CONTRACTION OF VASCULAR SMOOTH MUSCLE IN EXPERIMENTAL DIABETES: EFFECT OF EXTRACELLULAR CALCIUM

by

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This dissertation submitted by Mary Pruitt Owen has been examined and approved by an appointed committee of the faculty of the School of Graduate Studies of the Medical College of Georgia.

The signatures which appear below verify the fact that all required changes have been incorporated and that the dissertation has received final approval with reference to content, form, and accuracy of presentation.

This dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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DEDICATION

This dissertation is gratefully dedicated to my mother, Ruby H. Owen; to my father, the late Thomas C. Owen; to Mary George Jordan Waite; and to Dan Waite, Jr., whose inspiration, support, encouragement, and love over the years have allowed me to realize my dream of a career in science.

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INTRODUCTION

A. Statement of Problem

Generalized vascular disease is the single most important factor predisposing to morbidity and mortality in patients with diabetes mellitus (Marks, 1965). Vascular disease in diabetes mellitus involves all areas of the arterial system, from arteries to arterioles and capillaries. However, the nature of the process which leads to changes in the vascular system is not completely understood. Retinopathy, nephropathy, neuropathy, and increased incidence of cardiovascular disease are long-term complications associated with diabetes (Fagerberg, 1959; Siperstein, 1968; Marble, 1976; Herman *et al.*, 1977). Despite this, little is known concerning the reactivity of the diabetic vasculature to vasopressor substances. Few studies have examined the responsiveness of diabetic blood vessels to vasoactive substances. In addition, the results of these studies have been controversial. At the present time vascular reactivity in the diabetic state remains undefined.

B. Review of the Related Literature

Microangiopathy is the general term applied to capillary changes seen in the diabetic patient. Increased deposition of basement membrane and/or basement membrane-like material seems to be the landmark of diabetic microangiopathy. The

pathogenesis of the capillary wall thickening and particularly of the basement membrane is at present unclear. Based on morphological ultrastructural studies of quadriceps muscle capillaries in prediabetic and diabetic individuals, Siperstein *et al.* (1968) have suggested that diabetic microangiopathy is independent of the hyperglycemia of this disease and may arise as a manifestation of separate genetic entity that tends to occur in diabetes. On the other hand, Williamson *et al.* (1969) by means of a different tissue fixation technique, have concluded that the thickening of quadriceps muscle capillary basement membrane and of vascular changes elsewhere are secondary to carbohydrate intolerance or insulin deficiency. There is general agreement that once diabetes has been manifested, thickening of muscle capillary basement membrane correlates with the duration of the disease (Danowski *et al.*, 1965; Østerby, 1975).

The basis for the increased deposition of basement membrane and/or basement membrane-like material is currently under intense investigation. It is well-documented that in the kidney, glomerular epithelial cells participate in the synthesis of basement-like material. The morphological hallmark of increased synthesis is the tendency towards increased numbers of cisterae in the endoplasmic reticulum of kidney epithelial cells containing basement membrane-like material in diabetes (Østerby, 1975). It also is well-documented that the mesangial cells probably lay down basement membrane-like material in the mesangial regions. Embryological

studies seem to indicate that the endothelial cells also participate in the synthesis of basement membrane material (Østerby, 1975).

Basement membrane is glycoprotein in nature. The synthesis of its carbohydrate component does not require insulin so that it is possible that, in the presence of hyperglycemia, where glucose utilization has been impaired along insulin required pathways, this sugar may be shunted in larger than normal amounts to glycoprotein formation, with the subsequent overproduction of basement membrane material (Spiro, 1970). This concept correlates the elevated blood glucose levels with the development of the microangiopathy and clarifies the overproduction of a carbohydrate-containing material in a disease where there is an underutilization of glucose.

Spiro and Spiro (1971) found in alloxan-hyperglycemic rats an increased activity of glucosyltransferase, the enzyme responsible for attaching glucose and galactose to basement membrane polypeptide. In KK mice with genetic diabetes and human-like vasculopathy, Reddi *et al.* (1975) found increased activity of glucosyltransferase and prolyl-hydroxylase, the enzyme responsible for the conversion of proline into hydroxyproline. Incorporation of the amino acid glycine into protein by the renal cortex, another step in the formation of basement membrane, also was increased in the kidney cortex before the development of abnormal oral

glucose tolerance test and glomerulosclerosis. This suggests that hyperglycemia accelerates the formation of basement membrane material, but that in genetically transmitted diabetes, other factors may play an important role.

Lazarow and Speidel (1964) investigated basement membrane turn-over in alloxanized rats by incorporation of labeled amino acids into basement membrane and found that diabetic rats retain the labeled material for longer periods of time. This finding suggests that decreased basement membrane breakdown is the mechanism responsible for basement membrane thickness.

Recently, Fushimi and Tarui (1974) studied the activity of the β-N-acetylglucosaminidase enzyme (one of the lysosomal enzymes involved in the degradation of basement membrane glycoprotein) in normal and streptozotocin-diabetic rats. Eight weeks after induction of hyperglycemia, the kidney enzyme activity was markedly decreased in the diabetic rats as compared to the controls. Accumulation of glycoproteins in the kidney is commonly observed in the diabetic state. In contrast, the serum β-N-acetylglucosaminidase activity was found to be increased. Belfiore *et al.* (1972) found the enzyme activity to be increased in serum from diabetic patients with vasculopathy. They intimate that the increased β-N-acetylglucosaminidase activity may be in response to the metabolic need of degrading glycoproteins. These findings suggest that decreased removal of basement

membrane also may play an important role in the pathogenesis of the diabetic microangiopathy.

Macroangiopathy, the term applied to changes in large and medium size arteries, is two to three times more prevalent in diabetics than nondiabetics. The pathogenesis of the macroangiopathic lesions and its relationship to the onset of diabetes are again not clearly understood. The association between hyperlipemia and especially of hypertriglyceridemia and macrovascular disease in individuals with and without hyperglycemia or glucose intolerance has been confirmed in a variety of population studies (Bierman and Brunzell, 1978). However, the mechanisms to account for this relationship have yet to be clearly elucidated.

A working hypothesis of the pathogenesis of atherosclerosis consistent with a wide variety of experimental evidence is that atheroma formation begins with "injury" to the endothelium and exposure of the subendothelial tissue to increased concentrations of plasma constituents (Ross and Glomset, 1976). Platelets adhere, form microthrombi, and release their granular contents which, in conjunction with other plasma constituents including cholesterol-rich lipoproteins (Ross and Glomset, 1973) and insulin (Stout *et al.*, 1975), stimulate migration and focal proliferation of smooth muscle cells. The multipotential arterial smooth muscle cell is the predominate cell type in the intima and media of large arteries and appears to play a fundamental

role in the pathogenesis of atherosclerosis in both humans and experimental animals (Ross and Glomset, 1973). These cells not only migrate into the intima and proliferate early in atheroma formation, perhaps as a monoclonal response (Benditt and Benditt, 1973), but they accumulate intracellular lipid in the presence of increased concentrations of extracellular lipoprotein and they deposit extracellular connective tissue proteins. As the lesion progresses, it is characterized by increased numbers of lipid-laden smooth muscle cells, increased connective tissue matrix, and accumulation of extracellular lipid. A fibrous plaque is formed that encroaches on the lumen of the artery and may calcify. The artery can ultimately rupture which may lead to thrombosis and complete occlusion.

Why should atherosclerosis be accelerated and more severe in diabetes? One explanation could be that most of the risk factors (hyperglycemia, hypertriglyceridemia, hypercholesterolemia, hypertension, hyperinsulinemia, obesity, and dietary fat and cholesterol) identified for atherosclerosis in large population studies are intensified in diabetes (Bierman and Brunzell, 1978). Also, it has been suggested that genetic diabetes mellitus in humans represents a cellular abnormality intrinsic to all cells (Goldstein, 1971; Vracko and Benditt, 1974), resulting in decreased life span and increased cell turnover. If arterial endothelial and smooth muscle cells are intrinsically defective in

diabetes, then accelerated atherogenesis can be readily postulated on the basis of current concepts of pathogenesis.

Lundback (1976) has proposed that high serum levels of growth hormone may be responsible for the vascular changes in diabetes. The growth hormone hypothesis is based on the studies by Merimee *et al.* (1970, 1973) of micro- and macro-angiopathy in growth-hormone-deficient dwarfs with diabetes and in studies of the relationship between plasma growth hormone and retinopathy in diabetic patients (Knopf *et al.*, 1972; Pasa *et al.*, 1974).

Very little is known concerning the reactivity of the diabetic vasculature to vasopressor substances. Results from the few studies which have examined the responsiveness of diabetic blood vessels to vasoactive substances have been controversial. Szentivanyi and Pek (1973) found the nor-adrenergic reactions of conjunctival vessels normally sensitive only to α-antagonists to be inhibited by β-blocking agents in diabetic patients. It has been shown that the blood vessels in the isolated perfused hindquarters of alloxan-induced diabetic rats are abnormally sensitive to the constrictor effect of epinephrine, norepinephrine, and angiotensin II (Brody and Dixon, 1964). On the other hand, it has been reported that alloxan- or streptozotocin-induced diabetic rats exhibit an increased vascular responsiveness to infused angiotensin II but not to norepinephrine (Christlieb, 1974). More recently, it has been reported

that human diabetic subjects with no clinically detectable complications respond to the same extent as control patients to the pressor effect of angiotensin II and norepinephrine. However, a group of diabetic patients with evidence of retinopathy were more sensitive to the pressor effect of angiotensin II and norepinephrine than the control group (Christlieb *et al.*, 1976). The alterations noted in vascular reactivity to various vasopressor substances in the diabetic state may, in part, be related to the severity of the vascular disease, autonomic neuropathy, or some other unknown occurrence. At the present time, insufficient data is available to completely define vascular reactivity in the diabetic state.

Many factors play a role in the regulation of vascular muscle reactivity and tone. It has been well established that calcium is critical for the initiation of contraction in vascular muscle (Bohr, 1964b). Both an intra- and extracellular source of calcium are ultimately involved in the activation of contraction in vascular muscle (Somylo and Somylo, 1968, 1970). Agonists can modify the relationship between calcium and muscle contraction in order to alter vascular reactivity. However, the primary source of calcium affected is dependent upon the contractile agonist (Hiroaka *et al.*, 1968; Hudgins and Weiss, 1968; Greenberg *et al.*, 1973) and age of the animal (Cohen and Berkowitz, 1976).

Alloxan has been the "classical" experimental diabetogenic agent. More recently streptozotocin has become

increasingly popular as a diabetogenic agent. Orci *et al.* (1976) have shown by the use of an indirect immunofluorescence technique that both streptozotocin-treated hyperglycemic rats and patients with chronic juvenile-type diabetes mellitus have a reduced volume density of insulin-containing cells (β-cells) in the islets of Langerhans, while the volume density of both glucagon-containing cells (α-cells) and somatostatin-containing cells (δ-cells) is significantly increased. They also detected, in addition to this quantitative change in the α- and the δ-cell population, a qualitative change which consisted of a redistribution of the cells from their peripheral position in control islets to the more central part of the diabetic islet. However, the topographical proximity of α- and δ-cells was still maintained. Although streptozotocin appears to be more selective in destroying the β-cells of the pancreas and associated with less general toxic effects than alloxan (Arison, 1967; Junod *et al.*, 1967; Junod *et al.*, 1969; Hoftiezer and Carpenter, 1973), a recent study by Kazumi *et al.* (1978) showed that pancreatic islet cell tumors were induced in 73% of rats surviving nine months or longer following streptozotocin treatment. These latter investigators' findings suggest that pancreatic islet cell tumors induced by streptozotocin are insulin-secreting, and that streptozotocin has oncogenic effects on the rat pancreas.

The present studies were undertaken to more clearly define vascular reactivity in the diabetic state. Initial

attempts to elucidate the mechanism of altered reactivity were also undertaken. The streptozotocin-induced diabetic rat was used as the experimental animal model and vascular reactivity was measured in the thoracic aorta of the diabetic rat. Caution must be exercised in comparing any animal model to the full syndrome of clinical diabetes in humans. Caution also must be exercised in extrapolating information gained in the thoracic aorta to other areas of the arterial tree.

MATERIALS AND METHODS

A. Experimental Animals, Animal Care, and Housing

Male Holtzman albino rats (Holtzman Laboratories, Madison, WI) were used in the reported studies. The rats were housed individually in the environmentally controlled vivarium facilities of the Medical College of Georgia and maintained on a normal laboratory diet of Wayne Lab Blox. All rats were fed and watered *ad libitum*. The birth date and body weight of each animal were recorded on the arrival date.

B. Solutions

1. Ortho-Toluidine Glucose Determination Solutions

   a. Glucose "Stock" Standard Solution

      Glucose stock standard solution consisted of 10 mg anhydrous dextrose per ml of an aqueous 0.1% benzoic acid solution. This solution is stable for three years or longer when stored at -20°C.

   b. Glucose "Dilute" Standard Solutions

      Solutions of 50, 100, 150, 200, 250, and 300 mg per deciliter glucose were prepared from the stock standard solution. First the stock solution was brought to 25°C, then measured, and diluted with 0.1% benzoic acid to the volumes

indicated in Table 1. These solutions remain stable for several months when stored at 4°C.

c. Protein Precipitation Reagent

Trichloroacetic acid (CCl_3COOH) in a concentration of 3.0% w/v was used to precipitate serum protein. This solution is stable indefinitely at room temperature.

d. Color Reagent

The ortho-toluidine reagent (Sigma) used in the glucose determination consisted of a dilution of 6% (v/v) ortho-toluidine in glacial acetic acid containing thiourea.

2. Histological Solutions

a. Bouin's Fixative Fluid

Bouin's fluid, which was used as a tissue fixative, consisted of the following ingredients: picric acid (saturated aqueous solution), 75 ml; formalin, 25 ml; and glacial acetic acid, 5 ml.

b. Mayer's Hematoxylin Stain

Mayer's hematoxylin stain, which was used as a powerful nuclear stain, was composed of the following constituents: hematoxylin crystals, 1.0 gm; distilled water, 1000.0 ml; sodium iodate, 0.2 gm; ammonium alum, 50.0 gm; citric acid, 1.0 gm; and chloral hydrate, 50.0 gm.

TABLE 1

Glucose Dilute Standard Solutions

Ml Glucose Stock (10 mg/ml)	Total Volume (Dilute with 0.1% Benzoic Acid)	Final Glucose Concentration (mg/deciliter)
5	100	50
5	50	100
15	100	150
10	50	200
25	100	250
15	50	300

c. "Stock" Alcoholic Eosin Stain (1%)

The 1% stock alcoholic eosin stain was prepared by dissolving 1.0 gm of eosin Y (water soluble) in 20.0 ml of distilled water and then adding 80.0 ml of 95% alcohol.

d. "Working" Eosin Stain

The working eosin stain solution, which was used as a counterstain for the hematoxylin stain, was composed of one part of eosin stock solution and three parts of 80% alcohol with 0.5 ml of glacial acetic acid added to each 100 ml of stain solution just before use.

3. Organ Bath Solutions

a. $CaCl_2$ (2.50 mM) Organ Bath Solution

The composition of the solution containing 2.50 mM $CaCl_2$ used in the organ bath procedures is given in Table 2.

b. Altered $CaCl_2$ Organ Bath Solutions

The altered $CaCl_2$ organ bath solutions were prepared by a substitution of either 0.20, 0.40, or 0.80 mM $CaCl_2$ for 2.50 mM $CaCl_2$ in the solution formulated from Table 2.

4. Efflux Solutions

a. $CaCl_2$ (2.50 mM) Efflux Solution

This 2.50 mM $CaCl_2$ solution was identical to solution 3a above.

TABLE 2

$CaCl_2$ (2.50 mM) Organ Bath Solution Formula

Constituents	mM/Liter
NaCl	122
KCl	4.73
$NaHCO_3$	15.5
KH_2PO_4	1.19
$MgCl_2$	1.19
$CaCl_2$	2.5
Glucose	11.5
Na_2Ca-EDTA	0.026

b. $CaCl_2$-Free Efflux Solution

The 0 mM-$CaCl_2$ solution was prepared by an omission of $CaCl_2$ from the 2.5 mM $CaCl_2$ efflux solution.

c. $CaCl_2$-Free Plus Ethyleneglycol Tetraacetic Acid (EGTA) Solution

The 0 mM-$CaCl_2$ plus EGTA solution was prepared by an omission of $CaCl_2$ from, and an addition of EGTA (0.01 mM) to the 2.5 mM $CaCl_2$ efflux experiment solution.

C. Experimental Procedures

1. Induction of Experimental Diabetes

Diabetes was chemically induced with streptozotocin (Upjohn) in 42 to 43 day old male rats. The animals received a single I.V. injection of 65 mg/kg streptozotocin which was dissolved in saline acidified to pH 4.5 with citrate. Rats which were used as control animals were of the same sex and age and were injected with the vehicle (acidified saline solution). In addition, a series of control rats (age and sex matched) which received no type of injection were used. Preliminary results indicated no difference between the control group of rats which received an I.V. injection of the vehicle and the control rats which received no injection. Therefore, only the results obtained with the control group which received an I.V. injection

of the acidified saline solution are reported in the studies.

2. Determination of Hyperglycemia

Diabetes was judged by the classical criterion of hyperglycemia. The serum glucose level in each non-fasted animal was measured by the o-toluidine method (Dubowski, 1962) at the time of sacrifice. This method, based upon the condensation of glucose with primary aromatic amines in glacial acetic acid, is specific for aldohexoses. The reaction becomes essentially an equilibrium mixture of glycosylamine and the corresponding Schiff base. Since glucose is the predominantly abundant aldohexose in blood, the method is suitable for the measurement of glucose levels.

3. Histological Procedures

The rats were sacrificed by decapitation 31 weeks after injection. Samples of pancreas were removed from diabetic and control rats. These samples were then fixed in Bouin's solution, embedded in paraffin, stained with Mayer's hematoxylin, and counterstained with alcoholic eosin (Luna, 1968).

4. Organ Bath Procedures

a. Isolation of Aorta Vascular Tissue

The rats were sacrificed by decapitation either 14-20 or 28-35 days after injection. A section of

the thoracic aorta between the aortic arch and the diaphram was removed and placed in an oxygenated bathing solution. The aortae were cleaned of all excess fat and connective tissue and cut into 0.5 cm rings. Only one aortic ring was used from each animal. The vessels were placed on the tips of two 30 gauge stainless steel hypodermic needles which were bent into an L-shape. The needles then were separated so that the lower one was attached to a stationary glass rod and the upper one was attached to a Grass FT-03 force-displacement transducer. This procedure for ring preparation of blood vessels has been described by Hooker *et al.* (1977). Isometric contractions were recorded with a Beckman Type R411 dynograph.

Vessels were mounted in organ baths containing the 2.50 mM $CaCl_2$ organ bath solution (Table 2). Tissue bath solutions were oxygenated continuously (97% 0_2-3% $C0_2$) and maintained at a constant temperature of 37°C. The muscle preparations were equilibrated for 90 min under a resting force of 2 g. During the equilibration period the tissues were washed with fresh oxygenated solution every 30 min to prevent the accumulation of metabolic

end-products which have been shown to produce differential effects on drug induced contractions (Altura and Altura, 1970).

b. Protocol for Experiments Using Norepinephrine (NE) as Contractile Agonist

(1) Concentration-Effect Experiments

At the end of the initial equilibration period, a concentration effect relationship in 2.5 mM Ca^{++} organ bath solution was obtained for NE in the following four different groups of isolated aortic rings:

(a) from rats 14-20 days after vehicle injection (control),

(b) from rats 14-20 days after streptozotocin injection (diabetic),

(c) from rats 28-35 days after vehicle injection (control),

(d) from rats 28-35 days after streptozotocin injection (diabetic).

