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Department of Pharmaceutical Science
The University of British Columbia
Vancouver, Canada

Date May 19/93
Failure of the cardiovascular system, which is further exacerbated in the presence of hypertension, is considered the leading cause of death in diabetic individuals. Our aim in this study was to investigate the cardiac adrenergic neuronal changes induced by diabetes and hypertension, and then to correlate these changes with the contractile state of the myocardium. By using the radioactive agent, I-123 meta-iodobenzylguanidine (MIBG), which has the same uptake, storage and release mechanism as norepinephrine (NE), a non-invasive measurement of the integrity of the adrenergic nerve endings in heart and spleen was investigated.

One week following streptozotocin (STZ) injection (55 mg/kg), male Sprague-Dawley rats were divided into two groups. They were given subcutaneous injections of either deoxycorticosterone-acetate (DOCA, 25 mg/kg) or DOCA vehicle twice weekly for 3, 6, 9, or 12 weeks, and received 0.9% saline with 0.5% KCl to drink. Rats were designated by their respective treatments for each time period: CON, DOCA, STZ, and STZ-DOCA.

After 3, 6, 9, or 12 weeks of commencing DOCA or DOCA-vehicle treatment, MIBG was injected intravenously (15 mCi/mg). Five hours later the heart and spleen were dissected and counted with a gamma counter. Results were expressed as % Kg Dose and % Kg Dose/g. In similarly treated animals, after 12 weeks, the contractile function of the myocardium was studied using left ventricular papillary muscle preparations. Cardiac NE concentration was determined using HPLC with electrochemical detection.
Although cardiac NE stores in STZ and STZ-DOCA were significantly increased from CON, results showed decreased MIBG uptake into the diabetic hearts at all time points compared to CON. There were no differences in MIBG values at any time point between STZ and STZ-DOCA. DOCA, STZ, and STZ-DOCA demonstrated significant reduction in MIBG accumulation in their left ventricles from 3 to 12 weeks.

Length-tension studies revealed that with increasing preload tension the contractile performance in the STZ group was significantly diminished from CON between 4.5 g and 5.5 g. There were no significant differences between any of the other groups with increasing muscle length. None of the muscles responded well to the addition of either isoproterenol (1x10^{-10} to 1x10^{-4} M) or tyramine (1x10^{-4} to 1x10^{-2} M).

From these results we concluded that either: 1) MIBG does not provide an accurate indication of adrenergic integrity; or 2) there is no correlation between sympathetic function and myocardial function after 12 weeks of DOCA or DOCA-vehicle treatment in diabetic rats. We also concluded that DOCA-induced hypertension does not significantly affect the sympathetic nervous activity or negatively affect myocardial functioning in diabetic animals.
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LIST OF ABBREVIATIONS

BP  blood pressure
DOCA  deoxycorticosterone-acetate
ISO  isoproterenol
LV  left ventricle
LV/BW  left ventricular–body weight ratio
MIBG  meta-iodobenzylguanidine
NE  norepinephrine
PR  pulse rate
STZ  streptozotocin
TH  total heart
TYR  tyramine
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A special thanks to Nordion International Inc. for generously donating I-123 MIBG to us.
DEDICATION

I dedicate this thesis to my parents, Frank and Patricia, for their endless love and support, and to Geoffrey, who never stopped believing in me.
1.0 INTRODUCTION

It has been well established that diabetes mellitus is an independent risk factor for cardiovascular disease (Kannel et al., 1974). The etiology of cardiac disease in diabetes is multifactorial, and includes coronary atherosclerosis, microangiopathy, cardiomyopathy, and autonomic neuropathy. Of these maladies, the latter two will be discussed in the greatest detail as they are of the most interest in the current study. In addition, the role of hypertension in diabetes as well as the effect of sympathetic activity on diabetic heart function will be introduced. Finally, the diagnostic use of the radiolabeled tracer, meta-iodobenzylguanidine, and its role in examining adrenergic nerve endings in the heart will be discussed.

1.1 Diabetes and Heart Disease

A. Atherosclerosis

Coronary artery disease is a well known sequelae of macroangiopathy (Kannel and McGee, 1979). Histochemical studies have shown that atherosclerosis is caused by the deposition of Ca$^{2+}$, cholesterol crystals and increased amounts of glycoprotein (Ledet, 1968), which eventually lead to the hardening of the artery (see Tahiliani and McNeill, 1986). According to Kannel and McGee (1979), individuals with diabetes have a 2 to 3 fold increased risk of developing coronary artery disease. Risk factors which are likely to contribute to the development of atherosclerosis are hypertension, hypertriglyceridemia and hypercholesterolemia (Rubler, 1978).
B. Microangiopathy

Capillary basement membrane thickening has been recognized as the structural hallmark of microvascular damage (Tooke, 1989). In post-mortem ultrastructural studies performed on the basement membranes of capillaries from hearts of diabetic patients, it was revealed that the membranes were nearly three times as thick compared to those in normal hearts (Silver et al., 1977). Similarly, in experimental diabetes, capillary basement membrane thickening has been reported both in rats (Fischer et al., 1981) and monkeys (Yasuda et al., 1984). It is believed that this thickening is due to hyperglycemia and/or other hormonal and metabolic imbalances (Fischer et al., 1979; McGrath and McNeill, 1986). Williamson and Kilo (1983) have suggested that the alteration in the basement membrane may represent a proliferative response of the capillary endothelial cell to raised capillary pressure and/or injury.

C. Diabetic Cardiomyopathy

The increased risk of congestive heart failure in diabetes mellitus has been shown to involve a primary cardiomyopathy which is specific to the diabetic population (Thompson, 1988). Diabetic cardiomyopathy occurs in the absence of coronary atherosclerosis and hypertension (Rubler et al., 1972; Hamby et al., 1974; Neubauer and Christensen, 1976). This cardiac dysfunction is characterized by ventricular hypertrophy, interstitial fibrosis, focal necrosis, and impaired systolic and diastolic function (Hamby et al., 1974; Ahmed et al., 1975; Shapiro et al., 1981; Vadlamudi et al., 1982). Isolated muscles from diabetic rat hearts showed increased peak relaxation time, slowed relaxation rate and delayed relaxation (Fein et al., 1980).
The direct cause of diabetic cardiomyopathy is not fully understood, however, its onset is believed to be the result of both subcellular and biochemical alterations. Possible explanations include altered enzymatic activity of cardiac myosin ATPase as well as a decrease in the sequestration of Ca\(^{2+}\) into the sarcoplasmic reticulum (Penpargkul et al., 1981; Lopaschuk et al., 1983; Pierce and Dhalla, 1985). Decreases in sarcolemmal calcium pump activity have also been reported in the diabetic rat (Heyliger et al., 1987). Diabetic cardiomyopathy has been shown to be associated with abnormally enhanced sympathetic activity (Ganguly et al., 1986; Ganguly et al., 1987), a topic which will be discussed at length in a subsequent section.

D. Autonomic Neuropathy

Another factor which contributes to cardiac disease in the diabetic is autonomic neuropathy. This malady is associated with an increased incidence of morbidity and mortality. More specifically, some 17% to 40% of diabetics are diagnosed with autonomic neuropathy (Dryberg et al., 1981). Furthermore, approximately 50% of these patients die within 2.5 years after being diagnosed (Ewing et al., 1980). In addition, the death rate of diabetics having autonomic neuropathy is 2.5 times greater than in diabetics without such neuropathy (Ewing et al., 1976).

Some common clinical symptoms of neuropathy include postural hypotension, gastric fullness, impotence, and reduced beat to beat variation (Ewing, 1978). In addition, most patients diagnosed with autonomic neuropathy have evidence of other complications such as proliferative retinopathy, nephropathy, and peripheral neuropathy (Duchen et al., 1980).
Parasympathetic neuropathy often occurs before the onset of sympathetic neuropathy and it has been shown that the later will not occur in the absence of vagal denervation (Ewing, 1984).

The metabolic defects which occur in neuropathy are numerous. Due to the elevated levels of glucose there is an increase in the intracellular accumulation of sorbitol and fructose in neurons. These carbohydrates do not readily diffuse away from their site, and as a consequence, have been shown to slow motor nerve conduction velocity (Clarke et al., 1979). Reduction of myoinositol is another potential factor, as it is important for the normal structural integrity of the peripheral nerve (Clarke et al., 1979; Hosking et al., 1978). In addition, the synthesis of glucose and acetate into myelin fatty acids and cholesterol is reduced, which subsequently changes the myelin composition. More specific to the heart, segmental demyelination and axonal degeneration have been reported to occur (Hosking et al., 1977). In addition to metabolic disturbances, Maser et al. (1990) have suggested that diabetic autonomic neuropathy may be further exacerbated in the presence of hypertension and ischemia.

The existence of autonomic neuropathy in streptozotocin (STZ)–treated Wistar rats was first reported by Monckton and Pehowich (1980). In an ultrastructural study, they observed degenerative changes in the axonal plasma membranes and axoplasm of unmyelinated fibres only 24 hours after STZ injection (80 mg/kg). The authors speculated that perhaps a toxic rather than a diabetic cause produced the neuropathic changes observed. In a study by Tomlinson and Yusof (1983), rats treated 8 months previously with alloxan developed predominant parasympathetic
denervation of the heart, however, only moderate sympathetic denervation was measured.

The contrast in findings could be due to the severity of diabetes, or it is possible that the degenerative changes seen by Monkton and Pehowick (1980) were transient. Other factors which may influence the extent and onset of neuropathy are the strain of rat used and the duration of diabetes.

1.2 Diabetes and Hypertension

Hypertension is a well documented risk factor of diabetes mellitus (Garcia et al., 1974). Numerous clinical and experimental studies have demonstrated that hypertension increases the prevalence of cardiac complications that are associated with diabetes. The ultrastructure of the hypertensive–diabetic rat myocardium has been reported to possess a greater degree of myocyte alteration than is seen in diabetes or hypertension alone (Factor et al., 1983). This damage was manifested by capillary basement membrane thickening, interstitial accumulations of collagen and focal replacement fibrosis. It was also suggested that altered microcirculation may add to the deleterious effects observed under the two combined stresses.

