1956).

APPENDIX B. DETERMINATION OF PLASMA LDH ISOENZYME ACTIVITIES

Procedure

1. Using a clean capillary tube, 15-20 microliters of plasma were applied 2.0-2.5 cm from the center on the cathode side of a clean cellulose polyacetate electrophoresis strip (Gelman Instrument Company).
2. A Gelman rapid electrophoresis chamber was used to carry out the electrophoretic separation. Gelman high resolution buffer (Tris-barbital sodium barbital; pH 8.8; ionic strength 7.5) was used in the chamber.

   A constant voltage of 200 volts or 1.0 milliamp per strip was applied for 90 minutes using a Gelman DC power supply (Gelman Instrument Company).
3. A staining solution was prepared from the following reagents 30 minutes before the electrophoretic separation was complete.
   a. 1.0 ml of 1.0 M sodium lactate (60 percent syrup diluted 1:4 with 0.1 M phosphate buffer, pH 7.5);
   b. 3.0 ml of nitroblue tetrazolium (1.0 mg/ml in distilled water);
   c. 0.3 ml of phenazine methosulfate (1.0 mg/ml in distilled water);
   d. 1.0 ml of 0.1 M phosphate buffer (13.6 g KH_2PO_4 plus 3.3 g NaOH per liter of distilled water); and
   e. 10 mg of nicotinamide adenine dinucleotide (NAD^+).
4. Clean cellulose polyacetate (CPA) strips were floated on the staining solution until fully saturated. The staining strips were then placed wet side down on a clean glass slide and kept in a moisture chamber over wet filter paper.

5. After the electrophoretic separation of the plasma, the plasma CPA strips were inverted and placed on top of the staining strips on the glass slides. The moisture chamber was closed and incubated in the dark in an oven maintained at 30^{o} C for 25 minutes.

6. After the incubation, the combined staining strips and the plasma strips were removed from the chamber and soaked in a distilled water bath to wet the strips evenly.

7. The combined wet strips were scanned on a Densicord recording electrophoresis densitometer (Model 542, Photovolt Corp.).

8. The relative percentage of each LDH isoenzyme was determined by calculation of the areas beneath the curves of the densitometric tracings. The relative areas beneath the curves were calculated by determining the weight of the paper beneath the curve and dividing by the total weight of the paper beneath all the curves on that tracing.

9. The total plasma LDH activity was then multiplied by the relative percentage of each isoenzyme to obtain the activity in mU/ml for each isoenzyme.