Contractile responses to increasing concentrations of NE were obtained by a cumulative increase in the total concentration of NE in the bath. The tissue was allowed to be in contact with NE for 2 min before each successive concentration of NE was added. The

concentration range of NE employed was 1×10^{-10} M to 3×10^{-6} M. After the completion of the first concentration-effect curve, which was performed in 2.50 mM Ca^{++}, the medium was replaced with one of a different Ca^{++} concentration. The tissues were washed two times at 15 min intervals for 30 min and then allowed to re-equilibrate for 30 min in the altered Ca^{++} solution before the NE concentration-effect curve was obtained again. The sequence of the different Ca^{++} concentrations used were 2.50 mM, 0.20 mM, 0.40 mM, and 0.80 mM Ca^{++}. For all the different Ca^{++} concentrations used, the complete concentration-effect curve for NE was determined on the different groups of tissues.

c. Protocol for Experiments Using Potassium Chloride (KCl) as Contractile Agonist

(1) Concentration-Effect Experiments

At the end of the initial equilibration period, a concentration-effect relationship in 2.5 mM Ca^{++} organ bath solution was obtained for KCl in the following four different groups of isolated aortic rings:

(a) from rats 14-20 days after vehicle injection (control),

(b) from rats 14-20 days after streptozotocin injection (diabetic),

(c) from rats 28-35 days after vehicle injection (control),

(d) from rats 28-35 days after streptozotocin injection (diabetic).

Contractile responses to increasing concentrations of KCl were obtained by a cumulative increase in the total concentration of KCl in the bath. The tissue was allowed to be in contact with KCl for 2 min before each successive concentration of KCl was added. The concentration range of KCl employed was 1×10^{-3} M to 1×10^{-1} M.

After the completion of the first concentration effect curve, which was performed in 2.50 mM Ca^{++}, the medium was replaced with one of 0.20 mM Ca^{++} concentration. The tissues were washed two times at 15 min intervals for 30 min and then allowed to re-equilibrate for 30 min in the 0.20 mM Ca^{++} solution before the KCl

concentration-effect curve was obtained again. For both the 2.50 mM and 0.20 mM Ca^{++} concentrations used, the complete concentration-effect curve for KCl was determined on the different groups of tissues.

d. Protocol for Experiments Using Serotonin (5-HT) as Contractile Agonist

(1) Concentration-Effect Experiments

At the end of the initial equilibration period, a concentration-effect relationship in 2.50 mM Ca^{++} organ bath solution for 5-HT was obtained in the following four different groups of isolated aortic rings:

(a) from rats 14-20 days after vehicle injection (control),

(b) from rats 14-20 days after streptozotocin injection (diabetic),

(c) from rats 28-35 days after vehicle injection (control),

(d) from rats 28-35 days after streptozotocin injection (diabetic).

Contractile responses to increasing concentrations of 5-HT were obtained by a cumulative increase in

the total concentration of 5-HT in the bath. The tissue was allowed to be in contact with 5-HT for 2 min before each successive concentration of 5-HT was added. The concentration range of 5-HT employed was 1×10^{-7} M to 3×10^{-4} M.

After the completion of the first concentration-effect curve, which was performed in 2.50 mM Ca^{++}, the medium was replaced with one of 0.20 mM Ca^{++} concentration. The tissues were washed two times at 15 min intervals for 30 min and then allowed to re-equilibrate for 30 min in the 0.20 mM Ca^{++} solution before the 5-HT concentration-effect curve was obtained again. For both the 2.50 mM and 0.20 mM Ca^{++} concentrations used, the complete concentration-effect curve for 5-HT was determined on the different groups of tissues.

(2) Serotonin-Induced Contraction, Isoproterenol-Induced Relaxation Experiments

At the end of the initial equilibration period, the tissues were bathed

in a 2.50 mM Ca^{++} organ bath solution containing phentolamine (PA) (1.57 x 10^{-6} M), an α-receptor blocking agent, for 1 hr. Since Fleisch and Hooker (1976) reported the best relaxation obtained in the normal rat thoracic aortae was after a serotonin-induced contraction (EC85) the tissues then were contracted with 5-HT (EC85) and a concentration-relaxant effect relationship in the 2.50 mM Ca^{++} plus PA solution was obtained for isoproterenol (ISO) in the following two groups of isolated aortic rings:

(a) from rats 28-35 days after vehicle injection (control),

(b) from rats 28-35 days after streptozotocin injection (diabetic).

Relaxant responses to increasing concentrations of ISO were obtained by a cumulative increase in the total concentration of ISO in the bath after the tissues first had been contracted with 5-HT. The tissue was allowed to be in contact with ISO for 2 min before each successive concentration of

ISO was added. The concentration range of ISO employed was 1×10^{-10} M to 1×10^{-5} M.

e. Protocol for Experiments Using Phenylephrine (PE) was Contractile Agonist

(1) Concentration-Effect Experiments in 2.50 mM Ca^{++} Before and After α-Receptor Blockade with Phentolamine

At the end of the initial equilibration period, a concentration-effect relationship in 2.50 mM Ca^{++} organ bath solution for PE was obtained in the following two different groups of isolated aortic rings:

(a) from rats 28-35 days after vehicle injection (control),

(b) from rats 28-35 days after streptozotocin injection (diabetic).

Contractile responses to increasing concentrations of PE were obtained by a cumulative increase in the total concentration of PE in the bath. The tissue was allowed to be in contact with PE for 2 min before each successive concentration of PE was added. The concentration range of PE employed was 4.9×10^{-10} M to 1.5×10^{-5} M.

After the completion of the first concentration-effect curve, which was performed in 2.50 mM Ca^{++}, the tissues were washed two times at 15 min intervals for 30 min. The medium then was replaced with one of 2.5 mM Ca^{++} containing PA (1.57×10^{-6} M), an α-receptor blocking agent. The tissues were allowed to re-equilibrate for 60 min in the solution containing PA before the PE concentration-effect curve was obtained again.

The affinity constant (pD_2) for PE was calculated from the concentration-effect curve as described by Van Rossum (1963). The pD_2 value is a direct measure of the affinity of the agonist, PE, for the receptor. The affinity (pA_2) of the competitive antagonist, PA, also was calculated according to the procedure of Van Rossum (1963). The pA_2 value is related to the negative logarithm of the molar concentration of the competitive antagonist which causes a shift of the control

concentration-effect curve for the agonist by a factor of 2.

(2) Concentration-Effect Experiments in 0.20 mM Ca^{++} Before and After α-Receptor Blockade with Phentolamine

At the end of the initial equilibration period, a concentration-effect relationship in 0.20 mM Ca^{++} organ bath solution was obtained for PE in the following two different groups of aortic rings:

(a) from rats 14-20 days after vehicle injection (control),

(b) from rats 14-20 days after streptozotocin injection (diabetic).

Contractile responses to increasing concentrations of PE were obtained by a cumulative increase in the total concentration of PE in the bath. The tissue was allowed to be in contact with PE for 2 min before each successive concentration of PE was added. The concentration range of PE employed was 4.9×10^{-10} M to 1.5×10^{-5} M.

After the completion of the first concentration-effect curve, which was

performed in 0.20 mM Ca^{++}, the tissues were washed two times at 15 min intervals for 30 min. The medium was then replaced with one of 0.20 mM Ca^{++} containing PA (1.57×10^{-6} M), an α-receptor blocking agent. The tissues were allowed to re-equilibrate for 60 min in the solution containing PA before the PE concentration-effect curve was obtained again.

The pD_2 value for PE and the pA_2 value for PA were calculated from the concentration-effect curves by the procedures of Van Rossum (1963).

5. ^{45}Ca Efflux Experiments

a. Isolation of Aorta Vascular Tissue

The rats were sacrificed by decapitation 28-35 days after injection. A section of the thoracic aorta between the aortic arch and the diaphram was removed and placed in an oxygenated bathing solution. The aortae were cleaned of all excess fat and connective tissue and cut into 0.5 cm rings. Only one aortic ring was used from each animal. A 2 g weight was tied on each ring and the rings were suspended in a 2.50 mM Ca^{++} concentration solution. All tissue bath solutions were

oxygenated continuously (97% O_2-3% CO_2) and maintained at a constant temperature of 37°C. The muscle preparations were equilibrated for 90 min in 2.50 mM Ca^{++} efflux solution. During the equilibration period the tissues were transferred to a fresh oxygenated solution every 30 min to prevent the accumulation of metabolic end-products.

b. ^{45}Ca Loading and Efflux

At the end of the 90 min initial equilibration period the aortae were placed in a O-Ca^{++} solution with added $^{45}Ca^{++}$ (0.08-0.57 µM Ca^{++}, specific activity 0.26 µCi/ml), where they remained for a 60 min loading period. To prevent the accumulation of metabolic end-products during the loading period, the tissues were transferred to a fresh oxygenated solution 30 min after being placed in the solution. After the 60 min loading period, the aortae were dipped rapidly two times in a O-Ca^{++} solution and then transferred to tubes containing 3 ml of an aerated nonradioactive O-Ca^{++} plus EGTA solution such that the tissues entered a tube containing fresh O-Ca^{++} plus EGTA solution every 2 min for 60 min. The O-Ca^{++} plus EGTA solution was utilized to minimize the effects of $^{45}Ca^{++}$ re-uptake

(Goodman and Weiss, 1971; Goodman *et al.*, 1972) on the loss of $^{45}Ca^{++}$ and, in this manner, accentuate net efflux.

On the completion of the washout, each muscle was blotted lightly and placed in 1 ml of Soluene-100 (Packard) to solubilize the tissue. Aliquots of 0.1 ml were removed from each of the washout tubes. The solubilized tissue sample and each of the 30 aliquots for each tissue were diluted with 10 ml of Dimilume-30 (Packard) and radioactivity determined on a Packard Tri-Carb liquid scintillation spectrophotometer.

Washout data were expressed as the percentage of radioactivity remaining in the muscle after each 2 min interval and were plotted as desaturation curves (Bianchi, 1965; Weiss, 1966).

6. Body Weight and Heart Weight Experiments

The rats were weighed and sacrificed by decapitation either 14-20 or 28-35 days after injection. The heart was removed and wet weights obtained for the whole heart, the right ventricle, and the left ventricle. After drying in a Thelco drying oven to a stable weight, dry weights were determined for the two atria, the right ventricle, and the left ventricle. Calculated heart dry weight was obtained by the addition of the dry weights of the

two atria, the right ventricle, and the left ventricle.

D. Drug and Isotope Sources

The following drugs and isotope were used in these reported studies: streptozotocin (Upjohn, Kalamazoo, MI); l-norepinephrine bitartrate, serotonin creatinine sulfate, l-phenylephrine hydrochloride, and ethyleneglycol-bis(β-aminoethyl ether)N,N'-tetraacetic acid (Sigma, St. Louis, MO); phentolamine hydrochloride (Ciba Pharmaceutical, Summit, NJ); calcium chloride dihydrate and potassium chloride (Fisher Scientific, Fairlawn, NJ); and ^{45}Ca (New England Nuclear, Boston, MA).

E. Statistical Analysis

The results of the control and experimental groups were compared by the Student's t-test for unpaired data. Results within each group obtained by varying the calcium concentration were compared by the Student's t-test for paired data. Results of control groups in response to different drugs were compared by a one-way analysis of variance (anova). A one-way anova was also used to compare the results of diabetic groups in response to different drugs. A two-way anova was used to analyze the response of both control and diabetic groups to different drugs. The Student-Newman-Keuls (SNK) procedure was used

to measure differences among the means of responses to different drugs. A p-value of less than 0.05 was accepted as a significant difference. The EC50 values were calculated from the cumulative concentration-effect curves and expressed as the log EC50 (Fleming *et al.*, 1972).

F. Abbreviations and Symbols

The following abbreviations and symbols are being used throughout this dissertation:

NE - norepinephrine

KCl - potassium chloride

5-HT - 5-hydroxytryptamine (serotonin)

PE - phenylephrine

PA - phentolamine

ISO - isoproterenol

EDTA - ethylenediamine tetraacetic acid

EGTA - ethyleneglycol tetraacetic acid

min - minute

hr - hour

α - alpha

β - beta

δ - delta

g - gram

ml - milliliter

cm - centimeter

°C - degrees of temperature

% - percent

w/v - weight/volume

v/v - volume/volume

μM - micromolar (micromoles/liter)

mM - millimolar (millimoles/liter)

M - molar (moles/liter)

I.V. - intravenous

EC50 - median dose (effective concentration 50)

EC85 - effective concentration 85

S.E.M. - standard error of the mean

anova - analysis of variance

SNK - Student-Newman-Keuls

RV - right ventricle

LV - left ventricle

pD_2 - affinity constant for agonist

pA_2 - affinity constant for competitive antagonist

Wt. - weight

RESULTS

A. Serum Glucose Levels in Diabetic (Streptozotocin Injected) and Control (Vehicle Injected) Rats

The serum glucose level in each non-fasted animal was measured by the o-toluidine method (Dubowski, 1962) at the time of sacrifice. The serum glucose level was significantly elevated as early as 24 hrs following the single injection of streptozotocin (65 mg/kg). Blood glucose levels were 400 mg% or greater for the streptozotocin injected rats compared to a mean of 148 mg% for the control animals.

B. Histological Examination of Pancreatic Tissue Obtained from Diabetic (Streptozotocin Injected) and Control (Vehicle Injected) Rats

Examination of pancreatic tissue from 37 week old rats which had been diabetic for 31 weeks revealed almost complete disappearance of the cells of the islet of Langerhans which could be observed in pancreatic tissue from control rats of the same age (Figures 1-4).

C. Contractile Responses of Aortae Obtained from Diabetic (Streptozotocin Injected) and Control (Vehicle Injected) Rats to Contractile Agonists

1. Contractile Responses to Norepinephrine

a. Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 14-20 Days following Injection

Figure 1. *Light micrograph: Low magnification of pancreatic tissue obtained from a 37 week old control rat*

The arrows indicate islets of Langerhans which are irregularly scattered among numerous serous glandular acini. Hematoxylin and Eosin, X100.

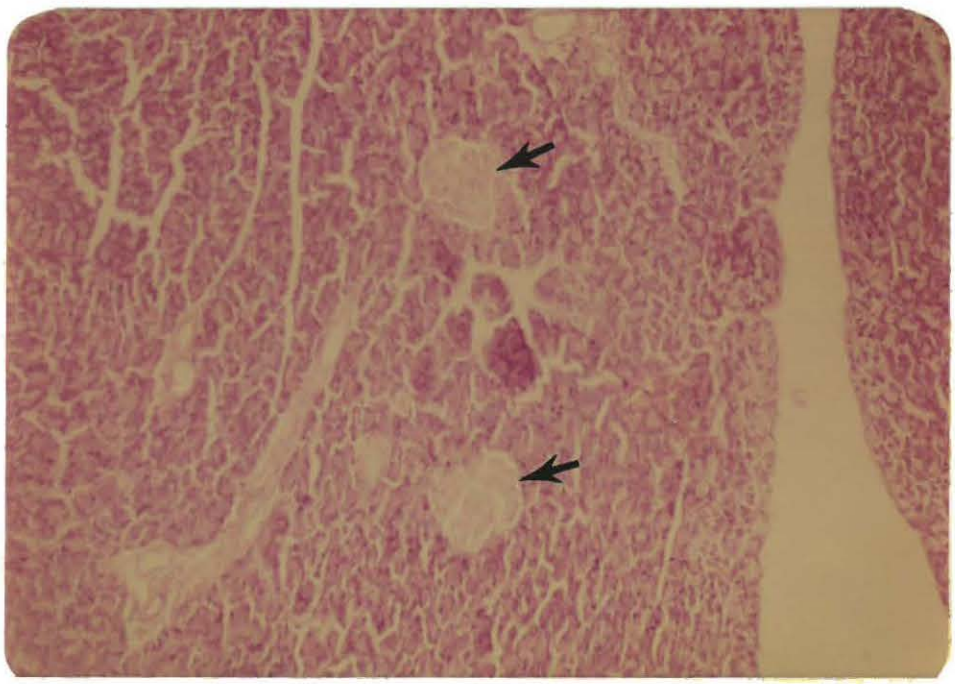


Figure 2. *Light micrograph: Low magnification of pancreatic tissue obtained from a 37 week old rat which had been diabetic for 31 weeks*

The arrows indicate islets of Langerhans which appear to be greatly decreased in volume. Hematoxylin and Eosin, X100.

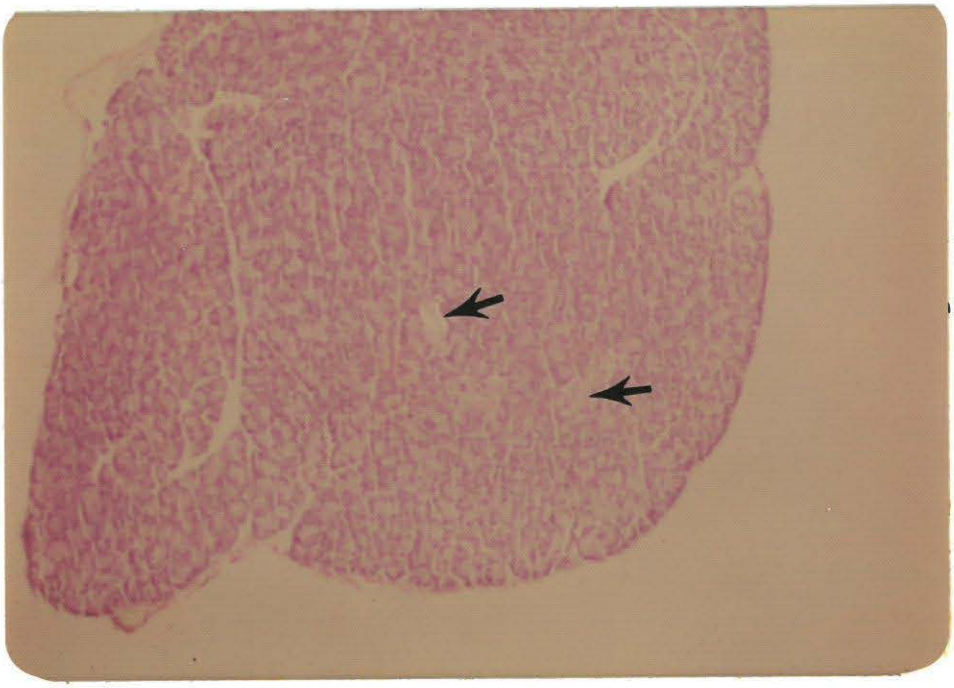


Figure 3. *Light micrograph: Higher magnification of pancreatic tissue obtained from a 37 week old control rat*

The arrows indicate the islets of Langerhans which are irregularly scattered among numerous serous glandular acini. Hematoxylin and Eosin, X400.