Several pathomechanisms of diabetic hypertension have been described, including sodium retention, nephropathy, and altered baroreflex sensitivity. In rats made diabetic with streptozotocin, neither marked vascular disease nor hypertension are present (Chobanian et al., 1982). However, it has been documented that some 40 – 80% of patients with long-standing diabetes are hypertensive (Christlieb, 1973). Therefore, in order to mimic the pathological
changes that are seen in the diabetic patient, it is important to produce diabetes mellitus complicated hypertension. Hypertension can be induced in STZ-diabetic rats through the subcutaneous injection of the mineralocorticoid, deoxycorticosterone-acetate (DOCA), in conjunction with a saline (NaCl) drinking solution (Hebden et al., 1990). In a study by Hebden et al. (1990), treatment of STZ-diabetic rats with DOCA was shown to mimic the morphological changes that are seen in the diabetic patient, namely hypertension and atherosclerosis. In addition, the animals were reported to have elevated levels of plasma lipids and cholesterol.

DOCA–NaCl hypertension is believed to involve an increased activity of the brain renin–angiotensin system. This alteration in activity is thought to cause an increase in sympathetic activity and vasopressin release (Schenk and McNeill, 1992). In addition, baroreflex sensitivity is attenuated (Nakamura et al., 1988).

1.3 Sympathetic Activity in Diabetes and Hypertension

Numerous clinical and experimental studies have demonstrated that the functional status of the cardiac sympathetic system of patients with diabetes mellitus is compromised (for review see Black and McNeill, 1991). As the function of the adrenergic neurons is mediated by the neurotransmitter, norepinephrine (NE), extensive research examining NE levels in the heart and other organs has been investigated.

Sympathetic activity in experimentally diabetic rats has been shown to be decreased (Yoshida et al., 1985) or increased (Ganguly et al., 1987). It has been proposed that hypoxia (Low et al., 1986); protein glycosylation (Brownlee et al.,
1984) and over activity of the aldose reductase pathway (Kador et al., 1985) are possible mechanisms which underlie this nervous abnormality. Kyoung et al. (1986) have reported that metabolic ketoacidosis and an increase in baroreceptor sensitivity are responsible for increasing the activity of the sympathetic nervous system.

The turnover of NE provides an accurate assessment of sympathetic activity. NE turnover has been reported to be both reduced (Yoshida et al., 1985), and increased (Ganguly et al., 1986; Hasking et al., 1986) in STZ-diabetic rats.

Plasma NE levels provide an indirect index of sympathetic activity. Plasma NE concentrations were shown to be increased in poorly controlled human diabetics (Christensen, 1974), and decreased in long term diabetics with neuropathy (Neubauer and Christensen, 1976). In patients with chronic myocardial failure (ie. congestive heart failure), reduced levels of tissue NE were accompanied by elevated plasma NE levels (Cohn et al., 1984). One must keep in mind that plasma NE is derived from circulating catecholamine levels, therefore, it does not provide an accurate indication of regional or localized changes in neuronal secretion.

In diabetes, cardiac NE concentration is described as being increased (Paulson and Light, 1981; Lucas and Qirbi, 1989; Fushimi et al., 1988; Ganguly et al., 1986), decreased (Neubauer and Christensen, 1976; Yoshida et al., 1985) or unchanged (Kaul and Grewall, 1980; Vadlamudi and McNeill, 1984). The discrepancies in these results may be due to strain differences, age at sacrifice, and variation in the method used to assay NE (Felton et al., 1982). In addition it has been
suggested that circadian rhythms may be responsible for producing variations in tissue NE levels, as has been shown to occur in blood (McCarty et al., 1981).

In the DOCA–salt hypertension model, cardiac NE concentration has been found to be decreased (Heller and Prohaska, 1984; Krakoff et al., 1967), while plasma NE levels are shown to be elevated (Eid and De Champlain, 1988). As in diabetes, NE turnover was reported to be elevated (Fujita et al., 1986). Sympathetic activity in DOCA–treated diabetic rats has not yet been investigated.

In the early stages of diabetes, before the development of myocardial failure, cardiac NE stores are predominantly increased. However, as the disease prevails, the tissue concentration of NE slowly diminishes. Felton et al. (1982) found that cardiac NE levels were elevated after a one month duration of diabetes, but returned to normal levels with increasing duration of diabetes. Similarly, NE concentrations in heart, foot, skin, brown fat tissue, and kidney were shown to be markedly increased by 6 weeks duration of diabetes, however, returned to base levels by 13 weeks (Fushimi et al., 1988) (Figure 1). Paulson and Light (1981) measured a significant increase in ventricular content merely 12 days post–injection of STZ. A similar trend in myocardial stores can be found with the onset of congestive heart failure. In studies using the cardiomyopathic Syrian Hamster model, intraneuronal concentrations of NE remained high in the early stages of myopathy, but eventually decreased with the onset of heart failure (Angelakos et al., 1972; Sole et al., 1975).

With the progression of myocardial disease, it is suggested that an alteration in sympathetic activity and/or degeneration of the nerve terminals are responsible for the changes in NE tissue concentration. Some investigators believe that in the
Figure 1. Increase in catecholamine concentration of various tissues in diabetic rats after induction of diabetes.

(reprinted from Fushimi et al., 1988)
development of heart failure an enhanced biosynthesis of NE exists. However, as time passes, the increase in NE turnover ultimately depletes the NE tissue stores (Hasking et al., 1986). Sole et al. (1975) hypothesize that decreased levels of NE, which accompany heart failure, may be secondary to a degenerative process at the nerve terminal. Another view proposed by Fushimi et al. (1988) is that an increase in synthesis of NE occurs early on in the disease process to compensate for the pathophysiologic changes occurring, however, with time, cellular degeneration occurs and the increase in NE synthesis is unable to replenish the depletion in NE stores.

1.4 Sympathetic Activity and Myocardial Function

There is some question as to whether a relationship exists between the storage of neurotransmitter and the functional state of the myocardium. Some reports suggest that sympathetic innervation plays an intricate role in the functioning status of the heart. Rushmer (1970) stated that although sympathetic innervation may contribute little to intrinsic myocardial function, it is an important mechanism for the elevation of cardiac output in response to physiological stress. Gaffney and Braunwald (1963) demonstrated that in noncompliant or dilated hearts that the length-tension relationship was compromised, indicating that sympathetic support is responsible for maintaining cardiac output. In agreement with these observations, Chidsey et al. (1964) found an absence of contractile response of the myocardium to tyramine in dogs with experimentally induced heart failure. However, when exogenous NE was introduced to the NE depleted isolated papillary muscles, the muscles contracted with the same magnitude as that observed in the control dogs. Chidsey et al. (1964) concluded that the depletion of NE stores may interfere with
the transmission of sympathetic nerve impulses to the heart, which in turn would adversely affect myocardial performance.

On the contrary, other investigators believe that cardiac NE stores, or sympathetic innervation, are not necessary to maintain a normal cardiac response. Using guanethidine to deplete NE stores in the left ventricle of rabbits, Maxwell et al. (1964) found only a minimal to moderate depression in active tension. In an experiment using papillary muscles of cats which had undergone cardiac denervation, it was discovered that the changes in rate of force development, peak force and time to peak tension in these animals were no different from control. This suggests that cardiac stores are not necessary for the fundamental operation of the myocardium (Spann et al., 1966).

1.5 Cardiac Adrenergic Assessment using MIBG

In 1981, Wieland et al. reported the potential of l-123 meta-iodobenzylguanidine (MIBG) as a myocardial imaging agent (Wieland et al., 1981a). In the same year, the first images of the primate adrenal medulla were described (Wieland et al., 1981b), and MIBG was shown to sequester in the chromaffin storage granules. Using MIBG, Sisson et al. (1981) demonstrated the scintigraphic localization of human pheochromocytomas in eight patients with known tumors. Today, MIBG scintigraphy is an effective technique used widely in the localization and management of pheochromocytomas.

MIBG has also been used in the study of the cardiac adrenergic system to explore the content of norepinephrine made available to the tissue. Numerous
studies have shown that MIBG is taken up by myocardial sympathetic nerves and acts in a manner that is qualitatively similar to norepinephrine (Dae et al., 1992). MIBG is a physiological analogue of norepinephrine and guanethidine (refer to Figure 2), and has the same uptake, storage, and release mechanisms as norepinephrine. MIBG is not metabolized by catechol-o-methyl-transferase or monoamine oxidase (Wieland et al., 1981a).

The uptake of MIBG into the heart is complex and involves both neuronal and extraneuronal components, which are designated Uptake 1 and Uptake 2, respectively. In adrenergic neuronal tissues, or Uptake 1, MIBG is taken into the nerve terminal via two different mechanisms, Type I and Type II, as depicted in Figure 3. Type I uptake, which is specific for norepinephrine, is sodium-dependent, ouabain sensitive, energy and temperature dependent, has high-affinity-low capacity, and is saturable (Jacques et al., 1984). This uptake mechanism typically predominates at concentrations less than 1 pmolar. The high affinity MIBG transporter, originally localized by Richards and Sadee (1986), was recently cloned and sequenced and was discovered to be identical to the norepinephrine transporter (Pacholczyk et al., 1991).

The second neuronal uptake mechanism is designated Type II, and is sodium-independent, ouabain insensitive, energy independent, temperature dependent, and non-saturable to 5 µmolar. Type II uptake is diffusional in nature and has been found to predominate at higher concentrations of MIBG (Tobes et al., 1985).

Once MIBG is within the cytoplasm of adrenergic cells, it is transported into the storage vesicles via the monoamine vesicular transporter (Glowniak et al., 1992).
Figure 2. Chemical Structures of norepinephrine, guanethidine, and metaiodobenzylguanidine.

(reprinted from Kline et al., 1981)
Figure 3. Proposed mechanism and site of localization of metaiodobenzylguanidine in adrenergic neuronal tissues. A. Type I, sodium dependent uptake; B. Type II, sodium-independent uptake; C. Vesicle uptake and storage.

(reprinted from Petry and Shapiro, 1990)
Nakajo et al. (1986) reported that MIBG is bound more firmly in the NE storage vesicles than in any other cardiac tissue compartment. In the same study, it was discovered that the intravesicular storage of MIBG in the hearts of Sprague–Dawley rats reached a maximum plateau value of 50% after four hours post injection.