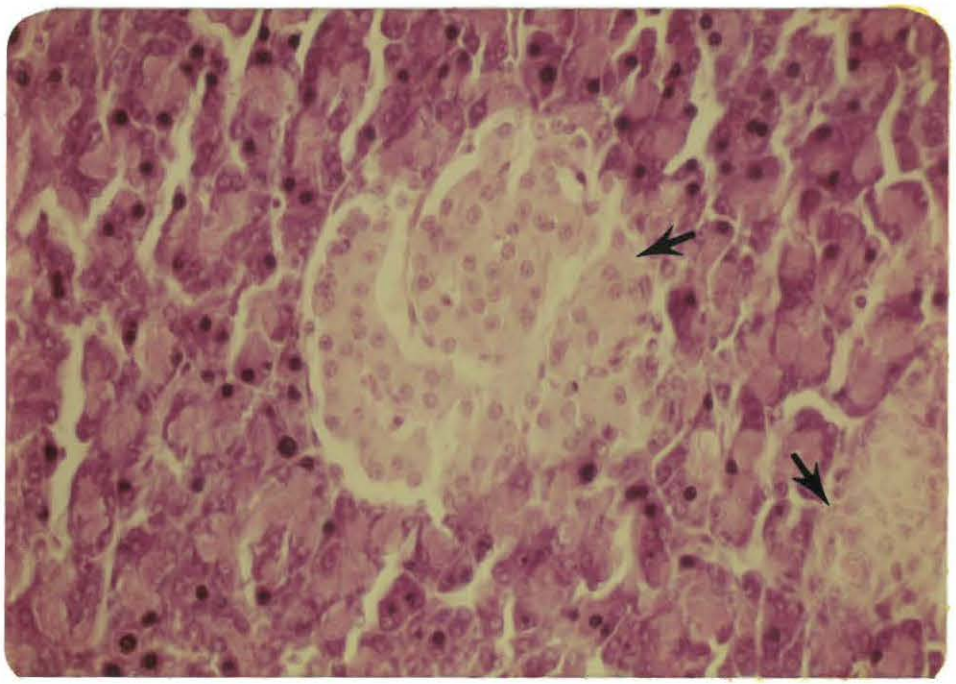
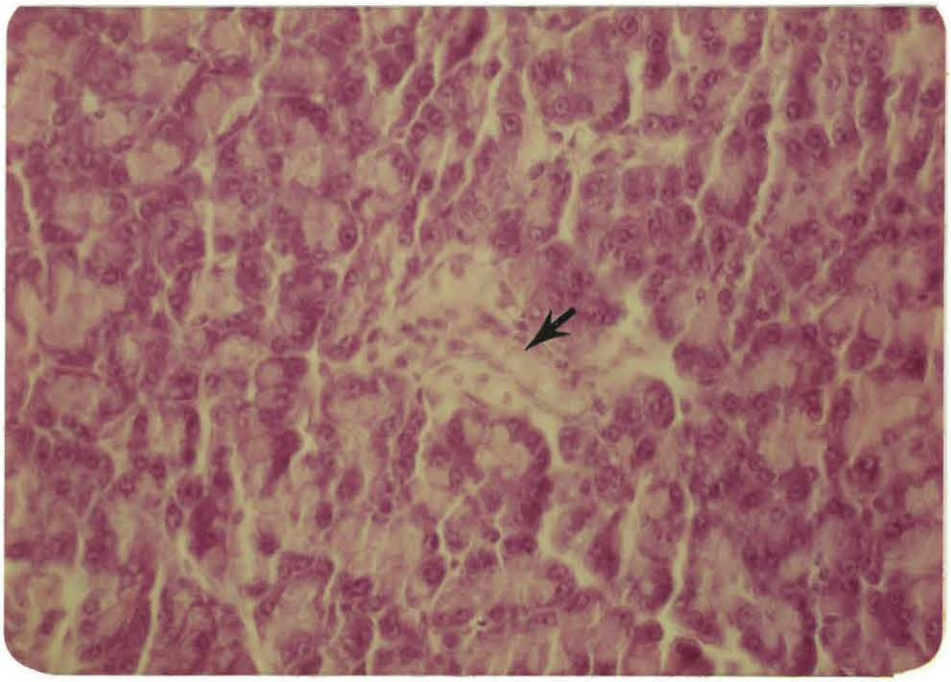


Figure 4. *Light micrograph: Higher magnification of pancreatic tissue obtained from a 37 week old rat which had been diabetic for 31 weeks*

The arrow indicates an islet of Langerhans which appears to be greatly decreased in volume. Hematoxylin and Eosin, X400.



Isolated aortic rings from control and diabetic rats contracted in response to NE over the concentration range of 1×10^{-10} M to 3×10^{-6} M in 2.50 mM Ca^{++}. As illustrated in panel D of Figure 5 and in Figure 6, there was no significant difference in the sensitivity of the diabetic rings (14-20 days) in response to NE when compared to the sensitivity of the control rings. The calculated mean log EC50 values were not significantly different in 2.50 mM Ca^{++} (Figure 6). In addition the maximum contractile force (mg force/mg tissue) developed in response to NE by the diabetic aortae was not significantly different from the maximum contractile force developed by the control aortae in the presence of 2.50 mM Ca^{++} (Figure 7).

b. Contractile Responses in Altered Extracellular Ca^{++} Concentration which Were Obtained from Rats 14-20 Days following Injection

Panels A, B, and C of Figure 5 illustrate the concentration-effect curves for NE in control and diabetic aortae (14-20 days) in the presence of 0.20, 0.40, and 0.80 mM Ca^{++}. The diabetic aortae were supersensitive to NE when the extracellular Ca^{++} concentration was

Figure 5. *Norepinephrine concentration-effect curves of aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of different external Ca^{++} concentrations (0.20, 0.40, 0.80, and 2.50 mM)*

Panels A, B, C, and D correspond to concentration-effect curves for NE in the presence of 0.20, 0.40, 0.80, and 2.50 mM Ca^{++}, respectively. Each point represents the mean of 6-9 different aortic rings. The vertical bars represent the standard error of the mean (S.E.M.).

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.

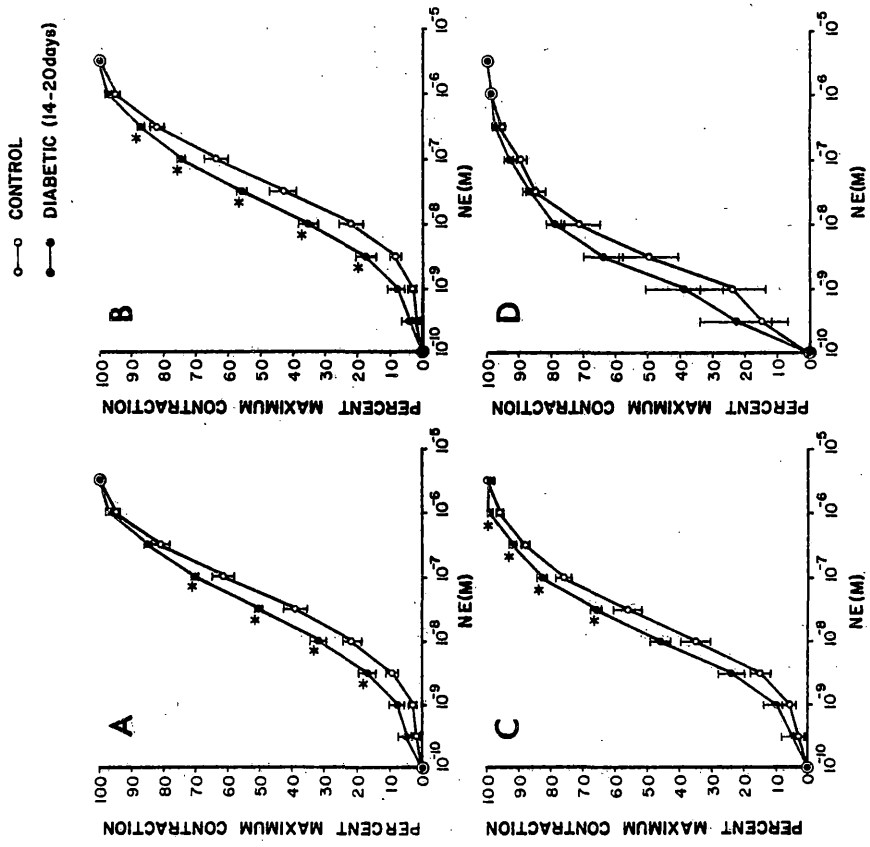

CONTROL
DIABETIC (14-20days)
A
B
C
D
PERCENT MAXIMUM CONTRACTION
NE(M)
0
10
20
30
40
50
60
70
80
90
100
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}

Figure 6. *Mean log EC50 for norepinephrine determined in aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of different external Ca^{++} concentrations (0.20, 0.40, 0.80, and 2.50 mM)*

Each point represents the mean of 6-9 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.

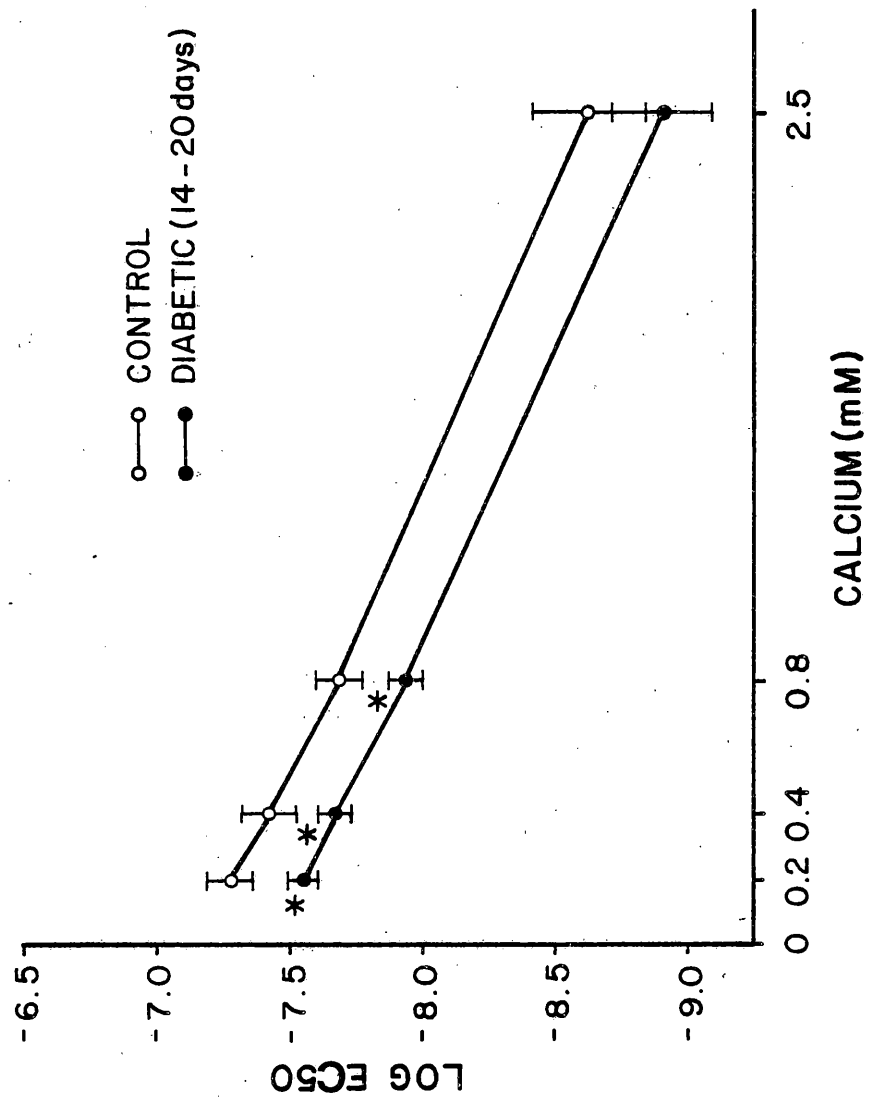
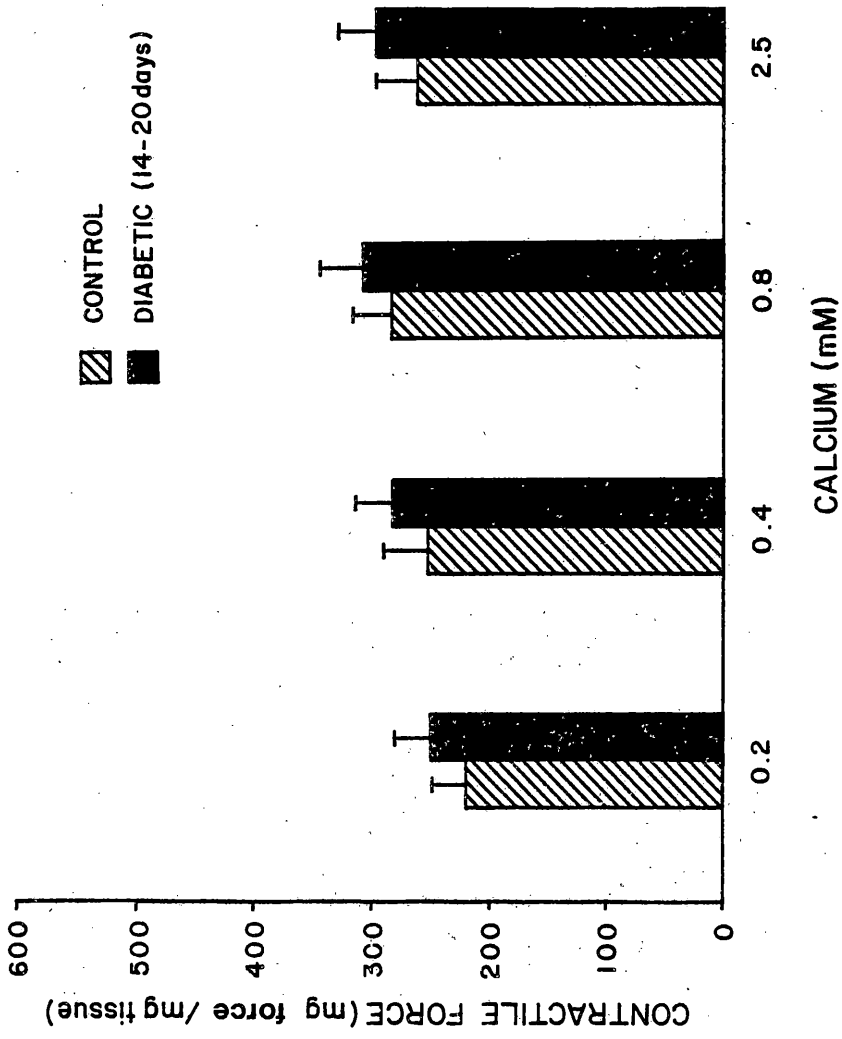

CONTROL
DIABETIC (14-20days)
LOG EC50
-6.5
-7.0
-7.5
-8.0
-8.5
-9.0
0
0.2
0.4
0.8
2.5
CALCIUM (mM)
*
*
*

Figure 7. *Maximum contractile force (mg force/mg tissue) in response to norepinephrine determined in aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of different external Ca^{++} concentrations (0.20, 0.40, 0.80, and 2.50 mM)*

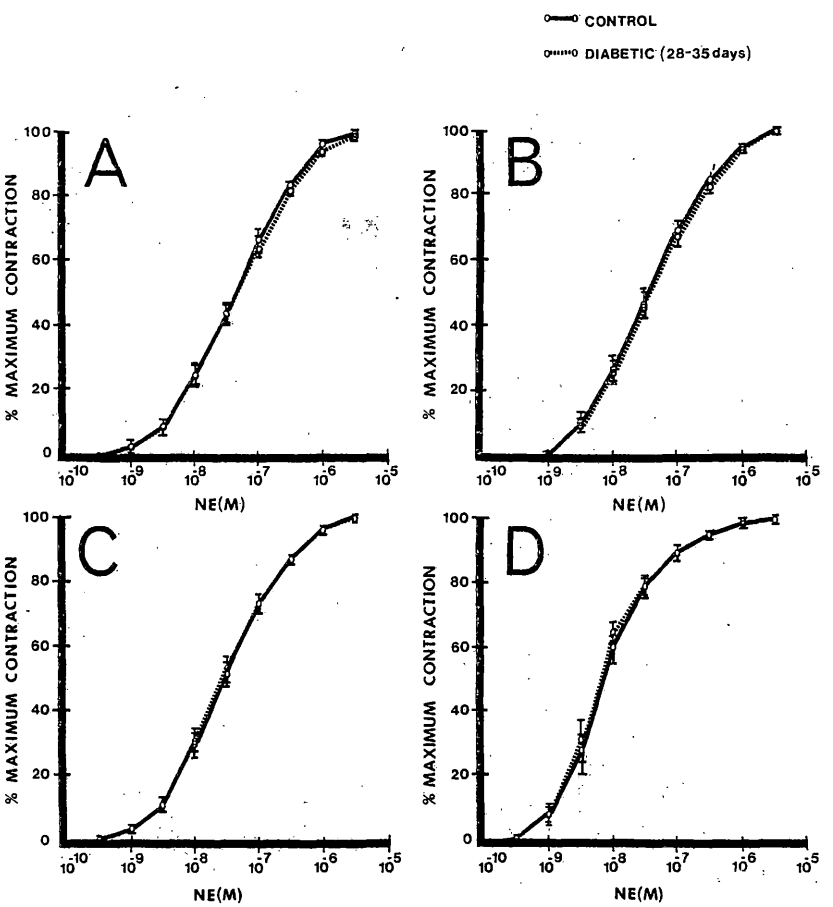
Each bar represents the mean of 6-9 different aortic rings. The vertical lines represent the S.E.M. There was no significant difference between diabetic and control tissues.


CONTRACTILE FORCE (mg force /mg tissue)
600
500
400
300
200
100
0
0.2
0.4
0.8
2.5
CALCIUM (mM)
CONTROL
DIABETIC (14-20 days)

reduced. This was evident by the significant ($p < 0.05$) decrease in the log EC50 values for aortae from the diabetic rats (Figure 6). In the presence of 0.20 mM Ca^{++} the log EC50 of the diabetic tissues was 7.54 $\pm$ 0.05 compared to a log EC50 of -7.27 $\pm$ 0.08 for the control tissues. In the presence of 0.40 mM Ca^{++} the log EC50 of the diabetic tissues was -7.68 $\pm$ 0.05 compared to a log EC50 of -7.42 $\pm$ 0.10 for the control tissues and in the presence of 0.80 mM Ca^{++} the diabetic tissue log EC50 was -7.93 $\pm$ 0.06 compared to a log EC50 of -7.67 $\pm$ 0.09 for the control tissue. The maximum contractile force developed by the 14-20 day diabetic aortae in response to NE was not significantly different from control aortae in the presence of 0.20, 0.40, and 0.80 mM Ca^{++}, as was the case in 2.50 mM Ca^{++} (Figure 7).

c. Contractile Responses in 2.50 mM Ca^{++} and in Altered Extracellular Ca^{++} Concentration which Were Obtained 28-35 Days following Injection

Figure 8 illustrates the concentration-effect curves for NE from control and diabetic aortae (28-35 days) in the presence of 0.20, 0.40, 0.80, and 2.50 mM Ca^{++}. The determined log EC50 values for NE of the diabetic aortae (28-35 days) in


CONTROL
DIABETIC (28-35 days)
A
B
C
D
% MAXIMUM CONTRACTION
NE(M)
0
20
40
60
80
100
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}

the presence of the various extracellular Ca^{++} concentrations were not significantly different from the values of the corresponding age-matched control aortae (Figure 9). Rats which were diabetic for 28-35 days did not show an increase in sensitivity to NE in the presence of 2.50 mM or an altered level of extracellular Ca^{++} concentration. When the response to NE, however, was expressed in terms of force developed by the aortae, the 28-35 day diabetic aortae developed a significantly greater force in response to the neurotransmitter than the control aortae. Figure 10 illustrates the maximum force developed by the control and diabetic (28-35 days) aortae in response to NE in the presence of various external Ca^{++} concentrations. Both in the presence of 2.50 mM and altered (0.20, 0.40, 0.80 mM) extracellular Ca^{++} concentration, the maximum contractile force developed to NE was significantly ($p < 0.05$) greater in the 28-35 day diabetic aortae than in the corresponding control tissues.

d. Maximum Contractile Response as a Function of Age and Duration of Diabetes

Since all rats were 42-43 days old at the time of injection, they were either 8 to 9 weeks

Figure 9. *Mean log EC50 for norepinephrine determined in aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of different external Ca^{++} concentrations (0.20, 0.40, 0.80, and 2.50 mM)*

Each point represents the mean of 8-9 different aortic rings. The vertical bars represent the S.E.M. There was no significant difference between diabetic and control tissues.

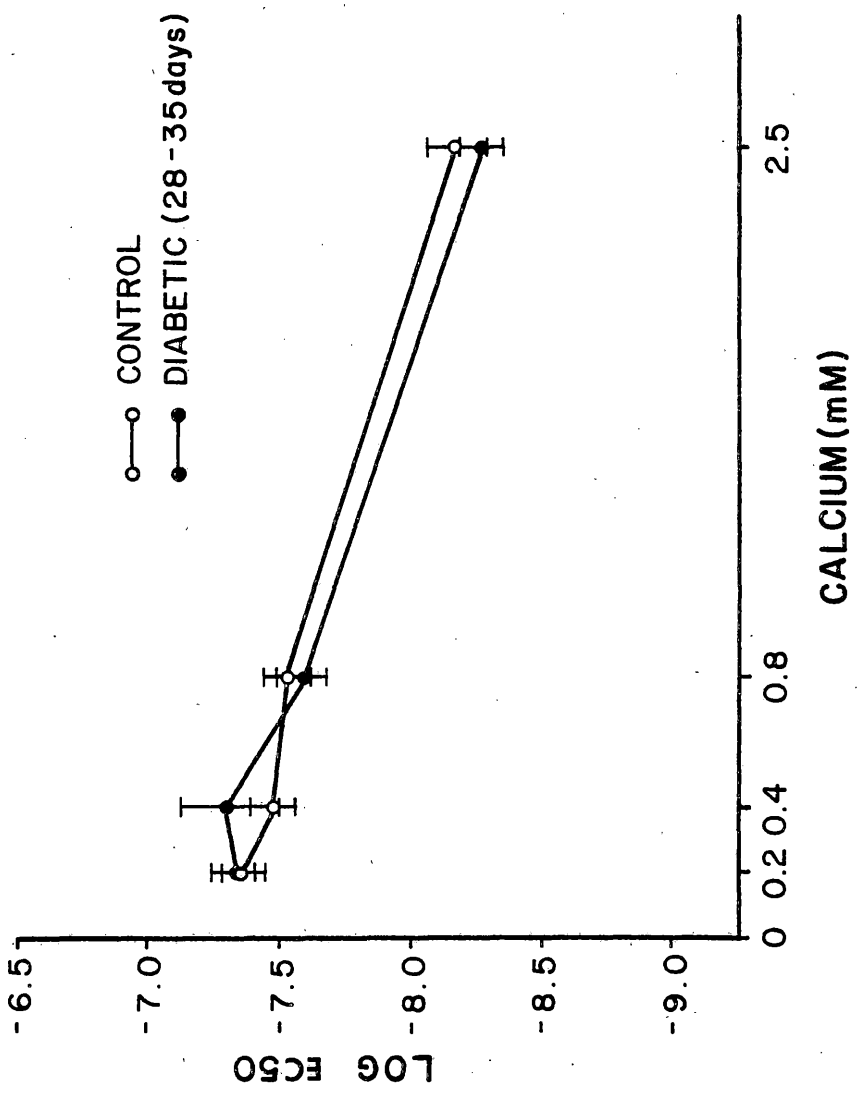
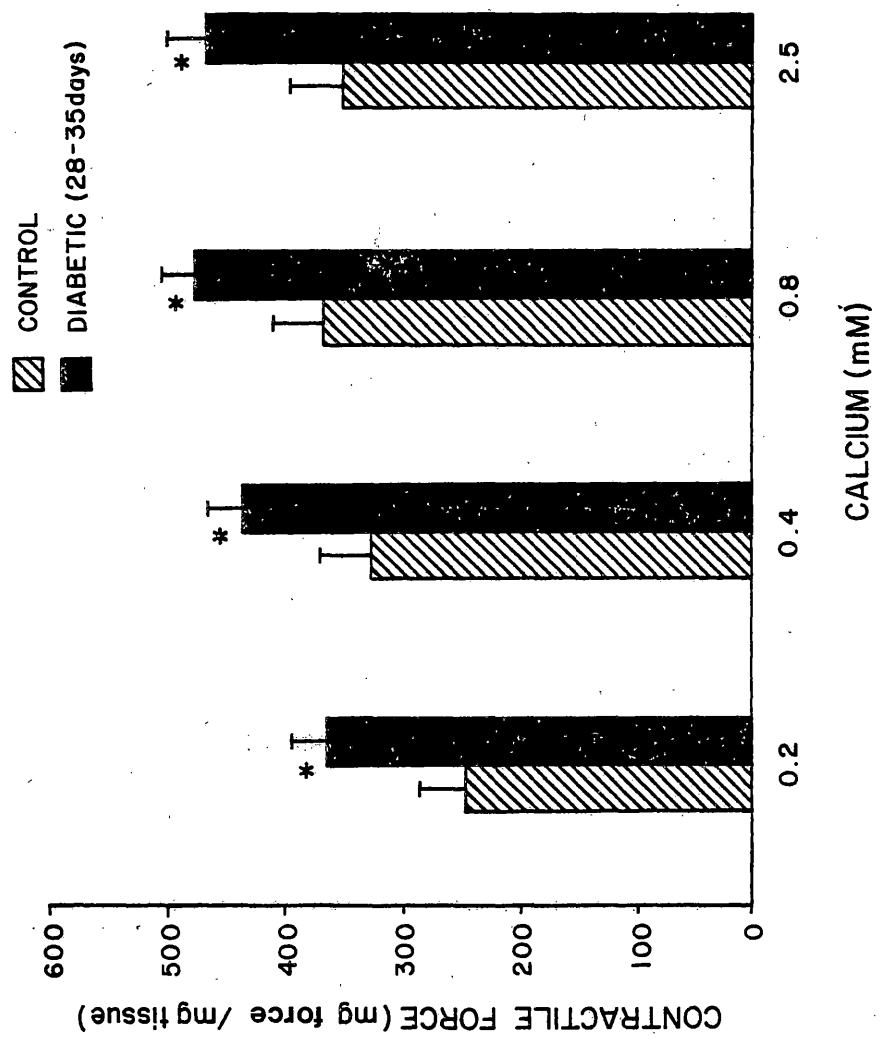

CONTROL
DIABETIC (28-35days)
LOG EC50
-6.5
-7.0
-7.5
-8.0
-8.5
-9.0
0
0.2
0.4
0.8
2.5
CALCIUM(mM)

Figure 10. *Maximum contractile force (mg force/mg tissue) in response to norepinephrine determined in aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of different external Ca^{++} concentrations (0.20, 0.40, 0.80, and 2.50 mM)*

Each bar represents the mean of 8-9 different aortic rings. The vertical lines represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.


CONTROL
DIABETIC (28-35days)
CONTRACTILE FORCE (mg force /mg tissue)
0
100
200
300
400
500
600
0.2
0.4
0.8
2.5
CALCIUM (mM)
*
*
*
*

old or 10 to 11 weeks old at the time of the experiment. Figure 11 illustrates the maximum force developed in response to NE for both age groups of the control and diabetic tissue in the presence of different extracellular Ca^{++} concentrations. There was a tendency for the older group of control aortae to develop a greater degree of contractile force than the younger group in each of the different Ca^{++} mediums. However, the difference between the two age groups of control tissue was not significant. In contrast to the control tissues, there was a significant difference between the maximum response to NE developed by the two groups of diabetic tissues. Aortae from the older diabetic rats developed a significantly greater degree of force than aortae from the younger diabetic rats at each of the different external Ca^{++} concentrations.

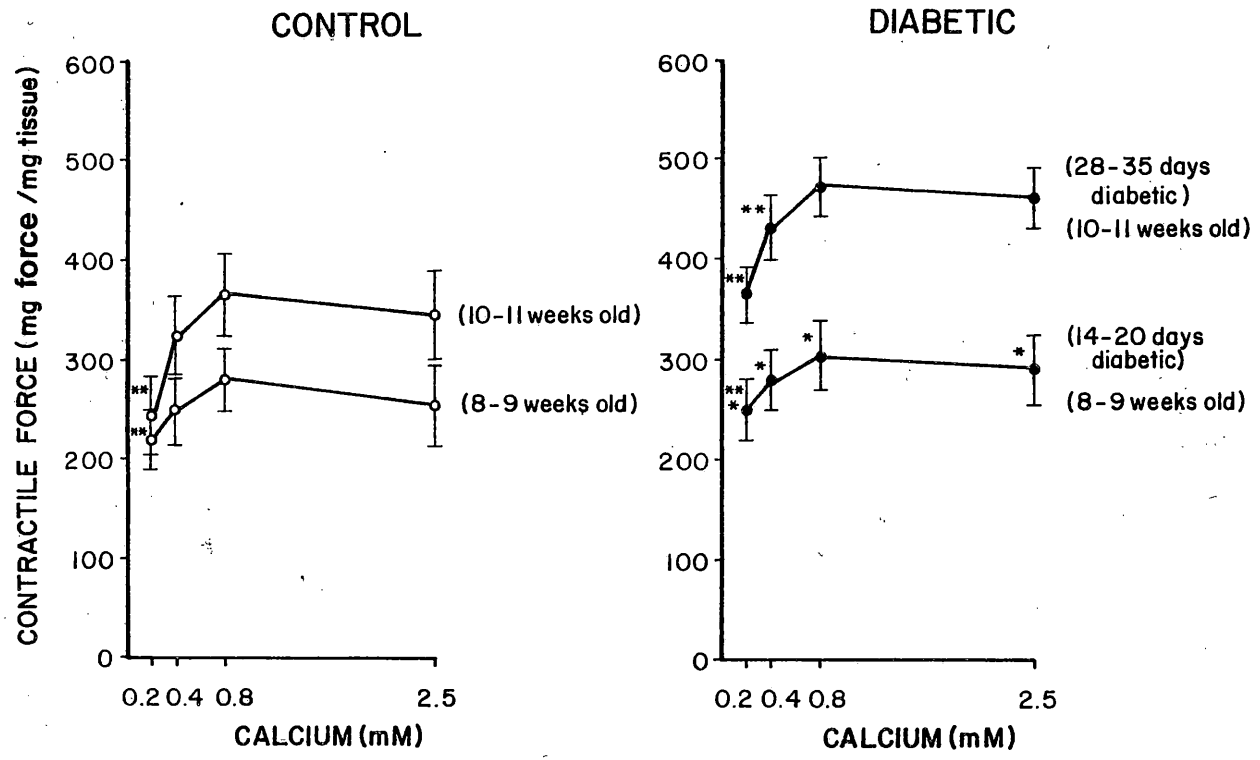
When the maximum contraction in response to NE in 2.50 mM external Ca^{++} was compared to the maximum contraction obtained from the same tissue in 0.20 mM external Ca^{++}, a significant decrease was detected in the control rats (8-9 weeks old and 10-11 weeks old) and in the younger diabetic rats (8-9 weeks old). The older

Figure 11. *Maximum contractile response to norepinephrine in control and diabetic aortae as a function of age and duration of diabetes in the presence of various external Ca^{++} concentrations (0.20, 0.40, 0.80, and 2.50 mM)*

Each point represents the mean of 6-9 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level between the two age groups.

** Denotes significant difference at least at the $p < 0.05$ level between the response of a tissue in 2.50 mM Ca^{++} and the response of that same tissue in a lowered Ca^{++} concentration.


CONTROL
DIABETIC
CONTRACTILE FORCE (mg force /mg tissue)
600
500
400
300
200
100
0
0.2 0.4 0.8
2.5
CALCIUM (mM)
(10-11 weeks old)
(8-9 weeks old)
(28-35 days diabetic)
(10-11 weeks old)
(14-20 days diabetic)
(8-9 weeks old)
**
*

diabetic rats (10-11 weeks old) exhibited a greater dependency on extracellular calcium as demonstrated by the significant decrease in contractile force development both in 0.40 and in 0.20 mM Ca^{++} when compared to 2.50 mM Ca^{++}.

2. Contractile Responses to Potassium Chloride

   a. Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 14-20 Days following Injection

      Isolated aortic rings from control and diabetic rats contracted in response to KCl over the concentration range of 1×10^{-3} M to 1×10^{-1} M in 2.50 mM Ca^{++}. As demonstrated in Figure 12, the diabetic rings (14-20 days) were significantly subsensitive in response to KCl when compared to the control rings. The log EC50 value for the diabetic tissues was -1.78 ± 0.04 compared to a log EC50 value of -1.93 ± 0.03 for the control tissues. The maximum contractile force developed by the 14-20 day diabetic aortae in response to KCl was not significantly different from the maximum contractile force developed by the control aortae in 2.50 mM Ca^{++} concentration (Figure 13).

Figure 12. *Potassium chloride concentration-effect curves of aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each point represents the mean of 7-8 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.

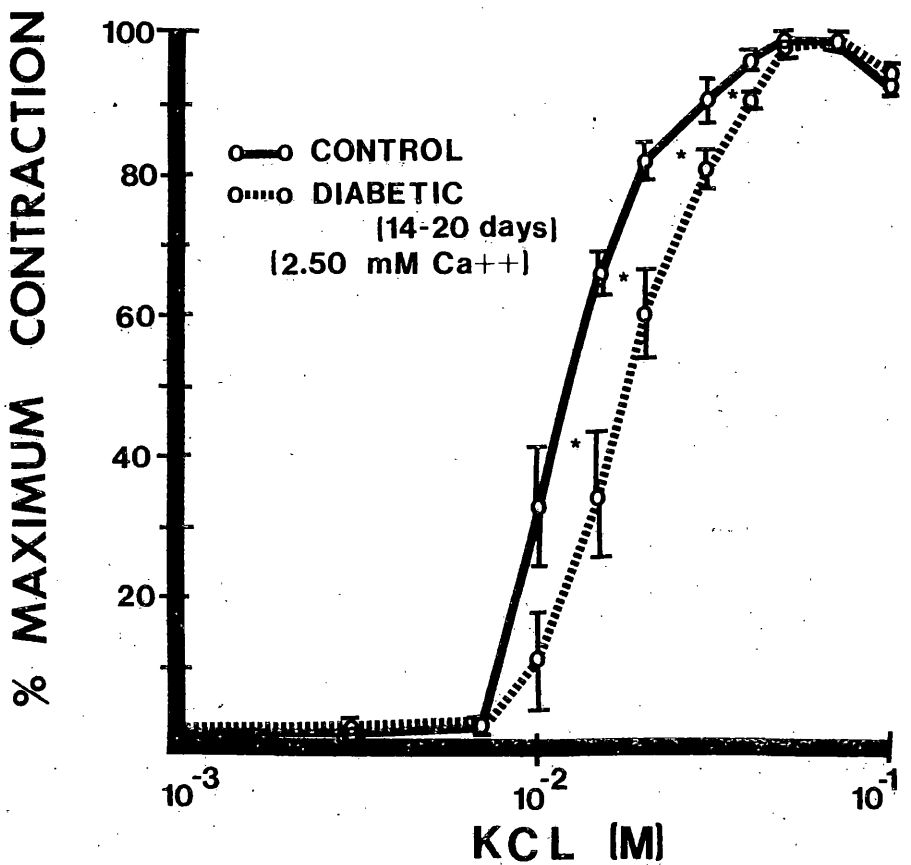
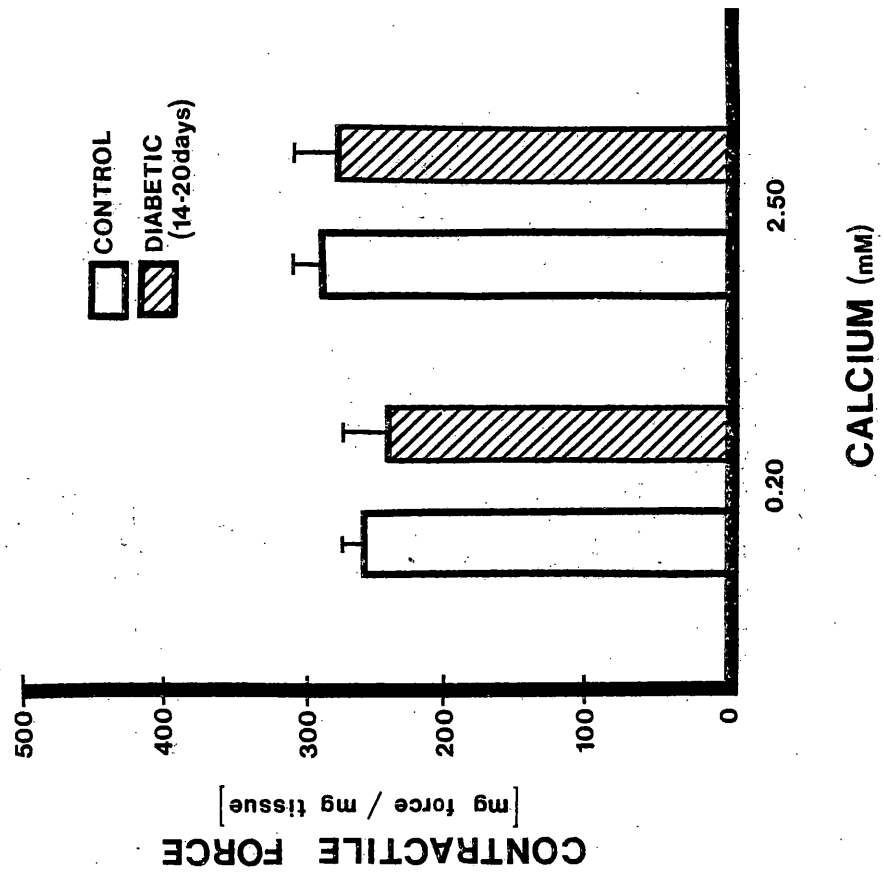

% MAXIMUM CONTRACTION
100
80
60
40
20
CONTROL
DIABETIC
(14-20 days)
(2.50 mM Ca++)
10-3
10-2
10-1
KCL (M)

Figure 13. *Maximum contractile force (mg force/mg tissue) in response to potassium chloride determined in aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 0.20 mM and 2.50 mM external Ca^{++} concentrations*

Each bar represents the mean of 7-8 different aortic rings. The vertical lines represent the S.E.M. There was no significant difference between diabetic and control tissues.


CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
CONTROL
DIABETIC
(14-20days)
0.20
2.50
CALCIUM (mM)

b. Contractile Responses in 0.20 mM Extracellular Ca^{++} Concentration which Were Obtained from Rats 14-20 Days following Injection

Figure 14 illustrates the concentration-effect curves for KCl in control and diabetic (14-20 days) aortae in the presence of 0.20 mM Ca^{++}. There was no significant difference in the sensitivity of the diabetic rings (14-20 days) in response to KCl when compared to the sensitivity of the control rings. Also, the maximum contractile force (mg force/mg tissue) developed in response to KCl by the diabetic tissue was not significantly different from the force developed by the control tissue in the presence of 0.20 mM Ca^{++} (Figure 13).

c. Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

The concentration-effect curves of Figure 15 demonstrate that there was a significant difference in the sensitivity of the diabetic aortae (28-35 days) in response to KCl when compared to the sensitivity of the control aortae. The diabetic aortae were subsensitive as indicated by a log EC50 of -1.70 ± 0.03 for the diabetic tissues and a log EC50 of -1.80 ± 0.03 for the

Figure 14. *Potassium chloride concentration-effect curves of aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 0.20 mM external Ca^{++} concentration*

Each point represents the mean of 7-8 different aortic rings. The vertical bars represent the S.E.M. There was no significant difference between diabetic and control tissues.