In extraneuronal compartments, such as vascular space and non-neuronal tissues, MIBG is taken up much the same way as NE. This mechanism, referred to as Uptake II, is characterized as having a low affinity–high capacity and is diffusional in nature. In a study by Jacques et al. (1984), it was reported that MIBG accumulates via the neuron–specific Uptake I mechanism up to 30 fold over its incorporation by Uptake II. Interestingly, in a recent study by Dae et al. (1992), it was discovered that the non-neuronal uptake mechanism is not significant in human hearts, whereas previous studies have shown it to be important in other animal species (Lightman and Iversen, 1969; Sisson et al. 1987b).

Sisson et al. (1987a) examined the relationship between nerve endings and MIBG uptake by subjecting Sprague–Dawley rat hearts to various interventions which disrupt sympathetic nerves. Pretreatment with 6-hydroxydopamine significantly reduced the uptake of MIBG to 31% of the control value. In addition, the administration of desmethylinipramine, an Uptake I blocker, led to a 50% inhibition of MIBG uptake. Similarly, in an experiment by Dae et al. (1989), where neuron disruption was produced by the application of phenol to the epicardial surface of dog hearts, the concentration of MIBG was reported to be reduced. These results suggest that MIBG uptake is dependent on the integrity of neuronal uptake in the heart.
Clinical studies performed over the past few years have provided useful information on the state of cardiac sympathetic innervation under various pathological conditions. Patients with congestive cardiomyopathy have been shown to have diminished myocardial retention of MIBG (Henderson et al., 1988). The uptake of MIBG in cardiac transplant patients was reported to be less than 10% of normals (Glowniak et al., 1989). Patients with severe diabetic autonomic neuropathy had significantly decreased cardiac uptake of MIBG (Sisson et al., 1987b; Glowniak et al., 1984). In addition, it has been suggested that there is a relationship between impairment of MIBG uptake and severity of heart failure. In recent studies using patients suffering from idiopathic dilated cardiomyopathy, it was discovered that there is a positive correlation between MIBG uptake and both peak positive left ventricular dp/dt (Merlot et al., 1992a), and left ventricular ejection fraction (Schofer et al., 1988). These studies imply that with the improvement of ventricular dysfunction, MIBG uptake increases.

In general, the activity of MIBG in the heart is thought to be a direct reflection of sympathetic nerve number, function and integrity. Furthermore, as MIBG is a structural analogue of NE, it provides qualitative information on myocardial catecholamine content. These characteristics of MIBG help make it a successful diagnostic tool in assessing the adrenergic system in various disease states.

1.6 Experimental Rationale

There are a number of pathophysiological changes that occur in the cardiovascular system of diabetics, such as congestive heart failure, cardiomyopathy, coronary artery disease and autonomic neuropathy. Hypertension,
which is prevalent among diabetics (Bell, 1989), is well known to exacerbate the cardiovascular complications that are associated with diabetes. In addition, diabetes is characterized by an abnormal sympathetic nervous system, which further aggravates any deleterious cardiovascular changes that are occurring.

Although the activity of the sympathetic nervous system in diabetes has been extensively examined, the results, as discussed previously, are highly inconsistent. Also, the relationship between the role of adrenergic nerves and NE stores and their role in maintaining the basal contractile state of the myocardium is not entirely clear. While some investigators believe that sympathetic innervation plays an integral part in cardiac function (Chidsey et al., 1964), other investigators report that a relationship does not exist (Maxwell et al., 1964).

The effect that hypertension has on the sympathetic system of the diabetic animal has not at present been investigated; nor has the relationship between cardiac NE stores and the contractile state of the myocardium in experimental diabetes. In this experiment, STZ-diabetic rats were administered DOCA-NaCl to induce hypertension so that we could examine their combined effects on the distribution of NE in the heart. To examine this, we employed a radioactive agent, MIBG, that preferentially localizes in the adrenergic nerve endings of the heart. Thus any structural or functional changes occurring in the nerves of the heart which are associated with diabetes and hypertension should be detected by alterations in MIBG uptake. In similarly treated animals, the contractile function of the myocardium was studied using a papillary muscle preparation.
From these studies, we were able to examine cardiac neuronal changes in control, diabetic, hypertensive, and diabetic–hypertensive rats. In addition, we were also able to correlate the neuronal changes with the contractile state of the myocardium.

It was our hope that MIBG would be able to provide an accurate assessment of cardiac adrenergic integrity in diabetic–hypertensive animals, and that the neuronal integrity, as measured with MIBG, was directly reflective of myocardial function. The specific objectives of this study were as follows:

1. To evaluate the retention of myocardial MIBG uptake in diabetic and non–diabetic hypertensive rats over 12 weeks.

2. To observe the relationship between adrenergic sympathetic integrity and myocardial function.

3. To examine the relationship between myocardial endogenous NE stores and MIBG accumulation in the heart.

4. To examine the effect that DOCA–induced hypertension has on the sympathetic system of the diabetic rat as measured by MIBG uptake.

5. To determine if MIBG can be used as an early marker in the assessment of cardiac dysfunction in diabetic animals.
2.0 MATERIALS AND METHODS

2.1 Induction of Diabetes Mellitus and Hypertension

Male Sprague-Dawley rats (Charles River, Montreal) weighing between 250 – 300 grams were housed 2–3 animals per cage in a room with a 12 hour light–dark cycle. They were given regular laboratory chow (Purina rat chow) and water ad libitum. Under light halothane anesthesia (Fluothane, Ayerst Labs, Vancouver), the rats were made diabetic via tail vein injections of STZ (Sigma, St. Louis, Mo., 55 mg/kg) or 0.9% saline (1 ml/kg). The presence of hyperglycemia was measured at 48 and 72 hours after injection with the use of a glucometer (Ames, Miles Lab.). Animals were accepted as diabetic if their blood glucose levels were 13 mmol/l or greater.

Seven days after the induction of diabetes, the rats were divided into two groups. They were given subcutaneous injections (25 mg/kg) of either deoxycorticosterone acetate (DOCA; Sigma, St. Louis, Mo.), a hypertensive–inducing agent, or DOCA vehicle (1.8 g NaCl, 1.8 g benzyl alcohol, 1.0 g carboxymethylcellulose, 0.8 g polysorbate 80; made up to 200 ml with distilled water) twice weekly. All animals received 0.9% NaCl with 0.5% KCl to drink ad libitum. Rats were designated by their respective treatments: CON (saline injected and received DOCA vehicle); STZ (STZ–injected and received DOCA vehicle); DOCA (saline–injected and received DOCA); and STZ–DOCA (STZ–injected and received DOCA). Experiments were conducted sequentially at 3, 6, 9, and 12 weeks after DOCA or DOCA vehicle injections (or 4, 7, 10, and 13 weeks post STZ or saline injections). Body weight was recorded twice a week during each experiment and
food and fluid intake were recorded once at the end of the last week of each experiment.

Systolic blood pressure (BP) and pulse rate (PR) were measured indirectly using an electro-sphygmanometer (Narco Bio-Systems), which consisted of a pneumatic pulse transducer, tail cuff, and graph recorder. The animal was restrained in an enclosed plastic semi-circle, with its tail able to extend freely. Directly under the body of the rat was a heated pad which was maintained at a temperature of 30°C. The tail-cuff was placed mid-length around the tail and the pulse transducer was taped to the underside of the bottom of the tail. Animals were stabilized for 15 minutes before measurements were taken. BP was determined as the level at which pulsations reappeared during the gradual deflation of the tail-cuff. Three consecutive measurements were taken at one minute intervals. PR was calculated by multiplying the arterial pulsations, which were recorded for 6 seconds, by 10. BP and PR measurements were taken every 3 weeks during the 12 week MIBG experiment. Although there has been some speculation as to the accuracy of the data obtained from this indirect method, in our laboratory, results from tail-cuff measurements have been between 5–8 mmHg higher than when measured via direct cannulation into the abdominal aorta.

2.2 Administration and Distribution Evaluation of I–123 MIBG

After 3, 6, 9, or 12 weeks of commencing DOCA or DOCA vehicle treatment, under sodium methohexital anesthesia (Eli Lilly, Toronto, Canada, 60 mg/kg, i.p.), PE10 polyethylene tubing was used to cannulate the jugular vein and MIBG (Nordion International Inc., Vancouver, B.C.) was injected intravenously (15 mCi/mg). Five
hours later, a sodium pentobarbital (M.T.C. Pharmaceuticals, Cambridge, Ont.) overdose was given (100 mg/kg, i.p.), and the heart and spleen were removed. The heart was dissected into its four chambers, which were then rinsed with saline, blotted dry, and weighed. Radioactivity of I-123 was measured using a gamma counter (Crystal 5400 Multidetector). Correction was made for decay. Results are expressed both as: i) % Kg Dose, which measures whole tissue content of MIBG, and ii) % Kg Dose/g, which normalizes for the weight of each tissue and provides the concentration of the tracer in the organ. Examples of how to obtain %Kg Dose/g and % Kg Dose are as follows:

Both expressions normalize for differences in body weight (kg), and thus take into consideration the varying biodistribution of MIBG between the different groups of animals.

These results were used to examine the uptake of MIBG in the left ventricle, and also the total heart, which is simply the combined uptake of MIBG into the four chambers of the heart. The left ventricle was chosen because diabetics frequently have impaired left ventricular function and also because left ventricular papillary muscles were used to measure heart function in this experiment. In addition, the presence of MIBG in the spleen was also measured in order to obtain a generalized picture of the sympathetic activity in each animal group.
2.3 Acute STZ–toxicity Study

In an ultrastructural study performed by Monckton and Pehowich (1980), degenerative changes in the axonal plasma membranes and axoplasm of unmyelinated fibres were observed 24 hours after STZ injection (80 mg/kg). These results suggested a toxic rather than a diabetic cause for the neuropathic changes seen. Therefore, to discredit this possibility, diabetes was induced in six male Sprague–Dawley rats (200–300g, Charles River, Montreal) using the method stated previously. Six control rats received saline injections via the tail vein. A glucometer was used 24 and 48 hours later to measure hyperglycemia. Animals were given water to drink ad libitum. Two days after the induction of diabetes, I–123 MIBG was injected into the jugular vein. The same protocol was used as discussed in the above section with respect to dissecting and counting the tissues. Results are expressed in % Kg Dose/g as there were no significant differences between organ weights.