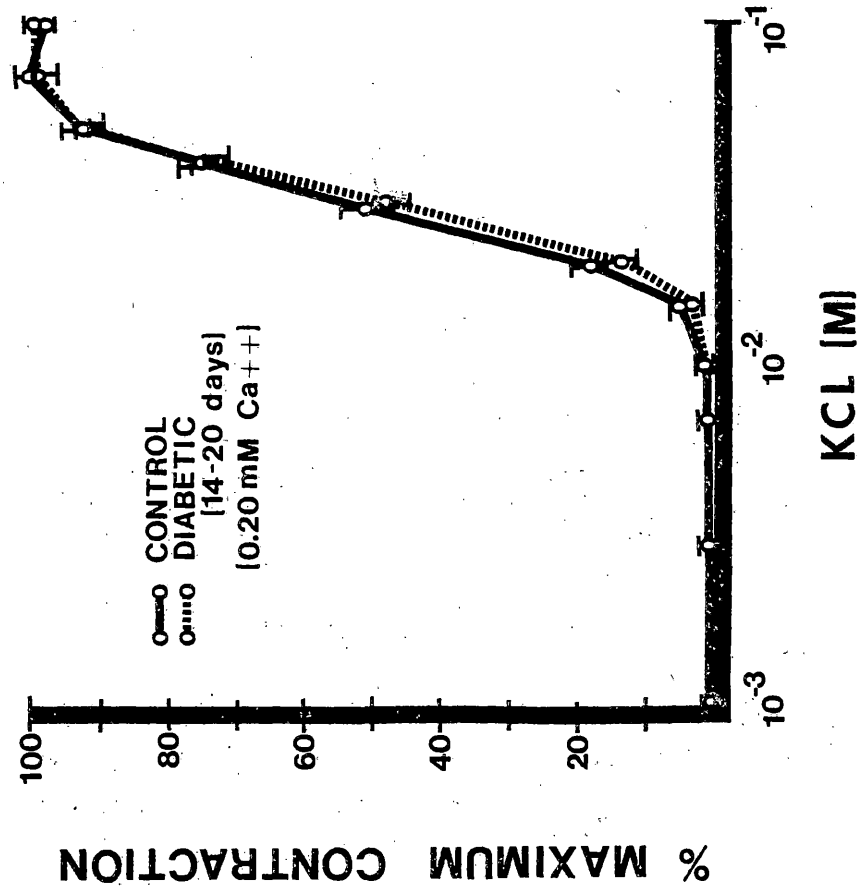
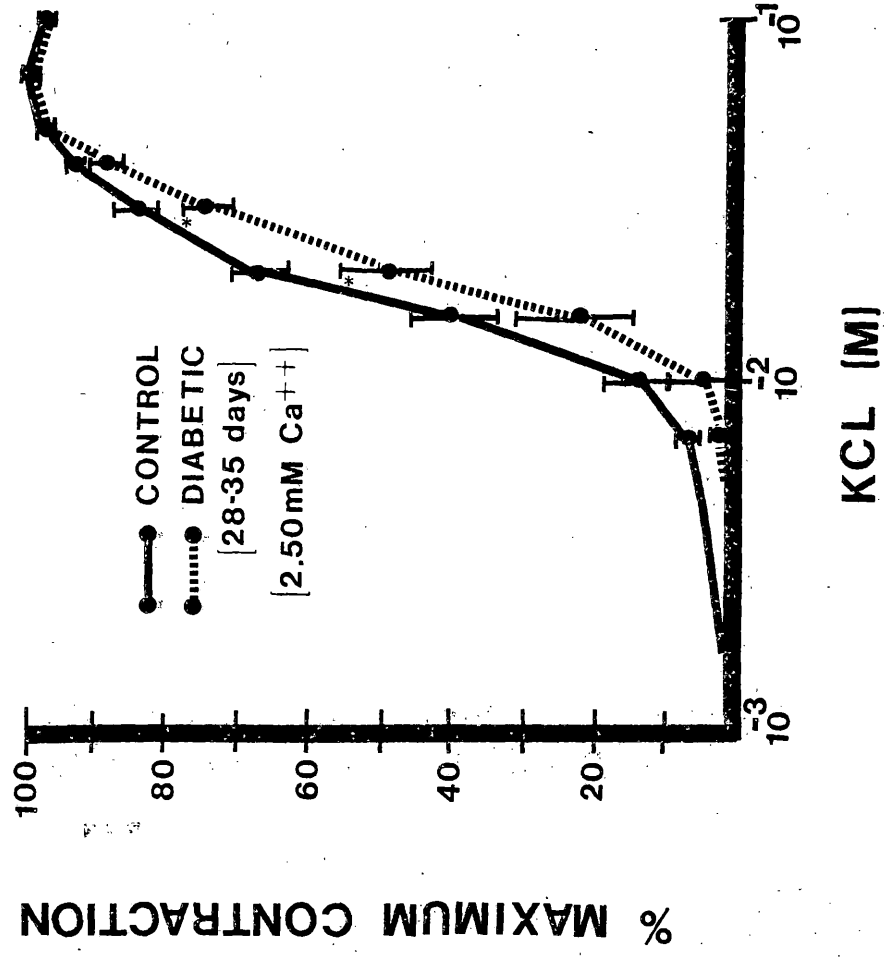

% MAXIMUM CONTRACTION
100
80
60
40
20
CONTROL
DIABETIC
[14-20 days]
[0.20 mM Ca++]
10^{-3}
10^{-2}
10^{-1}
KCL [M]

Figure 15. *Potassium chloride concentration-effect curves of aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each point represents the mean of 9-10 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.


% MAXIMUM CONTRACTION
100
80
60
40
20
CONTROL
DIABETIC
[28-35 days]
[2.50mM Ca++]
10-3
10-2
10-1
KCL (M)

control tissues. There was no significant difference in the maximum contractile force developed in response to KCl by the diabetic aortae (28-35 days) and in the maximum contractile force developed in response to KCl by the control aortae in the presence of 2.50 mM external Ca^{++} concentration (Figure 16).

d. Contractile Responses in 0.20 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

In contrast to the concentration-effect curves for KCl obtained from diabetic (28-35 days) and control aortae in 2.50 mM Ca^{++}, the concentration-effect curves for KCl in 0.20 mM Ca^{++} were not significantly different (Figure 17). In addition, the maximum contractile force developed in response to KCl by the diabetic aortae (28-35 days) was not significantly different from the maximum contractile force developed by the control aortae in the presence of 0.20 mM Ca^{++} concentration (Figure 16).

e. Maximum Contractile Response as a Function of Age and Duration of Diabetes

The maximum contractile force developed in response to KCl for both age groups (8-9 weeks

Figure 16. *Maximum contractile force (mg force/mg tissue) in response to potassium chloride determined in aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 0.20 mM and 2.50 mM external Ca^{++} concentrations*

Each bar represents the mean of 9-10 different aortic rings. The vertical lines represent the S.E.M. There was no significant difference between diabetic and control tissues.

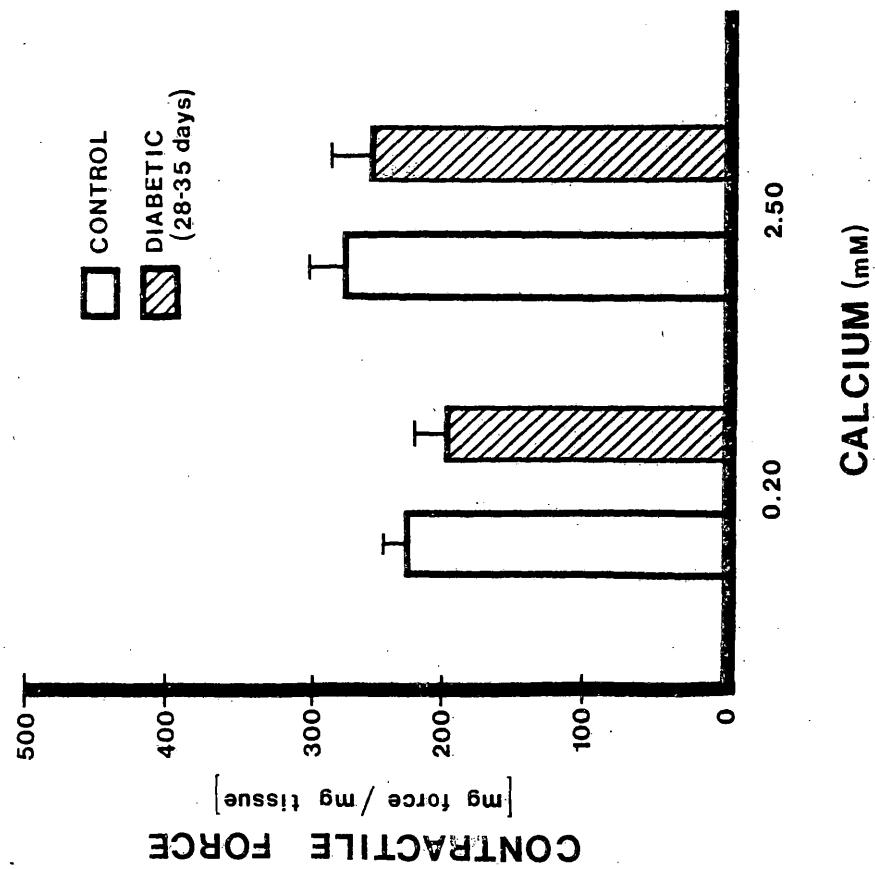
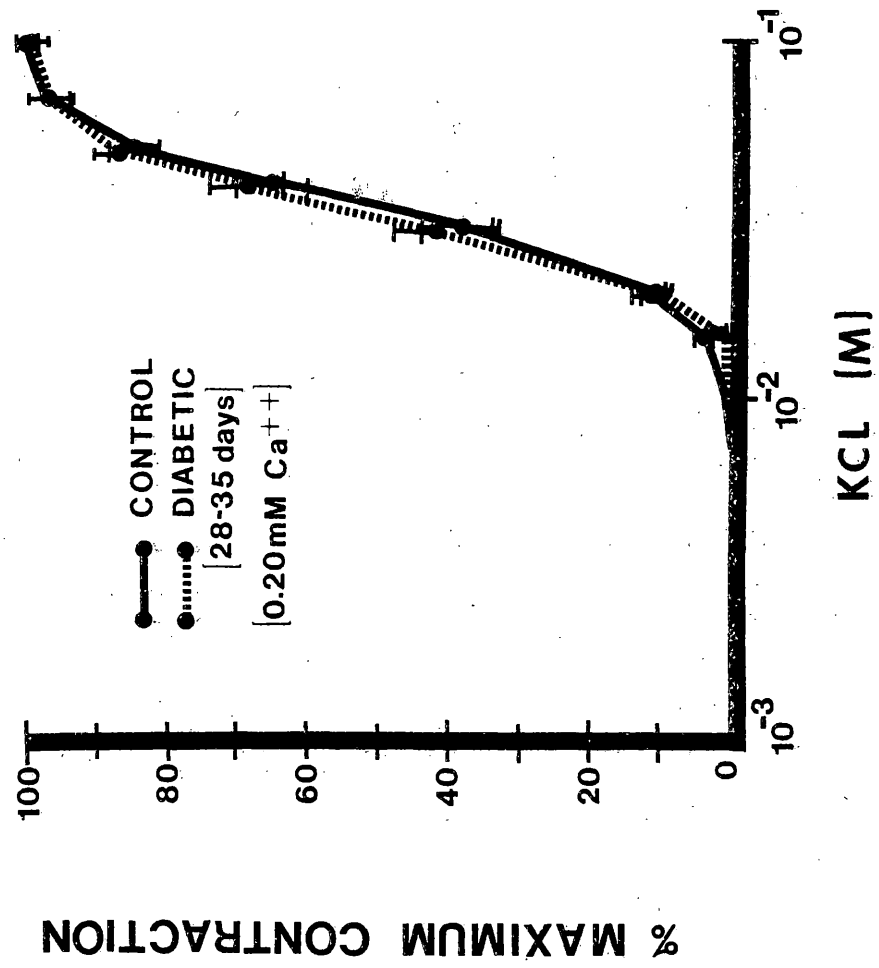

CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
0.20
2.50
CALCIUM (mM)
CONTROL
DIABETIC
(28-35 days)

Figure 17. *Potassium chloride concentration-effect curves of aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 0.20 mM external Ca^{++} concentration*

Each point represents the mean of 9-10 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.


% MAXIMUM CONTRACTION
100
80
60
40
20
0
10^{-3}
10^{-2}
10^{-1}
KCL (M)
CONTROL
DIABETIC
[28-35 days]
[0.20mM Ca^{++}]

old and 10-11 weeks old) of the control and diabetic tissue was not significantly different in either 0.20 mM or 2.50 mM Ca^{++} concentration (Figure 18). However, when the maximum contraction in response to KCl in 2.50 mM external Ca^{++} was compared to the maximum contraction in response to KCl obtained from the same tissue in 0.20 mM external Ca^{++}, a significant decrease was detected in the control rats (8-9 weeks old and 10-11 weeks old) and in the diabetic rats (8-9 weeks old and 10-11 weeks old).

3. Contractile Responses to Serotonin

a. Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 14-20 Days following Injection

Isolated aortic rings from control and diabetic rats contracted in response to 5-HT over the concentration range of 1×10^{-7} M to 3×10^{-4} M in 2.50 mM Ca^{++}. As demonstrated by the concentration-effect curves of Figure 19, the diabetic rings (14-20 days) were subsensitive in response to 5-HT when compared to the control rings. The log EC50 value was -5.02 ± 0.10 for diabetic rings and -5.34 ± 0.07 for control rings. However, the decrease in maximum contractile force observed in these same

Figure 18. *Maximum contractile response to potassium chloride in control and diabetic aortae as a function of age and duration of diabetes in the presence of 0.20 mM and 2.50 mM external Ca^{++} concentrations*

Each bar represents the mean of 7-10 different aortic rings. The vertical lines represent the S.E.M. There was no significant difference between the two age groups.

** Denotes significant difference at least at the $p < 0.05$ level between the response of a tissue in 2.50 mM Ca^{++} and the response of that same tissue in 0.20 mM Ca^{++} concentration.

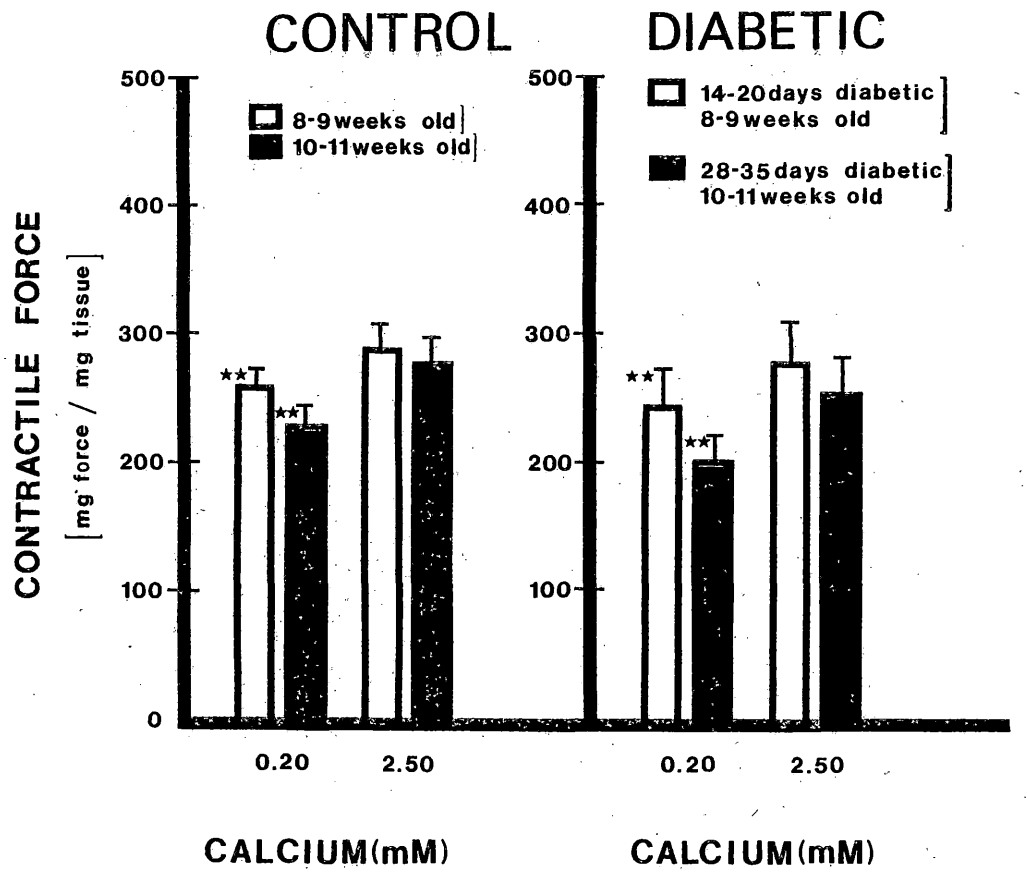
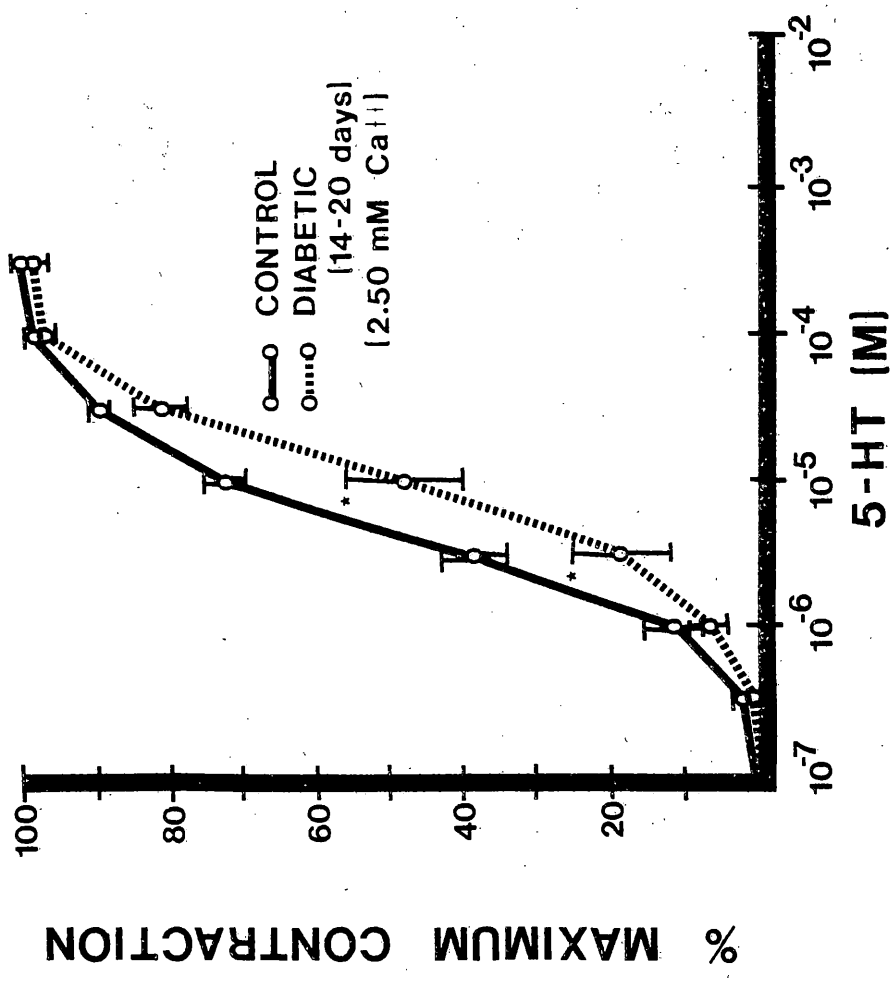

CONTROL
DIABETIC
8-9weeks old
10-11weeks old
14-20days diabetic
8-9weeks old
28-35days diabetic
10-11weeks old
CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
**
0.20
2.50
CALCIUM(mM)

Figure 19. *Serotonin concentration-effect curves of aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each point represents the mean of 8 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.