2.4 Papillary Muscle Experiments

A. Tissue Preparation

After 12 weeks treatment with DOCA or DOCA vehicle, the hearts were quickly removed under pentobarbital anaesthesia (100 mg/kg, i.p.). The apex of each heart was excised, wrapped in tinfoil, placed in liquid nitrogen, and then stored at –70°C. The samples were used for determination of cardiac NE concentration.

Both papillary muscles were removed from the left ventricle and placed in cold–oxygenated Chenoweth–Koelle (CK) buffer of the following composition (mM):
NaCl 120, KCl 5.6, MgCl₂.6H₂O 2.1, CaCl₂.2H₂O 2.2, NaHCO₃ 1.9 and glucose 10. Blood was collected from the chest wall cavity, and then centrifuged for 10 min at 4200 rpm (Beckman, Model J–6B). Plasma was separated and stored at −20°C for future analysis.

In order to induce transmural tissue stimulation, each papillary muscle was mounted between two cup–shaped gold plated electrodes (Grass Instruments, Co.). The other end of the tissue was attached to a Grass FT3 tension transducer connected to a Grass Model 7D polygraph eight–channel recorder. Tissues were perfused in a 20 ml tissue bath in CK buffer at 27°C, oxygenated with 95% O₂ – 5% CO₂. CK buffer was changed every 20 minutes. After an equilibration period of one hour, during which the muscle contracted at a resting force of 1 gram, the tissue was stimulated at supra maximum voltage (between 40V–80V) at a frequency of 0.2 Hz and a duration of 5 msec.

B. Length–tension Relationship

Under the above conditions, preload was lowered to 0 grams, and was then increased in 0.5 gram increments up to 10 grams. Preload, or muscle length, at each increment was not increased until the maximum force of contraction was obtained. This portion of the tissue bath experiment analyzed length–tension development in all 4 animal groups. Upon the completion of this experiment, tissues were maintained at a preload tension of 1 gram for 20 minutes.

C. Dose–response to Isoproterenol and Tyramine

At a preload resting tension of 0.2 g to 0.4 g, papillary muscle preparations were exposed to the inotropic agents isoproterenol (ISO) and tyramine (TYR).
ISO (Sigma, St. Louis, Mo.), a β-adrenergic agonist, was added cumulatively to the tissue bath to achieve concentrations ranging from $1 \times 10^{-10}$ to $1 \times 10^{-4}$ M. The maximal response to each dose of ISO was measured after 4 minutes. After the final concentration, the tissues were washed 3 times, and allowed to equilibrate for 15 minutes. TYR (Sigma, St. Louis), an indirect sympathomimetic, was then added to the bath in the concentration range $1 \times 10^{-4}$ to $1 \times 10^{-2}$ M. Tissues were given 5 minutes to respond to each successive dose, and the maximal response was recorded.

After the experiment, muscle length was measured, and the muscle was blotted dry and weighed. Cross-sectional area of each muscle was calculated by dividing its weight by its length. Tension was expressed as force per unit of cross-sectional area.

2.5 Catecholamine Determination

Tissue NE was assayed using HPLC with electrochemical detection. Previously frozen apex tissues were weighed and homogenized in a polytron homogenizer with saline, and the homogenate was centrifuged for 10 minutes at 3000 g at 4°C. As modified by Anton and Sayre (1962), the catecholamines were extracted from the supernatant with aluminum oxide in the presence of hydrogen potassium phosphate buffer (0.25 M). The pH was adjusted to 3.8 using phosphoric acid. Catecholamines were separated and quantified using a 5 μm particle size reverse-phase octadecylsilane column (15cm x 4.6mm). The system utilized a Waters M45 pump, a Waters UK6 injector, and a model 656 Electrochemical Detector (Metrohm). NE
levels were expressed as ug/g wet heart weight. All HPLC work was performed by the laboratory of Dr. R. Rangno (Saint Paul's Hospital, Vancouver).

2.6 Plasma Analyses

Plasma samples obtained from the 12 week tissue bath experiment were analyzed for glucose, insulin, cholesterol, and triglycerides. Plasma glucose, cholesterol, and triglycerides were determined using enzymatic colorimetric assay kits (all from Boehringer Mannheim, Diagnostica). Plasma insulin was determined using a radio–immunoassay kit (ICN Biomedicals Inc.).

2.7 Statistical Analyses

All results for any particular group were averaged and the standard error of the mean (SEM) was determined at each experimental time point. One way analysis of variance (ANOVA) followed by Fischers LSD test was used for comparing results from a given set of groups. A probability of less than 0.05 (p<0.05) was used.
3.0 RESULTS

3.1 General Features of Animals

A. Body Weight and LV/BW

STZ and STZ-DOCA groups exhibited significantly lower body weights than their control treated groups over a 12 week period (Figure 4). In addition, after 3 weeks, DOCA animals displayed lower body weights when compared to CON.

At the onset of each study, all rats weighed between 250–300 g. The left ventricular–weight to body–weight ratio (LV/BW) was determined using the weights of the left ventricles taken from the 4 MIBG studies. As shown in Figure 5, at all time points, the CON LV/BW values were significantly lower than the 3 remaining groups. Only at 12 weeks were DOCA and STZ-DOCA LV/BW ratios significantly elevated when compared to STZ.

B. Food and Fluid Consumption

Polydypsia was seen in the STZ-treated groups when compared to their respective control treated groups at 3, 6, 9, and 12 weeks (Figure 6). At all time points, DOCA had significantly higher fluid consumption compared to CON. Unexpectedly, there was no consistent elevation in food consumption in STZ and STZ-DOCA over the 12 weeks when compared to CON and DOCA (Figure 7). At 3 weeks, both STZ-treated groups ate more than the CON and DOCA groups, while at 6 weeks only STZ-DOCA consumed more than CON. At 9 weeks, food intake in the STZ group was significantly more than that observed in CON; however, at 12 weeks, neither of the STZ-treated groups consumed significantly more than CON.
Figure 4. Body weights of 13 week non-diabetic and diabetic rats.

STZ and STZ–DOCA values were significantly lower than CON and DOCA at all time points. DOCA values were significantly lower than CON from 3 to 12 weeks. (p<0.05)

n=10–12
Figure 5. Left ventricular-weight to body-weight ratios of non-diabetic and diabetic rats.

* p<0.05 when compared to CON
+ p<0.05 when compared to DOCA and STZ-DOCA

n=5-6
Figure 6. Fluid consumption measured over a 12 week period.

* p<0.05 when compared to CON
+ p<0.05 when compared to CON and DOCA

n = 6–12
Figure 7. Food consumption over a time period of 12 weeks.

* p<0.05 when compared to CON
+ p<0.05 when compared to DOCA
# p<0.05 when compared to all other groups

n = 6–12
C. Blood Pressure and Pulse Rate

Systolic blood pressure (BP) of CON and STZ groups remained relatively unchanged over the course of 12 weeks, ranging from 122 – 140 mmHg (Figure 8). At 3 weeks, BP of the DOCA group was significantly higher than the remaining groups, but by 6 weeks STZ–DOCA BP values were also greater than those of CON and STZ. BP of both DOCA and STZ–DOCA groups remained significantly elevated compared to CON and STZ from 6 to 12 weeks. At week 12 the BP of DOCA and STZ–DOCA were 196 mmHg and 206 mmHg, respectively.

After 3 weeks of DOCA or DOCA vehicle treatment, there were no differences in pulse rate (PR) between the 4 groups (Figure 9). At 6 weeks, the PR in the DOCA group was significantly higher than in both the STZ and STZ–DOCA groups. Also at 6 weeks, CON PR was significantly greater than that of STZ. STZ had significantly lower PR values than did DOCA at 9 weeks; however, by 12 weeks STZ PR values were lower than those measured in the remaining 3 groups.

3.2 Cardiac Norepinephrine

As shown in Figure 10, at 12 weeks, STZ and STZ–DOCA had significantly higher concentrations of heart NE compared to CON and DOCA. Although not significant, DOCA had a lower concentration of cardiac NE than did CON.

3.3 Plasma Analyses (measured at 12 weeks)

Table 1 shows plasma values of glucose, cholesterol, triglycerides, and insulin 13 weeks after the induction of diabetes. The plasma glucose levels in the STZ and
Figure 8. Indirect blood pressure measurements recorded over a period of 12 weeks using tail-cuff method.

* p<0.05 compared to CONTROL
+ p<0.05 compared to STZ

n = 6
Figure 9. Pulse rate measured over a period of 12 weeks using tail-cuff method.

* p<0.05 when compared to CON
+ p<0.05 when compared to DOCA
# p<0.05 when compared to all three groups

n = 6
Figure 10. Norepinephrine measured from the apex of the heart at 13 weeks diabetic.

* p<0.05 when compared to CON and DOCA
TABLE 1. Plasma glucose, insulin, cholesterol, and triglyceride levels measured after 12 weeks DOCA-treatment in diabetic and non-diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>DOCA</th>
<th>STZ</th>
<th>STZ-DOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose mmol/l</td>
<td>10.69±0.40</td>
<td>8.17±0.24</td>
<td>24.87±0.78*</td>
<td>16.82±2.35*</td>
</tr>
<tr>
<td>Insulin uU/ml</td>
<td>138.34±15.87*</td>
<td>30.08±3.64*</td>
<td>13.28±1.39</td>
<td>14.67±2.10</td>
</tr>
<tr>
<td>Cholesterol mmol/l</td>
<td>1.39±0.08</td>
<td>2.84±0.30+</td>
<td>1.54±0.11</td>
<td>2.43±0.36+</td>
</tr>
<tr>
<td>Triglyceride mmol/l</td>
<td>2.22±0.21</td>
<td>2.12±0.45</td>
<td>1.75±0.14</td>
<td>1.75±0.14</td>
</tr>
</tbody>
</table>

Values are means ± SE.
* p < 0.05 when compared to all other groups.
+ p < 0.05 when compared to CONTROL and STZ.

n = 10
STZ–DOCA injected groups were significantly elevated compared to their respective control groups. Interestingly, plasma glucose levels of STZ–DOCA were significantly lower when compared to STZ. Plasma insulin concentrations of STZ and STZ–DOCA were significantly lower than their respective controls (CON and DOCA), but were not significantly different from each other. Insulin values in the DOCA treated group were significantly lower than those measured in the CON group. The CON group insulin value of 138 ± 15.9 uU/ml is abnormally high. The most likely reason for this was that in the CON group, the radiolabelled pellet was lost when the test-tubes were decanted. The assay was then repeated in this group only, which may be why the CON value was unrealistically high. Another possible reason is that these values were fed values. CON group insulin values normally range from 40 to 50 uU/ml. Plasma cholesterol levels were elevated in both DOCA and STZ–DOCA treated groups when compared to CON and STZ. There were no statistical differences in plasma triglyceride levels among the 4 groups.