% MAXIMUM CONTRACTION
100
80
60
40
20
CONTROL
DIABETIC
(14-20 days)
(2.50 mM Ca++)
10-7
10-6
10-5
10-4
10-3
10-2
5-HT (M)

diabetic rings was not significantly different from the maximum contractile force generated by the control rings in the presence of 2.50 mM Ca^{++} concentration (Figure 20).

b. Contractile Responses in 0.20 mM Extracellular Ca^{++} Concentration which Were Obtained from Rats 14-20 Days following Injection

Contractile responses obtained from diabetic aortae (14-20 days) in response to 5-HT in 0.20 mM Ca^{++} and compared to contractile responses of controls in 0.20 mM Ca^{++} resembled the results obtained from diabetic (14-20 days) and control aortae in 2.50 mM Ca^{++}. The diabetic rings (14-20 days) were subsensitive in response to 5-HT when compared to the control rings (Figure 21). The log EC50 value was -4.67 ± 0.12 for diabetic rings and -5.15 ± 0.07 for control rings. There was not a significant difference between the maximal contractile force generated in 0.20 mM Ca^{++} by the diabetic tissues (14-20 days) and that force generated by the control tissues (Figure 20).

c. Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

The concentration-effect curves for 5-HT in 2.50 mM Ca^{++} obtained from diabetic (28-35

Figure 20. *Maximum contractile force (mg force/mg tissue) in response to serotonin determined in aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 0.20 mM and 2.50 mM external Ca^{++} concentrations*

Each bar represents the mean of 8 different aortic rings. The vertical lines represent the S.E.M. There was no significant difference between diabetic and control tissues.

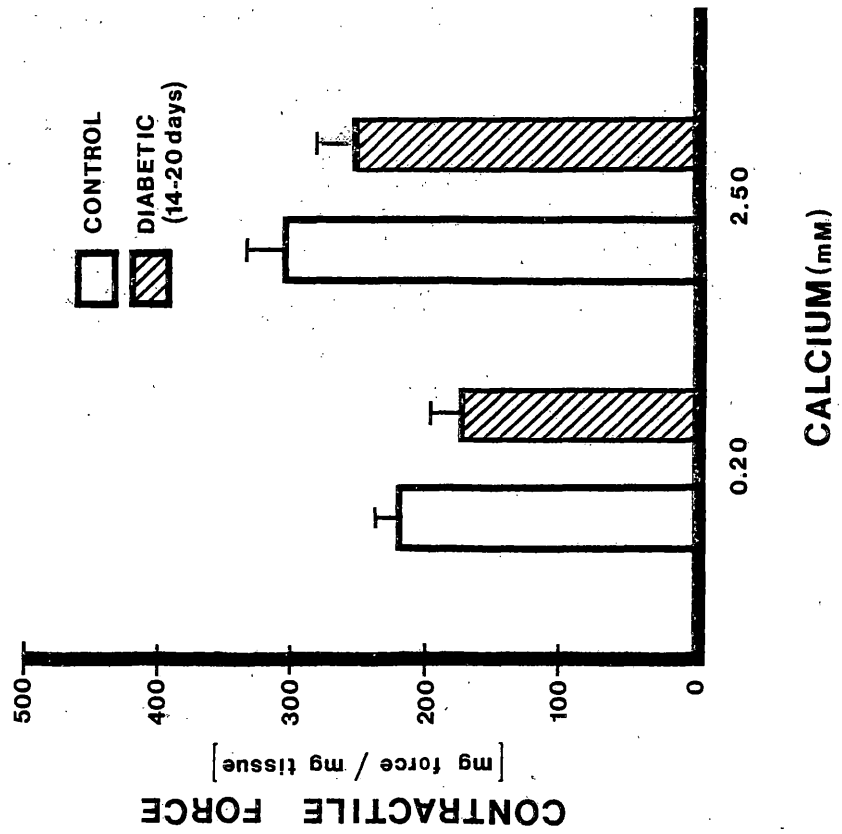
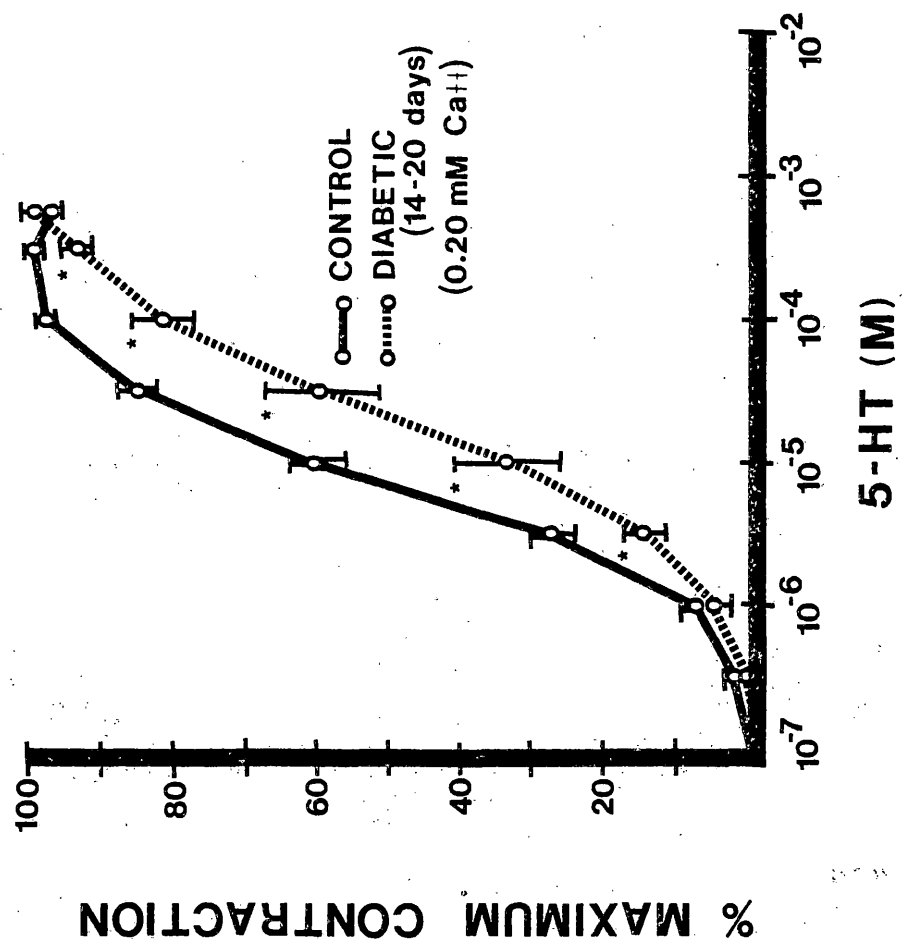

CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
0.20
2.50
CALCIUM (mM)
CONTROL
DIABETIC (14-20 days)

Figure 21. *Serotonin concentration-effect curves of aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 0.20 mM external Ca^{++} concentration*

Each point represents the mean of 8 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.


% MAXIMUM CONTRACTION
100
80
60
40
20
5-HT (M)
10^{-7}
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
CONTROL
DIABETIC
(14-20 days)
(0.20 mM Ca++)

days) and control aortic rings indicate a subsensitivity of the diabetic aortic rings (Figure 22). The concentration-effect curve for the diabetic tissues is shifted to the right. The log EC50 for the diabetic tissue was -5.38 $\pm$ 0.14 and the log EC50 for the control tissue was -5.86 $\pm$ 0.09. Figure 23 illustrates that the maximum contractile force generated in response to 5-HT by the diabetic tissue (28-35 days) was significantly less than the force generated by the control tissue in 2.50 mM Ca^{++}.

d. Contractile Responses in 0.20 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

Figure 24 illustrates the concentration-effect curves for 5-HT in control and diabetic (28-35 days) aortae in the presence of 0.20 mM Ca^{++}. There was no significant difference in the sensitivity of the diabetic rings (28-35 days) in response to 5-HT when compared to the sensitivity of the control rings. However, the maximum contractile force developed in response to 5-HT by the diabetic tissue was significantly less than the force developed by the control tissue in the presence of 0.20 mM Ca^{++} concentration (Figure 23).

Figure 22. *Serotonin concentration-effect curves of aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each point represents the mean of 8 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.

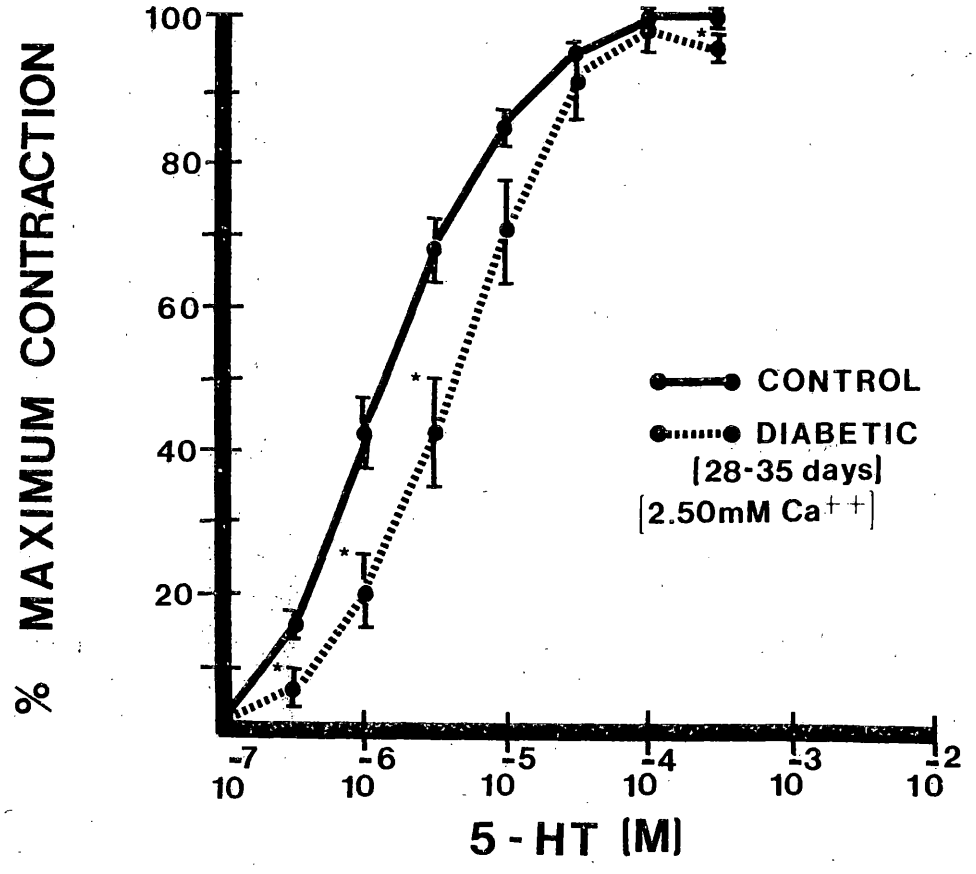

% MAXIMUM CONTRACTION
100
80
60
40
20
CONTROL
DIABETIC
[28-35 days]
[2.50mM Ca++]
10-7
10-6
10-5
10-4
10-3
10-2
5 - HT (M)

Figure 23. *Maximum contractile force (mg force/mg tissue) developed in response to serotonin determined in aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 0.20 mM and 2.50 mM external Ca^{++} concentrations*

Each bar represents the mean of 8 different aortic rings. The vertical lines represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.

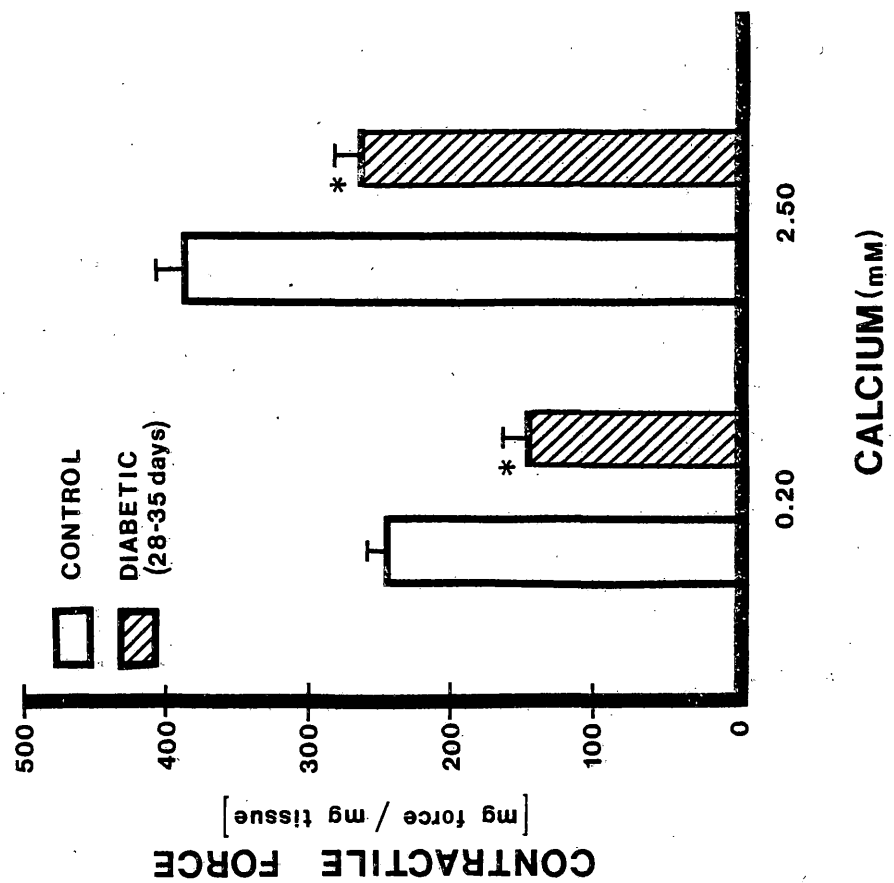
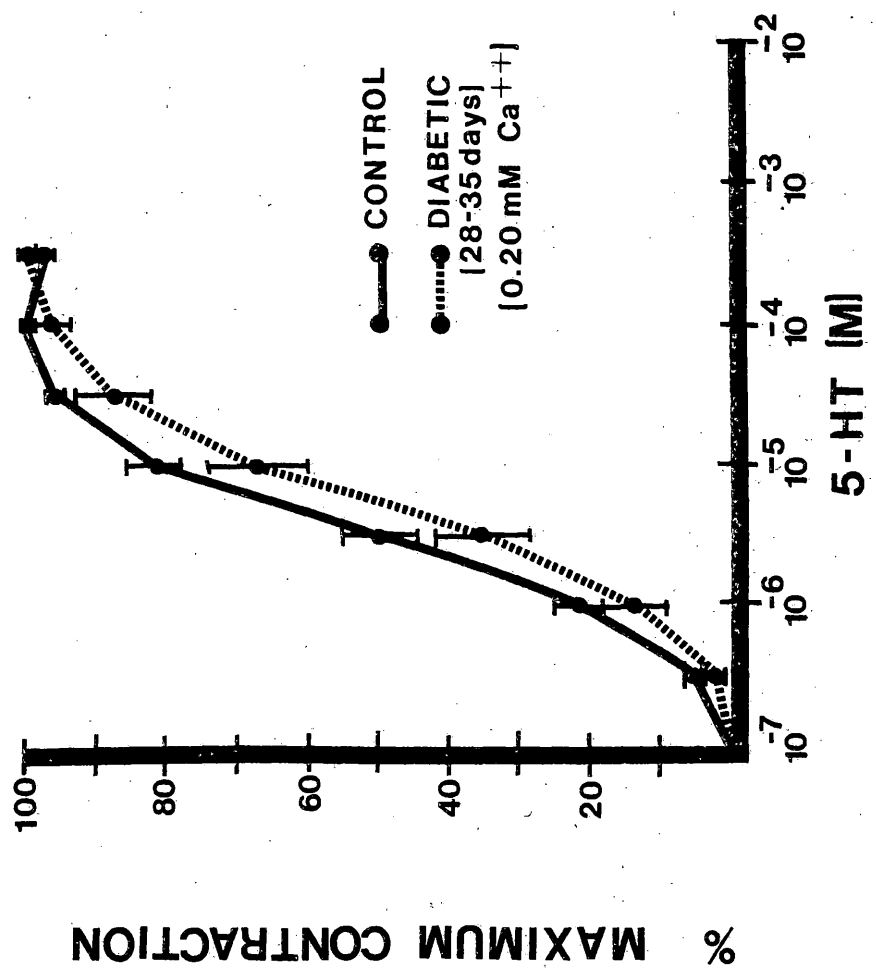

CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
CONTROL
DIABETIC (28-35 days)
*
*
0.20
2.50
CALCIUM (mM)

Figure 24. *Serotonin concentration-effect curves of aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 0.20 mM external Ca^{++} concentration*

Each point represents the mean of 8 different aortic rings. The vertical bars represent the S.E.M. There was no significant difference between diabetic and control tissues.


% MAXIMUM CONTRACTION
100
80
60
40
20
10^-7
10^-6
10^-5
10^-4
10^-3
10^-2
5-HT (M)
CONTROL
DIABETIC
(28-35 days)
(0.20 mM Ca++)

e. Maximum Contractile Response as a Function of Age and Duration of Diabetes

Figure 25 illustrates the maxiumum contractile force developed in response to 5-HT for both age groups of the control and diabetic tissue in the presence of 0.20 and 2.50 mM extracellular Ca^{++} concentration. The older group (10-11 weeks old) of control aortae developed a significantly greater degree of contractile force than the younger group (8-9 weeks old) of control aortae in the presence of 2.50 mM Ca^{++} concentration. There was no significant difference between the contractile force developed in response to 5-HT by the younger (8-9 weeks old) and the older (10-11 weeks old) control groups in the presence of 2.50 mM Ca^{++}. Neither was there a significant difference between the contractile force developed in response to 5-HT by the older (10-11 weeks old) and younger (8-9 weeks old) diabetic aortae in either 0.20 mM or 2.50 mM external Ca^{++} concentration.