3.4 Acute MIBG Study

MIBG uptake measured in the heart 2 days after STZ (or saline) injection indicated that STZ did not exhibit a toxic effect on the adrenergic nerve endings (Figure 11). There were no measurable differences (p<0.05) when comparing the concentration of MIBG in the tissues of the CON group and the STZ–treated group.

3.5 MIBG Uptake

As discussed earlier, MIBG results are expressed as total content of MIBG in the organ (% Kg Dose) as well as in concentration per organ (% Kg Dose/g).
Figure 11. Acute STZ toxicity study performed 2 days post-injection of STZ. No significant difference was measured between the two groups LV=left ventricle; TH=total heart

n=6
Depending on the weight of the tissue, the concentration of MIBG per gram of tissue could be increased (with a small organ) or decreased (with a very large organ). In general, after dividing by tissue weight, the concentration of MIBG was shown to increase, particularly in the STZ-treated groups (due to their lower tissue sizes). Mean organ weights of left ventricle, total heart, and spleen measured at each time point are listed in Table 2.

For each experiment the absolute values of MIBG uptake in both heart and spleen of all four groups are compared. These results are expressed as both % Kg Dose and % Kg Dose/g. When examining the uptake of MIBG over a 12 week period, the values obtained for DOCA, STZ, and STZ–DOCA are all expressed as a % of the CON value. In other words, the mean absolute values of each group were divided into the mean absolute value of the CON group, and CON was made 100%. This calculation normalized each experiment and accounted for any technical variations that may have occurred. These results were also expressed in both % Kg Dose and % Kg Dose/g.

A. **Left Ventricle**

   **% Kg Dose**

   The total amount of MIBG uptake into the left ventricle, expressed in absolute values, is shown in Figure 12. DOCA values were significantly lower than CON at 6, 9, and 12 weeks. MIBG values in STZ and STZ–DOCA were significantly lower than both CON and DOCA at 3, 6, and 9 weeks; however, at 12 weeks, they were only significantly lower from CON.
TABLE 2. Organ Weights.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>DOCA</th>
<th>STZ</th>
<th>STZ–DOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>3 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Ventricle</td>
<td>0.980±0.003</td>
<td>1.046±0.037</td>
<td>0.790±0.040</td>
<td>0.767±0.026</td>
</tr>
<tr>
<td>Heart</td>
<td>1.219±0.040</td>
<td>1.301±0.045</td>
<td>0.994±0.053</td>
<td>0.947±0.025</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.730±0.037</td>
<td>0.734±0.066</td>
<td>0.570±0.037</td>
<td>0.512±0.016</td>
</tr>
<tr>
<td><strong>6 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Ventricle</td>
<td>0.914±0.052</td>
<td>1.165±0.034</td>
<td>0.732±0.032</td>
<td>0.717±0.033</td>
</tr>
<tr>
<td>Heart</td>
<td>1.222±0.069</td>
<td>1.475±0.041</td>
<td>0.966±0.041</td>
<td>0.948±0.039</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.780±0.048</td>
<td>1.007±0.059</td>
<td>0.434±0.051</td>
<td>0.393±0.043</td>
</tr>
<tr>
<td><strong>9 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Ventricle</td>
<td>1.042±0.025</td>
<td>1.217±0.041</td>
<td>0.790±0.020</td>
<td>0.776±0.085</td>
</tr>
<tr>
<td>Heart</td>
<td>1.287±0.026</td>
<td>1.467±0.052</td>
<td>0.962±0.018</td>
<td>0.923±0.091</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.863±0.056</td>
<td>0.903±0.072</td>
<td>0.492±0.032</td>
<td>0.463±0.068</td>
</tr>
<tr>
<td><strong>12 Weeks</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left Ventricle</td>
<td>1.244±0.012</td>
<td>1.349±0.064</td>
<td>0.956±0.060</td>
<td>1.281±0.165</td>
</tr>
<tr>
<td>Heart</td>
<td>1.648±0.005</td>
<td>1.690±0.079</td>
<td>1.294±0.080</td>
<td>1.519±0.131</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.368±0.115</td>
<td>1.327±0.069</td>
<td>0.879±0.082</td>
<td>1.148±0.122</td>
</tr>
</tbody>
</table>

Values are means ± SE.
Organ weights are obtained from MIBG experiments.
* p < 0.05 when compared to CONTROL.
+ p < 0.05 when compared to DOCA.
n = 6
Figure 12. Absolute values of I-123 MIBG uptake into the left ventricle measured over a period of 12 weeks.

* p<0.05 when compared to CON
+ p<0.05 when compared to DOCA

n=5–6
When measuring changes in MIBG content in the left ventricle over time, a general decrease was seen in the DOCA group, which ranged from 82 ± 9.9% of CON at 3 weeks to 43 ± 7.0% at 12 weeks (Figure 13). There was a decreasing trend in both STZ and STZ–DOCA from 3 weeks to 9 weeks. Values ranged from 53 ± 4.4% at 3 weeks to 32 ± 4.5% at 9 weeks in the STZ group, and from 49 ± 3.1% at 3 weeks to 26 ± 3.2% at 9 weeks in the STZ–DOCA group.

% Kg Dose/g

At all time points, the absolute values, expressed as concentrations of MIBG, were shown to be significantly lower in all 3 groups compared to CON (Figure 14).

Left ventricular uptake of MIBG in the DOCA group was 77 ± 8.5% of CON at 3 weeks, but decreased significantly to 39 ± 6.0% by 12 weeks (Figure 15). Similarly, there was a decrease in MIBG uptake in both STZ–treated groups. MIBG measured in the STZ group decreased significantly from 66 ± 3.8% at 3 weeks to 49 ± 4.3% at 12 weeks. The uptake in the STZ–DOCA group fell from 63 ± 4.3% (at 3 weeks) to 32 ± 2.9% (at 12 weeks).

The values of MIBG accumulation expressed as content and concentration were compared to determine if dividing the % Kg Dose by the weight of the left ventricle would change the concentration of MIBG significantly. Indeed, we discovered that at 3 weeks, the concentrations of MIBG in STZ and STZ–DOCA groups, 66 ± 3.8% of CON and 63 ± 4.3% of CON respectively, were significantly elevated compared to the content measured. MIBG contents of STZ and STZ–DOCA were 53 ± 4.4% and 49 ± 3.1% respectively. The statistics are not shown on the Figures.
Figure 13. Total content of I-123 MIBG measured in the left ventricle over a period of 12 weeks. (p<0.05)

DOCA: 3–9; 3–12; 6–12
STZ: 3–6; 3–9; 3–12
STZ–DOCA: 3–9; 3–12 6–9

n=5–6
Figure 14. Absolute values of I-123 MIBG uptake into the left ventricle measured over a period of 12 weeks.

* p<0.05 when compared to CON

n=5–6
Figure 15. Concentration of I-123 MIBG measured in the left ventricle over a period of 12 weeks. (p<0.05)

DOCA: 3–9; 3–12; 6–12
STZ: 3–6; 3–9; 3–12
STZ–DOCA: 3–9; 3–12; 6–9; 6–12

n=5–6
B. **Total Heart**

**% Kg Dose**

When comparing the absolute values of MIBG in the heart at each time point, STZ and STZ–DOCA had a significant decrease in MIBG uptake from 3 to 12 weeks when compared to CON, and from 3 to 9 weeks compared to DOCA (Figure 16). The total content of MIBG measured in the DOCA group was significantly lower than CON at weeks 6, 9, and 12.

With increasing time, there was an overall decrease in MIBG uptake into the total heart of the DOCA group (Figure 17). At 3 weeks the uptake was 86 ± 10.6% of CON, whereas by 12 weeks it was only 44 ± 6.4% of CON. In both STZ–treated groups there was no significant difference between the 9 and 12 week MIBG values. However, both groups did have a significant decrease in MIBG content from 3 weeks to 9 weeks. At these time points, STZ MIBG content fell from 54 ± 4.4% to 34 ± 5.1% while STZ–DOCA values decreased from 50 ± 3.0% to 28 ± 3.3% of CON.

**% Kg Dose/g**

The absolute values of MIBG concentration in all 3 groups were significantly lower when compared to CON at all time points (Figure 18). At 3 weeks, MIBG uptake in the STZ–DOCA group was significantly lower than the DOCA group; however, at 6 weeks, only STZ had significantly lower concentrations of MIBG compared to DOCA.

The concentration of MIBG in the DOCA group decreased in a linear manner from 3 to 12 weeks (Figure 19). The uptake was 81 ± 8.6% of CON at 3 weeks and decreased to 43 ± 6.0% by 12 weeks. Uptake in the STZ group fell significantly from
Figure 16. Absolute values of I-123 MIBG uptake into the heart measured over a period of 12 weeks.

* p<0.05 when compared to CON
+ p<0.05 when compared to DOCA

n=5-6
Figure 17. Total content of I-123 MIBG measured in adrenergic nerve endings over a period of 12 weeks (p<0.05).

DOCA: 3–12; 6–12
STZ: 3–6; 3–9; 3–12
STZ–DOCA: 3–6; 3–9; 3–12; 6–9

n=5–6
Figure 18. Absolute values of I-123 MIBG uptake into the heart measured over a period of 12 weeks.

* p<0.05 when compared to CON
+ p<0.05 when compared to DOCA

n=5–6
Figure 19. Concentration of I-123 MIBG measured in the total heart over a period of 12 weeks.