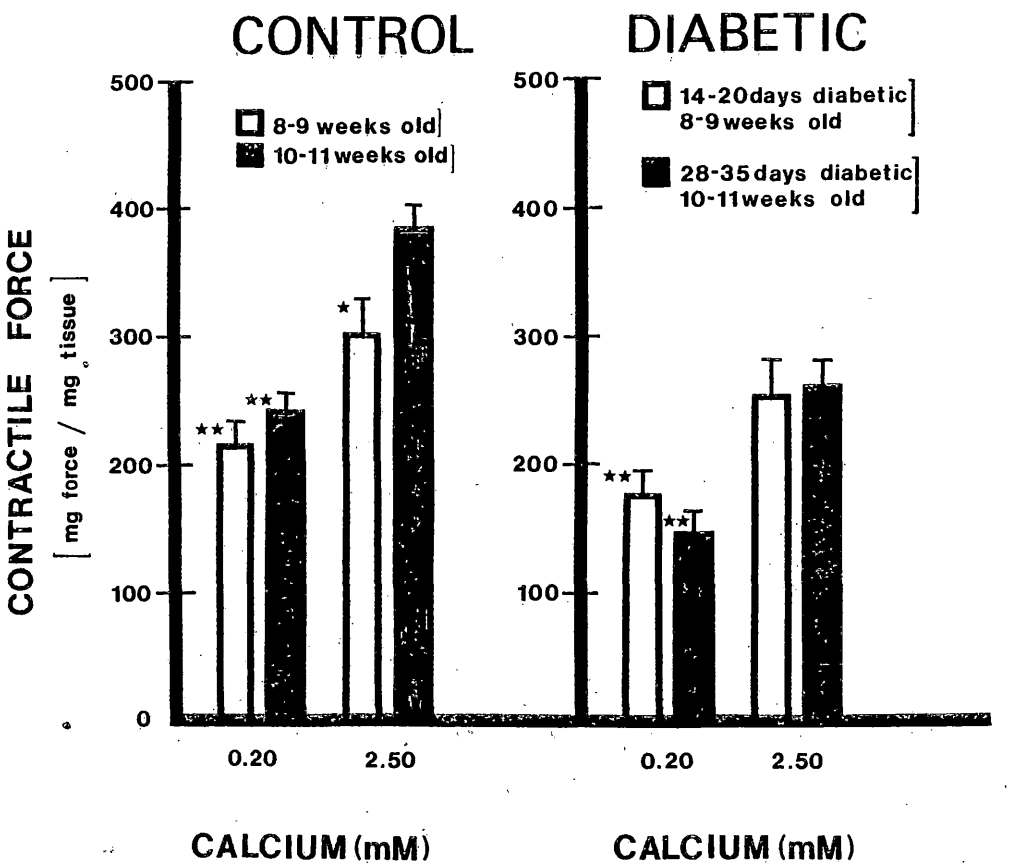
When the maximum contraction in response to 5-HT in 2.50 mM external Ca^{++} was compared to the maximum contraction obtained from the same tissue in 0.20 mM external Ca^{++}, a significant decrease was detected in the control

Figure 25. *Maximum contractile response to serotonin in control and diabetic aortae as a function of age and duration of diabetes in the presence of 0.20 and 2.50 mM external Ca^{++} concentrations*

Each bar represents the mean of 8 different aortic rings. The vertical lines represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level between the two age groups.

** Denotes significant difference at least at the $p < 0.05$ level between the response of a tissue in 2.50 mM Ca^{++} and the response of that same tissue in 0.20 mM Ca^{++} concentration.


CONTROL
DIABETIC
8-9 weeks old
10-11 weeks old
14-20 days diabetic
8-9 weeks old
28-35 days diabetic
10-11 weeks old
CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
0.20
2.50
CALCIUM (mM)
CALCIUM (mM)

rats (8-9 weeks old and 10-11 weeks old) and in the diabetic rats (8-9 weeks old and 10-11 weeks old).

4. Contractile Responses to Phenylephrine

a. Contractile Responses in 0.20 mM Ca^{++} which Were Obtained from Rats 14-20 Days following Injection

Isolated aortic rings from control and diabetic rats contracted in response to PE over the concentration range of 4.9×10^{-10} M to 1.5×10^{-5} M in 0.20 mM Ca^{++} (Figure 26). The log EC50 of -6.94 ± 0.10 for the diabetic tissue was not significantly different from the log EC50 of -7.19 ± 0.07 for the control tissue. In addition, the maximum contractile force developed in response to PE by the diabetic aortae was not significantly different from the contractile force developed by the control aortae in the presence of 0.20 mM Ca^{++} (Figure 27).

b. Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

Figure 28 illustrates the concentration-effect curves for PE obtained from control and diabetic (28-35 days) aortae in the presence

Figure 26. *Phenylephrine concentration-effect curves of aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 0.20 mM external Ca^{++} concentration*

Each point represents the mean of 7-8 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.

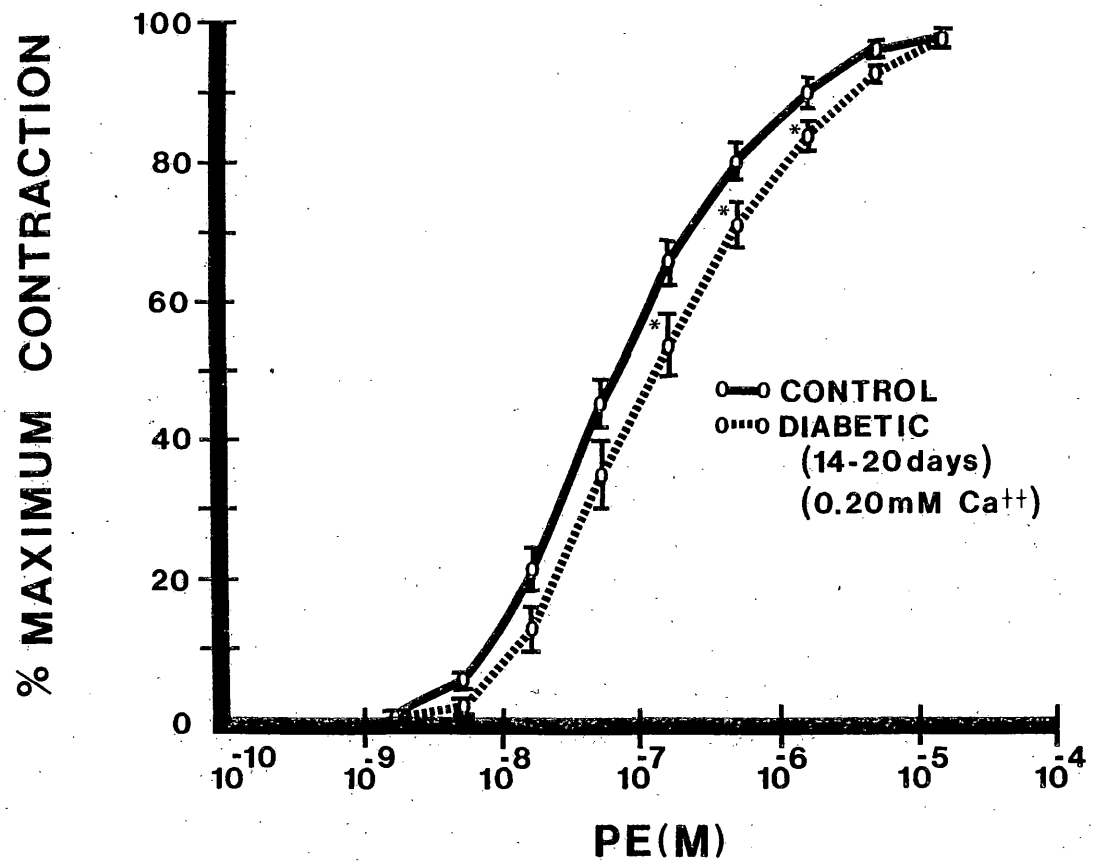

% MAXIMUM CONTRACTION
100
80
60
40
20
0
10^-10
10^-9
10^-8
10^-7
10^-6
10^-5
10^-4
PE(M)
CONTROL
DIABETIC
(14-20 days)
(0.20mM Ca++)
*

Figure 27. *Maximum contractile force (mg force/mg tissue) in response to phenylephrine determined in aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 0.20 mM external Ca^{++} concentration*

Each bar represents the mean of 7-8 different aortic rings. The vertical lines represent the S.E.M. There was no significant difference between diabetic and control tissues.

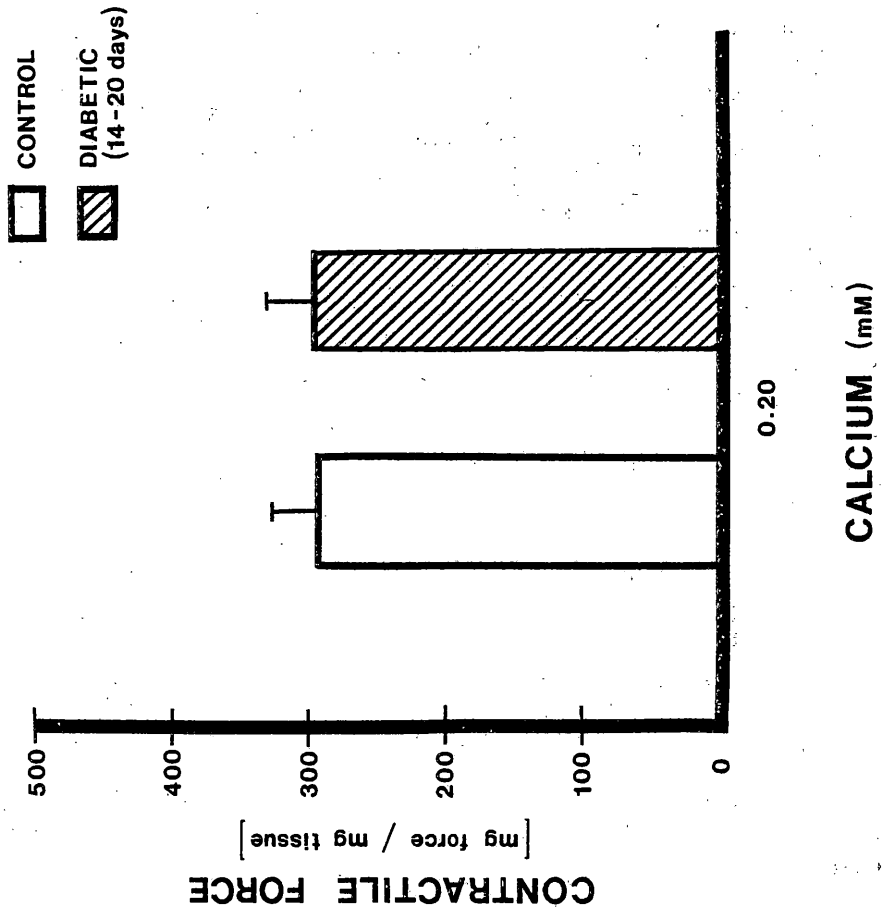
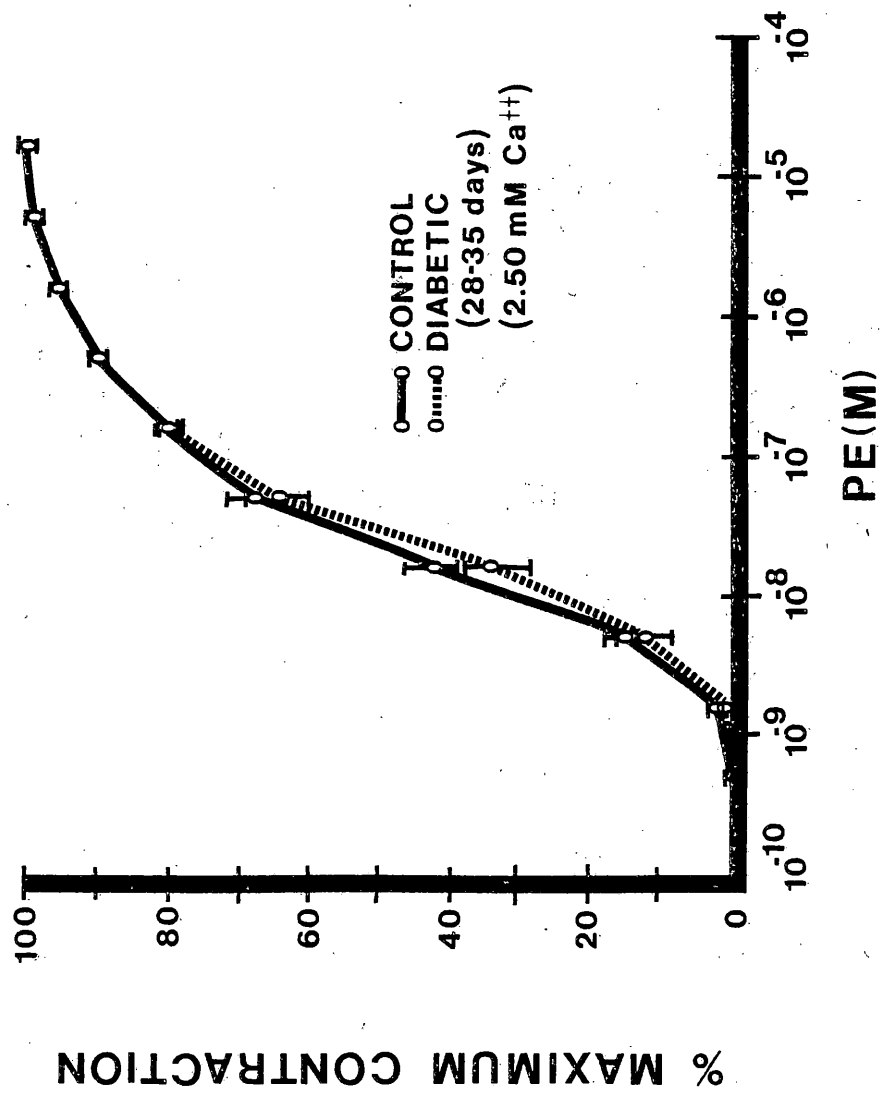

CONTROL
DIABETIC
(14-20 days)
CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
0.20
CALCIUM (mM)

Figure 28. *Phenylephrine concentration-effect curves of aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each point represents the mean of 10-12 different aortic rings. The vertical bars represent the S.E.M. There was no significant difference between diabetic and control tissues.


% MAXIMUM CONTRACTION
100
80
60
40
20
0
CONTROL
DIABETIC
(28-35 days)
(2.50 mM Ca++)
10-10
10-9
10-8
10-7
10-6
10-5
10-4
PE(M)

of 2.50 mM external Ca^{++} concentration. The log EC50 value for the diabetic tissues (-7.59 $\pm$ 0.09) was not significantly different from the log EC50 value for the control tissues (-7.67 $\pm$ 0.06). Also the maximum contractile force developed in response to PE by the diabetic aortae was not significantly different from the contractile force developed by the control aortae in the presence of 2.50 mM Ca^{++} concentration (Figure 29).

5. Comparisons of Maximum Contractile Responses to Norepinephrine, Potassium Chloride, Serotonin, and Phenylephrine

   a. Maximum Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 14-20 Days following Injection

      The mean maximum contractile force developed to the agonists NE, KCl, and 5-HT in the presence of 2.50 mM extracellular Ca^{++} did not differ significantly either for the diabetic tissues (14-20 days) or for the control tissues when one-way analysis of variance (anova) analyses were employed. Also a two-way anova demonstrated that the maximum contractile force developed to the agonists did not differ between the control and diabetic groups (Figure 30).

Figure 29. *Maximum contractile force (mg force/mg tissue) in response to phenylephrine determined in aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each bar represents the mean of 10-12 different aortic rings. The vertical lines represent the S.E.M. There was no significant difference between diabetic and control tissues.

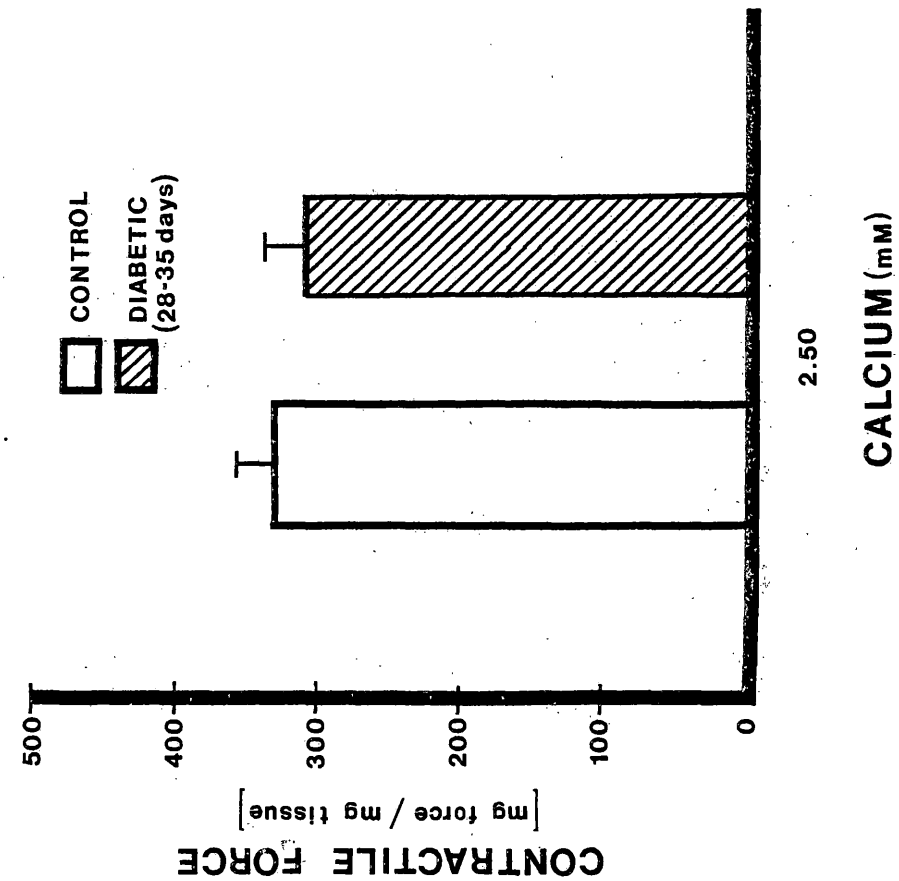
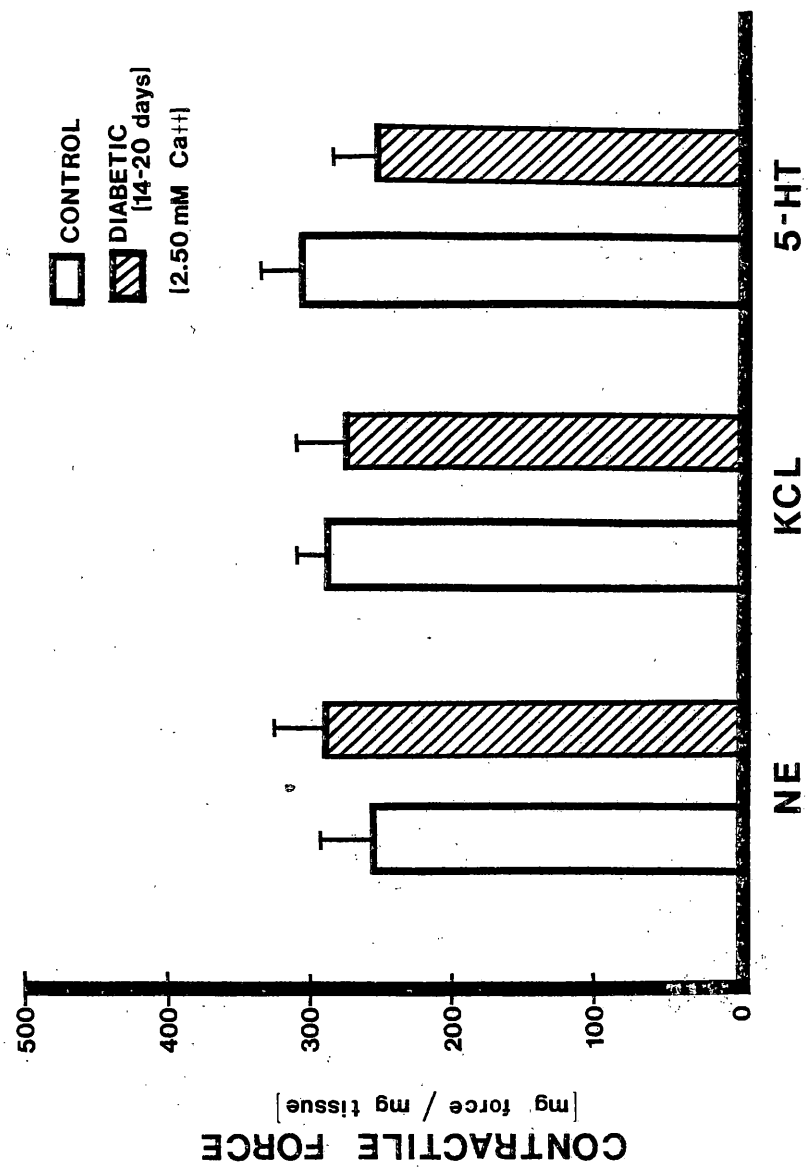

CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
CONTROL
DIABETIC
(28-35 days)
2.50
CALCIUM (mM)

Figure 30. *Maximum contractile force (mg force/mg tissue) in response to norepinephrine, potassium chloride, and serotonin determined in aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each bar represents the mean of 6-8 different aortic rings. The vertical lines represent the S.E.M. A two-way anova demonstrated there was no significant difference in the contractile force developed to the drugs in either the control or diabetic tissues.