(p<0.05)

DOCA: 3–9; 3–12; 6–12
STZ: 3–6; 3–9; 3–12
STZ–DOCA: 3–9; 3–12; 6–9; 6–12

n=5–6
67 ± 3.8% at 3 weeks to 45 ± 6.8% at 9 weeks. There was a slight, although not significant, increase in concentration of MIBG at 12 weeks (50 ± 4.0%). Similar to DOCA, STZ–DOCA exhibited a steady decrease in myocardial MIBG concentration. Values ranged from 65 ± 4.1% at 3 weeks to 35 ± 2.8% at 12 weeks.

When comparing values between MIBG content and concentration in the heart, a significant difference was measured at 3 weeks in the STZ–DOCA group. While the content of MIBG was 50 ± 2.9% of CON, the concentration was greater at 65 ± 4.1% of CON. This was simply due to the relatively small heart sizes of this group at 3 weeks.

C. Spleen

% Kg Dose

When expressed as total content, the absolute values of MIBG in the spleen were significantly lower in all 3 groups when compared to CON at each time point (Figure 20). With the exception of 12 weeks, STZ and STZ–DOCA values were significantly less than DOCA.

In the DOCA group, the total uptake of MIBG increased slightly from 3 to 6 weeks, then fell significantly from 6 weeks (80 ± 1.6%) to 12 weeks (60 ± 9.0%) (Figure 21). The content of MIBG in both STZ and STZ–DOCA groups increased significantly from 6 to 12 weeks. The values increased from 32 ± 5.3% of CON to 50 ± 5.1% for STZ, and from 31 ± 4.2% to 51 ± 5.5% for STZ–DOCA.
Figure 20. Absolute values of I-123 MIBG uptake into the spleen measured over a period of 12 weeks.

* p<0.05 when compared to CON
+ p<0.05 when compared to DOCA

n=5–6
Figure 21. Total content of I-123 MIBG measured in the spleen over a period of 12 weeks. (p<0.05).

DOCA: 6–12
STZ: 6–12
STZ–DOCA: 6–12; 9–12

n=5–6
% Kg Dose/g

With the exception of 9 weeks, all 3 groups had significantly lower concentrations of MIBG uptake than did CON (Figure 22). At 9 weeks, only DOCA and STZ values were less than CON.

When expressed as concentration, there was no change in the uptake of MIBG at any time point in either of the 3 groups (Figure 23).

At weeks 6 and 9, both STZ and STZ–DOCA MIBG values were significantly elevated when expressed as tissue concentration (Figure 23) compared to tissue content (Figure 21). This was due to the small spleen sizes in these groups at 6 and 9 weeks.

3.6 Papillary Muscle Experiments

A. Papillary Muscle Characteristics

As shown in Table 3, left ventricular papillary muscle length was significantly shorter in STZ and STZ–DOCA groups compared to DOCA. DOCA had a marked increase in wet weight when compared to the STZ group. However, when the cross-sectional area of each muscle group was calculated, there were no statistical differences measured between any of the groups.

B. Length–Tension Relationship

Figure 24 shows that in all 4 groups there were progressive increases in tension with elevated preload (or increasing muscle length). In general, STZ had a lower g/mm² response than the other groups, however, only the values from 4.5 g
Figure 22. Absolute values of I-123 MIBG uptake into the spleen measured over a period of 12 weeks.

* p<0.05 when compared to CON
+ P<0.05 when compared to DOCA

n=5–6
Figure 23. Concentration of I–123 MIBG measured in the spleen over a period of 12 weeks.

There is no significant difference in any group over the 12 week period.

n=5–6
TABLE 3. General Features of Papillary Muscles.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>DOCA</th>
<th>STZ</th>
<th>STZ-DOCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML (mm)</td>
<td>7.64±0.26</td>
<td>8.08±0.41</td>
<td>6.83±0.27*</td>
<td>7.14±0.32*</td>
</tr>
<tr>
<td>MW (mg)</td>
<td>8.60±0.56</td>
<td>9.39±0.92</td>
<td>7.39±0.47*</td>
<td>8.15±0.56</td>
</tr>
<tr>
<td>XS (mm²)</td>
<td>1.08±0.07</td>
<td>1.10±0.08</td>
<td>1.07±0.07</td>
<td>1.14±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE
n = 14-18
ML = muscle length
MW = muscle weight
XS = cross-sectional area
* p < 0.05 when compared to DOCA
Figure 24. Force of contraction measured against increasing preload tension in left ventricular papillary muscle.

mm2 = cross-sectional area of the muscle

* p<0.05 when compared to CON

n=7–13
to 5.5 g were significantly lower than CON. There were no significant differences in tension generated with increasing preload between STZ–DOCA and DOCA or between STZ–DOCA and STZ. However, there was an overall increase in the active tension generated from the STZ–DOCA group at higher preload tensions.

C. **Dose–response to Isoproterenol and Tyramine**

The dose–response for both ISO and TYR was expressed both as force per unit cross-sectional area (g/mm\(^2\)) and as a percentage of the maximum response generated by each individual group. As illustrated in Figure 25 there were no significant differences in the g/mm\(^2\) response of any of the groups when ISO was added to the tissue bath in increasing doses. Interestingly, the force of contraction in the STZ–DOCA group was somewhat elevated when compared to the remaining 3 groups, however, due to the large standard error bars the increase was insignificant. When the % maximum response for each animal group was calculated (Figure 26), the greatest force of contraction in the CON group was shown to occur after the addition of the highest dose of ISO. At this point the CON group value was significantly higher than DOCA, STZ and STZ–DOCA groups. In addition, % maximum response of the CON group was significantly lower than the other 3 groups up until a concentration of 1 \(\times\) \(10^{-7}\)M was achieved in the bath. DOCA, STZ, and STZ–DOCA groups displayed a decrease in % maximum response with increasing concentration of ISO. Unfortunately, it appears that all muscles were contracting at close to the maximum even before ISO was added to the bath. The CON group was already at 80.7 ± 3.6% of their maximum response before the addition of ISO, while DOCA, STZ, and STZ–DOCA were at 89 ± 2.5%, 90 ± 2.1%, and 94 ± 1.1% of their maximum force of contraction, respectively.
Figure 25. Measurement of contractile force in response to the addition of increasing concentration of Isoproterenol.

There is no significant difference between any of the values.

n=7–13
Figure 26. Percentage of the maximum contractile force generated in each group after the addition of increasing concentrations of Isopreterenol.

* p<0.05 when compared to CON

n=7–13
Similar results to those seen with ISO are seen in the TYR dose-response curve when expressed as g/mm$^2$. Figure 27 also illustrated that there were no significant differences in the force of contraction in any of the groups with increasing concentration of TYR. Once again, the STZ–DOCA values were generally higher, although not significantly, than the other 3 groups. When the dose-response curve was expressed as a % of the maximum response (Figure 28), the CON values were shown to be significantly lower than the remaining groups up to the concentration of 0.008 M. All tissues showed an increase in force of contraction with an increase in dose, although this response was seen best in the CON group. As seen prior to the addition of ISO, the tissues were already responding close to maximally before the TYR was introduced to the bath. The CON group value was 76 ± 2.5% of maximum, whereas DOCA, STZ, and STZ–DOCA were already 85 ± 3.3%, 87 ± 2.5%, and 88 ± 1.4% of maximum respectively.
Figure 27. Measurement of contractile force in response to the addition of increasing concentration of tyramine.

There is no significant difference between any of the values.

n=7–13
Figure 28. Percentage of the maximum contractile force generated in each group after the addition of increasing concentrations of tyramine.

* p<0.05 when compared to CON
n=7–13
4.0 DISCUSSION

4.1 Body Weights

Our observation of decreased weight gain in the DOCA group over the 12 week period was in agreement with previous findings (Yamamoto et al., 1985; Hebden et al., 1990; Dai and McNeill, 1992; Dai et al., 1993). The mechanism behind this phenomenon remains unclear.

4.2 Plasma Insulin and Glucose Analyses

The glucose values in the 13 week diabetic STZ group were significantly elevated from the STZ–DOCA values, confirming earlier results reported from our laboratory. Both Hebden et al. (1990) and Dai and McNeill (1992) reported this anomaly at various time points when using the DOCA–salt induced hypertension model. More interesting is the fact that the insulin levels in these two groups were almost identical, ruling out the possibility of elevated insulin levels in the STZ–DOCA group. In a recent study by Dai et al. (1993) it was suggested that the subsequent decrease in insulin secretion observed after DOCA treatment is secondary to its effects on glucose metabolism. It has been postulated that DOCA may have an effect on glucose metabolism by either directly inhibiting it’s production, increasing it’s utilization, or by indirectly enhancing the metabolic effects of the existing insulin (Dai and McNeill, 1992). This latter suggestion may also help to explain why the DOCA group insulin values were significantly lower compared to the CON group.
4.3 MIBG Uptake

MIBG is a useful tool in determining if there exists a structural or functional imbalance in adrenergic innervation. By measuring MIBG values, one can ultimately evaluate the role that altered innervation plays in the pathophysiology of diabetes. In this experiment, the uptake of MIBG into the myocardium was used to investigate the relationship between sympathetic integrity and myocardial function in diabetes.

At present, there is no widely accepted non-invasive test in which to measure cardiac sympathetic nervous function. Unfortunately, by the time most long-standing diabetics seek medical attention for cardiovascular complications, they have already developed substantial, irreversible autonomic damage in their myocardium. Therefore, the availability of an agent that would aid in the early diagnosis of cardiac complications due to sympathetic alterations would be of phenomenal benefit in the diabetic population.

Although a fair amount of research has been conducted on the use of MIBG to map the adrenergic nervous system in man, very little information has been published on the distribution of MIBG in the hearts of patients with diabetes. The results of these experiments unanimously report that patients with diabetes complicated autonomic neuropathy have little or no measurable concentration of MIBG in their myocardium (Glowniak et al., 1984; Sisson et al., 1987; Fagret et al., 1991). Kahn et al. (1988) suggest that not all neurons of the heart are equally impaired in patients with generalized diabetic autonomic neuropathy (DAN), because many hearts have small measurable concentrations of MIBG. Furthermore, they speculate that the consequential imbalance of adrenergic stimulation may likely
be the cause of fatal arrhythmias, a phenomenon that is unusually high in these patients (Watkins et al., 1980).

In a recent study by Mantysaari et al. (1992), myocardial MIBG accumulation was studied in diabetic patients with and without DAN. Although both groups had a diminished initial uptake of MIBG into the myocardium compared to the control group, only the group with DAN experienced difficulty in retaining the MIBG 6 hours after it's injection. They believe that by 6 hours, the MIBG accumulated in the heart reflects the amount of functioning sympathetic neuronal tissue.

At present there is no published literature on the distribution of MIBG in experimentally induced diabetes. On the one hand, there is really very little need to use animals to study the distribution of MIBG in diabetic hearts, as MIBG itself has been proven to be a safe, effective tool when used in actual patients. The majority of MIBG is excreted unchanged in the urine after 24 hours (Mangner et al., 1986), and the use of the common radiolabel, I–123, has relatively low Auger electrons and a useful half-life of 13.1 hours (Shapiro and Gross, 1987). However, the use of MIBG in experimentally induced animal models can provide valuable information that would be difficult to attain in a human model. For example, in our experiment we were able to compare the accumulation of MIBG in the hearts of 4 different test groups; Control, Hypertensive, Diabetic, and Diabetic–hypertensive, which enabled us to examine the effects of both pathologies on sympathetic function. In addition, we were able to directly measure the NE concentration in the hearts of each group, and compare these to the MIBG values obtained; a comparison which is difficult to explore in patients. Finally, we were able to perform mechanical tests from hearts of each group, which provided us with insight on the relationship between sympathetic
nervous function and myocardial function. Each one of these tests was able to contribute valuable information on the efficacy and usefulness of this radiolabelled compound.

When using the expressions % Kg Dose and % Kg Dose/g to measure the accumulation of MIBG in tissue, it is important to recognize that organ sizes play an instrumental part in obtaining the end value. For example, the LV/BW ratios in Figure 5 show that the ventricle sizes of STZ, DOCA, and STZ–DOCA were significantly increased compared to the CON group, which may be one of the reasons why the MIBG concentrations in these 3 groups were significantly lower than CON. The fact that organ sizes can appear to either increase or decrease the density of nerve endings in a tissue validates the need to look at the total content of MIBG within the tissue. Although the left ventricle of the DOCA group was significantly larger than CON at 6 weeks, and it's concentration was shown to be significantly lower than CON at this time point, the content of MIBG in the DOCA group was also significantly lower than CON at 6 weeks. This indicates that the decreased accumulation of MIBG observed in the DOCA group was not entirely due to a dilution effect.

Therefore, although at times, discussing MIBG content and concentration may present some confusion, it is imperative to study the results of both in order to get a true picture of the organ's adrenergic integrity.

In this study, the accumulation of MIBG into the hearts of diabetic animals was in accordance with the findings reported in diabetic patients. We discovered a significant decrease in MIBG uptake in the left ventricle and total heart at each
experimental time point in both diabetic groups when compared to the control group. From the results obtained in the STZ-toxicity study, we can be somewhat confident that this decrease in MIBG uptake in the diabetic groups was not due to the toxic effect of STZ itself. In general, there were no differences between the absolute MIBG values of the STZ group and the STZ-DOCA group, a finding which we did not expect. Also in the diabetic groups, at weeks 3, 6, and 9, the MIBG content measured in the left ventricle and heart was found to be significantly lower compared to DOCA. Interestingly, the concentration of MIBG in the heart and left ventricle of the DOCA group was also significantly reduced when compared to the CON group at all time points. These results suggest that an alteration in sympathetic function existed in these 3 groups, but occurred to a greater extent in the diabetic groups.

One of the aims of this study was to observe the changes in MIBG uptake into the hearts of DOCA, STZ, and STZ-DOCA groups over a 12 week period. These results, expressed as a % of the control group, showed a marked reduction of MIBG accumulation over time. This suggests that there was a progressive impairment of cardiac adrenergic activity with increasing length of disease. The results from the left ventricle mimicked those from the entire heart, when expressed as both content and concentration. The slight increase in MIBG content measured in both diabetic groups at 12 weeks, albeit insignificant, was unexpected. Instead, we predicted that the accumulation of MIBG in the hearts of the diabetic animals would continue to decrease.

The accumulation of MIBG in the spleen provided some interesting insight. The spleen was chosen because it is highly innervated with adrenergic nerves (Nakajo et
al., 1986), therefore, we thought it would be able to represent any abnormalities in
generalized sympathetic activity in each of the treated groups. In general, the uptake
of MIBG in DOCA, STZ, and STZ–DOCA, expressed in content and concentration,
was significantly lower than the CON group at all times. However, when measuring
the change in MIBG uptake from 3 to 12 weeks in each group, the uptake remained
relatively constant. These results suggest that the stress of diabetes and
hypertension do diminish the sympathetic function of other organs outside the heart,
however, there is no alteration in function over 13 weeks.

There are several possible explanations as to why there are decreases in MIBG
uptake in the hearts of DOCA, STZ, and STZ–DOCA from 3 to 12 weeks. One of the
most obvious reasons would be that a disruption of sympathetic innervation, or
autonomic neuropathy, had occurred. This would explain the gradual decrease in
MIBG uptake in the diabetic groups. However, the concentrations of NE found in the
apex of the hearts of STZ and STZ–DOCA were significantly elevated compared to
both CON and DOCA, making it doubtful that neuropathy was the sole reason for
the observed decreases.

Another possible explanation for the decrease in MIBG uptake could be that
the uptake I function of the heart was impaired. Several studies have reported that in
patients with moderate to severe heart failure, there are decreases in NE uptake
(Rose et al., 1985; Petch and Nayler, 1979). In a recent study by Merlot et al.
(1992b), they discovered that patients with idiopathic dilated cardiomyopathy had
significantly reduced levels of MIBG uptake in heart. In our experiment, there were
elevated levels of NE in apices of the diabetic groups; therefore, it doesn't appear
that the uptake I mechanism was significantly impaired.
Another factor which may cause the appearance of decreased MIBG accumulation in the nerve terminals is an increase in neuronal release of MIBG. In an experiment using DOCA–hypertensive rats, Krakoff et al. (1967) reported a defect in the storage and retention of NE in the granules of the sympathetic nerves. If NE had difficulty in binding to the granules, perhaps the same can be said for MIBG. Similarly, in an experiment by Bouvier and de Champlain (1985), the release rate of NE in DOCA–treated rats was almost doubled compared to the control group. Although we were not entirely certain that our animals suffered from cardiomyopathy, there is a great deal of evidence that suggests that cardiomyopathic hearts have difficulty in retaining MIBG. In patients with congestive cardiomyopathy, Henderson et al. (1988) found that there was an accelerated clearance of MIBG from their hearts. Taki et al. (1990) discovered that there is a positive correlation between MIBG washout from the heart and septal thickness. Since in this experiment, ventricular hypertrophy was observed in the 3 groups which displayed decreases in MIBG, the correlation proposed by Taki et al. (1990) may help to explain our results. Ganguly et al. (1987, 1988) support the view that in diabetic–cardiomyopathic rats, there is an increase in the release of NE from the nerve endings.

Another plausible explanation is that MIBG uptake is competitively inhibited by high levels of circulating catecholamines. As previously discussed, in diabetes and hypertension there are a number of factors that could lead to increased levels of circulating NE such as increased sympathetic tone, impaired neuronal uptake, or increased neuronal release of NE. In a study by Nakajo et al. (1983), it was reported that there exists an inverse relationship between the cardiac accumulation of MIBG
and circulating catecholamine levels. Our results certainly support this hypothesis. Although we did not measure circulating NE levels, we did measure NE concentration in the heart. The decreased levels of MIBG that we measured in STZ, and STZ–DOCA were accompanied by elevated cardiac NE stores. In addition, the significantly reduced cardiac NE stores found in the DOCA group reflect the fact that the MIBG accumulation in this group was greater than that found in both diabetic groups. However, other investigators oppose this view. In a study by Merlot et al. (1992a), patients with moderate heart failure had similar circulating catecholamine levels compared to the control patients, but they still had a significant reduction in MIBG uptake. Schofer et al. (1988) also found no competition between elevated plasma NE levels and MIBG uptake in patients with idiopathic dilated cardiomyopathy. Both Sisson et al. (1988) and Dae et al. (1989) found corresponding decreases in NE content when MIBG uptake was decreased. This explanation still remains a controversial topic, but is mentioned as a distinct possibility for decreasing MIBG uptake in a phenomenal number of papers.

It is possible that any one or a combination of all 4 of the above explanations may account for the reduction in MIBG accumulation. It remains a mystery as to why the combination of diabetes and hypertension did not exacerbate the decrease in uptake of MIBG. Unfortunately, the physiological effects that the DOCA–Salt hypertension model produces are poorly understood.

4.4 Myocardial Mechanics

The use of isolated ventricular papillary muscle allows for the direct study of myocardial contractile performance (Sonnenblick, 1965). In this experiment we were
interested in correlating sympathetic neural function to myocardial function; therefore, we utilized the method of transmural stimulation (TNS) to evoke a contraction in the heart. When stimulating only the nerve endings within the tissue, the contraction generated is directly related to the NE released within the heart, although the condition of the muscle fibre itself also plays a fundamental role in the contractile performance of the heart.

In TNS studies, the effect that hypertrophy has on the contractile force of the myocardium has generated a great deal of controversy. In a study by Kerr et al. (1961), in hypertrophied left ventricular papillary muscles from rat, the maximum tension developed was significantly greater per unit weight than that developed in normal papillary muscles. Similarly, Bing et al. (1988) observed an increase in the active tension generated in papillary muscles of SHR rats compared to control preparations. They propose, along with Wendt-Gallitelli et al. (1979), that in hypertrophied hearts there is an increase in contractile unit density, which in turn augments the per unit performance of papillary muscle. However, the length-tension relationship measured in DOCA-treated hypertrophied hearts was shown to be no different from that of it's age-matched control (Heller, 1978). Heller (1977) also found no difference in active tension between SHR and age matched WKY preparations. She concluded from this experiment, that neither hypertension, nor the accompanying hypertrophy, has any influence on the contractile state of the myocardium. Fein et al. (1980) measured the combined effects of renovascular hypertension and diabetes on the contractile function of the myocardium. Significant increases in both muscle weight and muscle length in both the hypertensive and hypertensive-diabetic (HD) groups compared to their respective controls were observed. They reported a significant reduction in active tension when increasing
the length of the left ventricular papillary muscle in the HD rats compared to the diabetic and control groups. This decrease was thought to be the result of a decrease in myosin ATPase, as there was a complete conversion of myosin isoenzyme V1 to the less active V3. In a more recent study by Fein et al. (1990), active length–tension properties of diabetic and control rats were compared. There were no significant differences between muscle length and muscle weight between these groups after 30 weeks. Interestingly, there was also no difference in active tension measured. The difference in all of these findings could be due to a number of variables such as the length of duration of hypertension and/or the different strains and treatments used in order to induce hypertension. In this present study, similar to Heller (1978), the elevated BP and ventricular hypertrophy observed in the DOCA group did not affect the contraction of the left ventricular papillary muscles.

After normalizing for cross-sectional area we observed a definite decrease in the force of contraction in the STZ group with increasing muscle length. Although the developed tension generated by STZ was markedly lower than all remaining groups, it was only significantly lower from CON between the preload tensions 4.5 to 5.5 g. The fact that DOCA treatment augmented the contractile performance of the STZ group compared to STZ alone was an interesting finding. It appeared that DOCA treatment somehow offset the decrease in myocardial contraction known to be prevalent in diabetes.

These results are in agreement with a study performed by Dai and McNeill (1992), who measured myocardial performance using a working heart preparation. After 12 weeks of DOCA or DOCA vehicle treatment, they discovered that at high filling pressures, the STZ group had a significantly lower rate of ventricular force
development (+dp/dt), rate of ventricular relaxation (−dp/dt), and left ventricular developed pressure (LVDP) when compared to the CON group. In addition, they also observed that DOCA-treatment did not negatively affect the contractile performance of the diabetic rats, but rather, appeared to delay the onset of myocardial dysfunction in the STZ-treated group.

In both of these experiments there were no significant differences in myocardial function between the STZ group and the STZ–DOCA group, which further indicates that DOCA–induced hypertension does not exacerbate myocardial dysfunction in STZ–diabetic rats.

CON, DOCA, and STZ–DOCA groups exhibited steady increases in contractile force with increasing muscle length, with no measurable differences between them. There was a marked, although not significant, increase in the contractile response in the STZ–DOCA group at higher preload tensions compared to CON and DOCA. One possible explanation for this finding may be because DOCA–treatment is associated with an increase in sympathetic activity (Schenk and McNeill, 1992), which is illustrated by an increase in NE turnover (Fujita et al., 1986). Therefore, if more NE is released from the nerve endings, it stands to reason that the resultant muscle contraction should be augmented, provided that there were no functional alterations at either the receptor or sub-receptor level. On the otherhand, one possible reason why the DOCA–treated animals did not display significantly elevated length–tension responses compared to the remaining groups may be due to the pharmacological effects of DOCA. It is believed that the elevation in sympathetic activity in DOCA–treated animals is secondary to the action that DOCA plays on the renin–angiotensin system. Thus, in an isolated tissue preparation, the central effects
of DOCA are obliterated, which may perhaps, in turn, diminish the elevation in sympathetic activity.

Chidsey et al. (1966) found a significant correlation between ventricular NE concentration and the maximum active tension developed by the papillary muscles of hearts undergoing mitral valve replacement. We were unable to obtain such a correlation in our experiment. The significantly elevated concentrations of NE found in the hearts of the STZ and STZ–DOCA groups were not reflected in their contractile performance. In addition, the contractile performance of the diabetic group did not indicate that DAN had occurred. If in fact there was severe structural damage of the nerve endings, the developed tension in the diabetic groups would likely have been significantly lower than their respective controls beginning at a low preload tension.

To obtain a better picture of the NE stores available to be liberated from the nerve endings of our 4 groups we applied tyramine (TYR) to the papillary tissues. TYR releases NE from the stored site that is not accessible to nerve impulses (Weiner et al., 1962). However, to be certain that the contractile response observed after the addition of TYR was not due to an alteration in the β-receptor pathway, we also applied isoproterenol (ISO) to the tissues.

It has been reported that higher concentrations of TYR are needed in order to elevate the release of endogenous NE stores from the hearts of STZ–diabetic rats (Foy and Lucas, 1978; Ganguly et al., 1987). These results suggest that there is a decrease in granular storage of NE. However, as discussed earlier, it seems that the
length of duration and severity of diabetes are the key factors in determining the cardiac stores of NE.

It is well known that STZ-diabetes is associated with a decrease in β-receptors. Savarese and Berkowitz (1979) discovered a 28% decrease in ventricular β-adrenoceptors two months after the induction of diabetes by STZ injection. In addition, a decreased β-receptor density without an alteration in receptor affinity has been reported (Heyliger et al., 1982; Ramanadham and Tenner, 1987). The explanations for the reduction in β-adrenoceptors are numerous. Some suggestions for the reduction are: increases in plasma catecholamine levels, decreases in plasma T3 levels, and impaired body weight gain (Nishio et al., 1988). Cros et al. (1986) proposed that there is a functional uncoupling of the myocardial β-receptor from adenylate cyclase activation.

Unfortunately, the results we obtained after the application of ISO and TYR to the tissue baths were not in good agreement with previous findings, and were inconclusive. After normalizing for the cross-sectional area of the muscles, there was no difference in active tension generated between the 4 groups with the addition of increasing doses of either ISO or TYR to the bath. Although we expected to find a significant decrease in papillary muscle contractility in the diabetic groups, our results were not entirely unexpected considering the order of methods carried out in this study. The drugs were added to the tissues after the length–tension experiment was completed, or after the muscles were exposed to 10 grams of preload tension. Therefore, it is possible that the diminished response that we observed was due to extensive tissue damage or fatigue, irrespective of the NE stores available for release or the receptor sensitivity. This reasoning can also be
used to explain the unusual results we found when measuring the % maximum response generated in the tissues.

4.5 MIBG Uptake and Myocardial Function

The objective of this investigation was to determine whether a relationship exists between the integrity of the cardiac adrenergic nervous system and the functional state of the myocardium in diabetes–complicated hypertension.

Our results showed that there was a diminished accumulation of MIBG in the nerve endings of STZ, DOCA and STZ–DOCA groups over a period of 12 weeks, indicating that autonomic nervous dysfunction had occurred. However, the myocardial mechanical studies demonstrated that there was a significant decrease in contractile performance only in the STZ group. Based on these findings, there are 2 main conclusions that can be made: 1) MIBG does not accurately depict structural changes that occur in adrenergic nerves, or 2) there is no significant correlation between diminished nerve integrity and cardiac function.

That MIBG uptake provides an accurate assessment of cardiac adrenergic integrity needs further consideration. In this study, there was no indication that DAN was present by 12 weeks; however, there were significant decreases in MIBG uptake in both STZ–diabetic groups. Furthermore, the fact that we could not pinpoint the exact mechanism for the progressive decreases in MIBG uptake was somewhat disconcerting. It would be interesting to conduct a study where in addition to the measurement of MIBG uptake into the diabetic tissues, a histochemical or freeze–fracture study was also performed to study the adrenergic
neurons. This would definitely help to clarify what a reduction in MIBG accumulation means to the structural integrity of the nerve. Our results do bear some similarity to those of Mantaysaari et al. (1992), who recently reported a decrease in MIBG accumulation in the hearts of diabetic patients without DAN. They could only suggest that these results were reflective of an unknown mechanism.

After 12 weeks of DOCA or DOCA vehicle treatment, the observed alterations in sympathetic function, as measured by MIBG uptake, did not appear to significantly affect the functioning of the myocardium. This might lead us to believe that under resting conditions, sympathetic activity does not markedly influence heart function. However, another distinct possibility as to why there was no correlation between adrenergic neuronal integrity and myocardial function may have been due to the length of the study. Although we measured a decrease in MIBG accumulation in DOCA, STZ, and STZ–DOCA groups over 12 weeks, it is possible that the degree to which sympathetic activity was altered in these groups was not enough to directly effect the functioning of the heart. Other investigators have reported that cardiac NE stores are significantly increased before the development of myocardial failure (Felton et al., 1982; Fushimi et al., 1988). As we too had significantly elevated cardiac NE stores in the diabetic groups, perhaps we were measuring the onset of myocardial dysfunction.

It is extremely difficult to compare the results obtained from this study to those reported in clinical studies. Clinical experiments usually consist of patients whose sympathetic nervous system has already been severely compromised (i.e., patients with severe autonomic neuropathy, Shy–Drager syndrome or idiopathic
cardiomyopathy), whereas in our experiment it was not apparent that the animals suffered from debilitating sympathetic failure.

One of the objectives of this experiment was to determine if MIBG could be used as an early marker in the diagnosis of cardiac dysfunction in diabetic animals. However, it appears that the changes in MIBG uptake and myocardial dysfunction do not occur in parallel. In similarly treated rats, Dai and McNeill (1992) found no decrease in myocardial function at 4 and 10 weeks post STZ-injection. In this study; however, at 4 and 10 weeks diabetic, the animals were shown to have a significant decrease in MIBG uptake. Therefore, in experimental diabetes, it seems that MIBG uptake alone may not be adequate to detect the development of cardiac dysfunction.
5.0 SUMMARY AND CONCLUSIONS

1. The content of MIBG uptake in STZ and STZ–DOCA animals was significantly reduced compared to the CONTROL group at 3, 6, 9, and 12 weeks.

2. Over the 12 week period, a progressive decrease in MIBG accumulation was measured in DOCA, STZ, and STZ–DOCA groups.

3. At 12 weeks, there was a significant decrease in contractile performance in the STZ group compared to CON.

4. DOCA–Salt hypertension does not appear to negatively affect the structural adrenergic integrity or the contractile performance of the diabetic rats.

5. There was an inverse relationship between cardiac NE stores and MIBG uptake in STZ and STZ–DOCA groups.

6. There was no direct correlation between the decrease in MIBG uptake, cardiac NE concentration and myocardial contractile performance, indicating that either:
   a) MIBG does not accurately indicate structural or functional changes in adrenergic nerves; or
   b) there is no relationship between diminished nerve integrity and heart function.

7. The study of MIBG uptake alone may not be adequate for early detection of myocardial dysfunction in experimental animal diabetes.
6.0 REFERENCES