CONTROL
DIABETIC (14-20 days)
(2.50 mM Ca++)
CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
NE
KCL
5-HT

b. Maximum Contractile Responses in 0.20 mM Ca^{++} which Were Obtained from Rats 14-20 Days following Injection

After a one-way anova revealed a difference between the maximum contractile force developed in response to NE, KCl, 5-HT, and PE in the diabetic aortae, the Student-Newman-Keuls (SNK) test demonstrated a significant difference ($p < 0.05$) only between the mean maximum contractile force generated by PE and 5-HT (Figure 31). A one-way anova revealed no significant difference between the maximum force developed in response to NE, KCl, 5-HT, and PE in the control aortae.

c. Maximum Contractile Responses in 2.50 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

The SNK test showed that the mean maximum contractile force developed by the diabetic aortae (28-35 days) in response to NE was significantly greater ($p < 0.01$) than the mean maximum contractile force developed in response to either KCl, 5-HT, or PE (Figure 32). Also, the mean maximum contractile responses to KCl, 5-HT, and PE did not differ significantly. A one-way anova revealed no significant difference

Figure 31. *Maximum contractile force (mg force/mg tissue) in response to norepinephrine, potassium chloride, serotonin, and phenylephrine determined in aortae from rats diabetic for 14-20 days and corresponding age-matched control rats in the presence of 0.20 mM external Ca^{++} concentration*

Each bar represents the mean of 6-8 different aortic rings. The vertical bars represent the S.E.M. The SNK test revealed a significant difference ($p < 0.05$) only between the mean maximum contractile force generated by PE and 5-HT in the diabetic aortae.

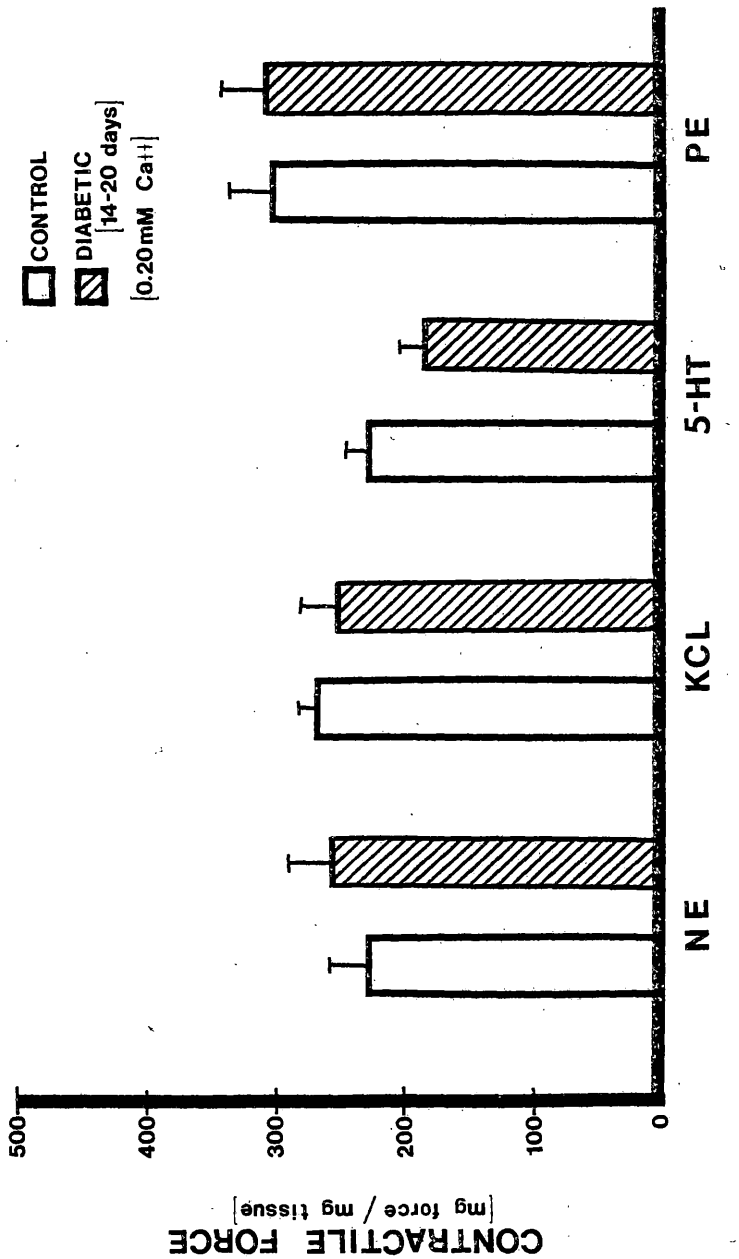
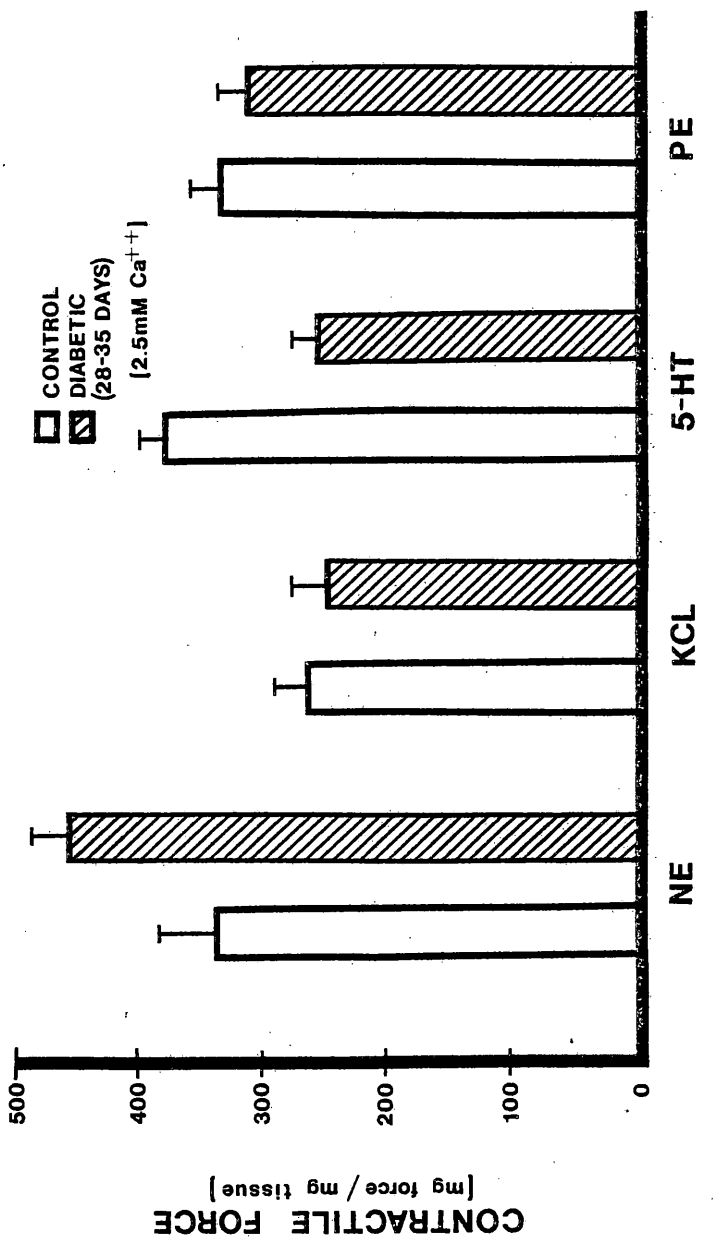

CONTROL
DIABETIC [14-20 days]
[0.20mM Ca++]
CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
NE
KCL
5-HT
PE

Figure 32. *Maximum contractile force (mg force/mg tissue) in response to norepinephrine, potassium chloride, serotonin, and phenylephrine determined in aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Each bar represents the mean of 8-12 different aortic rings. The vertical lines represent the S.E.M. The SNK test revealed the mean maximum contractile force developed by the diabetic aortae in response to NE was significantly greater ($p < 0.01$) than the mean maximum contractile force developed in response to either KCl, 5-HT, or PE.


CONTRACTILE FORCE
[mg force / mg tissue]
500
400
300
200
100
0
NE
KCL
5-HT
PE
CONTROL
DIABETIC
(28-35 DAYS)
[2.5mM Ca++]

between the maximum contractile responses developed in the control aortae to NE, KCl, 5-HT, and PE.

d. Maximum Contractile Responses in 0.20 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

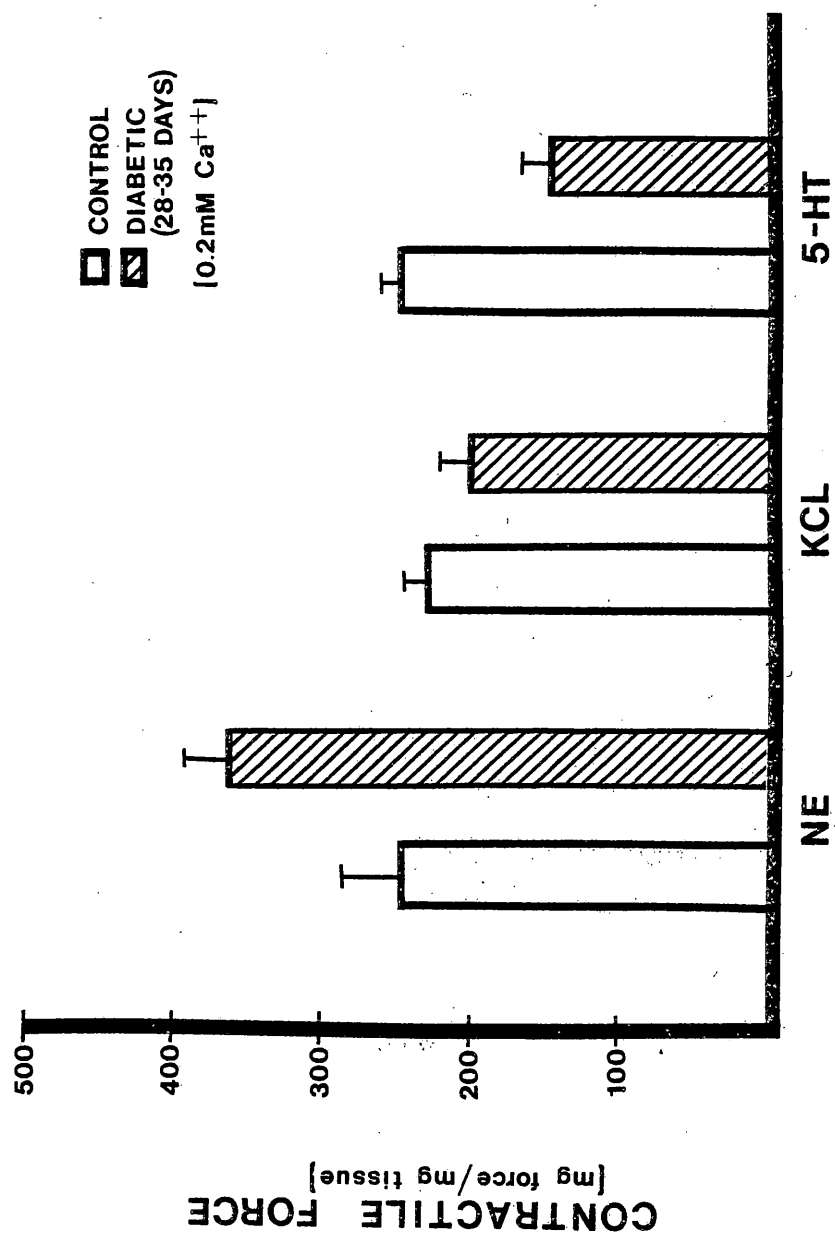
The mean maximum contractile force developed by the diabetic aortae (28-35 days) in response to NE was significantly greater ($p < 0.01$) than the mean maximum contractile force developed in response to either KCl or 5-HT in the presence of 0.20 mM Ca^{++} (Figure 33). The SNK test also demonstrated that the mean maximum contractile force developed by the diabetic aortae (28-35 days) in response to KCl and 5-HT did not differ significantly. A one-way anova showed no significant difference between the maximum contractile responses developed in the control aortae to NE, KCl, and 5-HT.

D. Affinity Constant (pD_2) to Phenylephrine and Affinity Constant (pA_2) of Phentolamine Determined in Aortae Obtained from Diabetic (Streptozotocin Injected) and Control (Vehicle Injected) Rats

1. Affinity Constant (pD_2) of Phenylephrine and Affinity Constant (pA_2) of Phentolamine in 0.20 mM Ca^{++} which Were Obtained from Rats 14-20 Days following Injection

Figure 33. *Maximum contractile force (mg force/mg tissue) in response to norepinephrine, potassium chloride, and serotonin determined in aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in 0.20 mM external Ca^{++} concentration*

Each bar represents the mean of 8-10 different aortic rings. The vertical lines represent the S.E.M. The SNK test revealed the mean maximum contractile force developed by the diabetic aortae in response to NE was significantly greater ($p < 0.01$) than the mean maximum contractile force developed in response to either KCl or 5-HT.


CONTRACTILE FORCE
[mg force/mg tissue]
500
400
300
200
100
NE
KCL
5-HT
CONTROL
DIABETIC
(28-35 DAYS)
[0.2mM Ca++]

The pD_2 of phenylephrine (6.94 ± 0.10) obtained from the diabetic aortae (14-20 days) was not significantly different from the pD_2 of phenylephrine (7.19 ± 0.07) obtained from the control aortae in the presence of 0.20 mM external Ca^{++} (Table 3). The phenylephrine-phentolamine antagonism was described in this study by the calculated pA_2 or affinity constant of phentolamine. The calculated pA_2 for phentolamine obtained from diabetic aortae was 7.70 ± 0.07, which was not significantly different from the calculated pA_2 for phentolamine obtained from control aortae (7.89 ± 0.08).

2. Affinity Constant (pD_2) of Phenylephrine and Affinity Constant (pA_2) of Phentolamine in 2.50 mM Ca^{++} which Were Obtained from Rats 28-35 Days following Injection

The pD_2 of phenylephrine (7.59 ± 0.09) obtained from the diabetic aortae (28-35 days) was not significantly different from the pD_2 of phenylephrine (7.67 ± 0.06) obtained from the control aortae in the presence of 2.50 mM Ca^{++} concentration (Table 3). Neither was the calculated pA_2 for phentolamine obtained from the diabetic aortae (7.97 ± 0.06) significantly different from the calculated pA_2 for phentolamine obtained from the control aortae (8.07 ± 0.05).

Table 3

Affinity Constant (pD_2) of Phenylephrine and Affinity Constant (pA_2) of Phentolamine Determined in Aortae from Rats Diabetic for 14-20 Days and Corresponding Age-Matched Control Rats in the Presence of 0.20 mM External Ca^{++} Concentration and from Rats Diabetic for 28-35 Days and Corresponding Age-Matched Control Rats in the Presence of 2.50 mM External Ca^{++} Concentration

Values represent the mean $\pm$ S.E.M. N denotes number of different aortic rings contained in a group. There was no significant difference between the pD_2 of phenylephrine of control and diabetic aortic rings in each age group.

There was also no significant difference between the pA_2 of phentolamine obtained from control and diabetic aortic rings in each age group.

Table 3

Group	N	External Ca^{++} Concentration			
		0.20 mM Ca^{++}		2.50 mM Ca^{++}	
		pD_2	pA_2	pD_2	pA_2
Control (8-9 weeks old)	7	7.19±0.07	7.89±0.08		
Diabetic (14-20 days diabetic) (8-9 weeks old)	8	6.94±0.10	7.70±0.07		
Control (10-11 weeks old)	10			7.67±0.06	8.07±0.05
Diabetic (28-35 days diabetic) (10-11 weeks old)	12			7.59±0.09	7.97±0.06

E. Relaxant Responses of Aortae Obtained from Diabetic (28-35 Days following Streptozotocin Injection) and Control (28-35 Days following Vehicle Injection) Rats to Isoproterenol in the Presence of 2.50 mM External Ca^{++} Concentration and Phentolamine

When relaxant responses were plotted as a percent of assumed maximal possible relaxation, that is, relaxation of the contracted tissue (5-HT-induced contraction) back to the baseline tension, the degree of relaxation caused by isoproterenol was markedly increased with the diabetic state. Aortae from diabetic rats relaxed to 130.6 $\pm$ 5.4% of the assumed maximal relaxation, whereas tissues from control rats produced a relaxation equivalent to 84.9 $\pm$ 12.1% of the assumed maximal relaxation (Figure 34).

F. ^{45}Ca Efflux from Aortae Obtained from Diabetic (28-35 Days following Streptozotocin Injection) and Control (28-35 Days following Vehicle Injection) Rats

When the data from the ^{45}Ca washout experiments were expressed as the percentage of radioactivity remaining in the muscle after each 2-min interval and were plotted as desaturation curves, there was no significant difference between the data from the diabetic (28-35 days) rats and the data from the control rats (Figure 35). Plots of percentage radioactivity in the tissues versus time were curvilinear and were resolved by end-tail subtraction (Riggs, 1970) into three linear components of loss from

Figure 34. *Concentration-effect curves for the relaxant effects of isoproterenol on aortae from rats diabetic for 28-35 days and corresponding age-matched control rats in the presence of 2.50 mM external Ca^{++} concentration*

Relaxant responses were plotted as percent of the assumed maximal possible relaxation, that is, relaxation of the contracted aortic ring (5-HT-induced contraction) back to the baseline tension.

The aortic rings were contracted with 5-HT (EC85). Diabetic tissues were contracted with 2.25×10^{-5} M 5-HT and control tissues were contracted with 1×10^{-5} M 5-HT.

Each point represents the mean of 6-8 different aortic rings. The vertical bars represent the S.E.M.

* Denotes significant difference at least at the $p < 0.05$ level from control aortae.

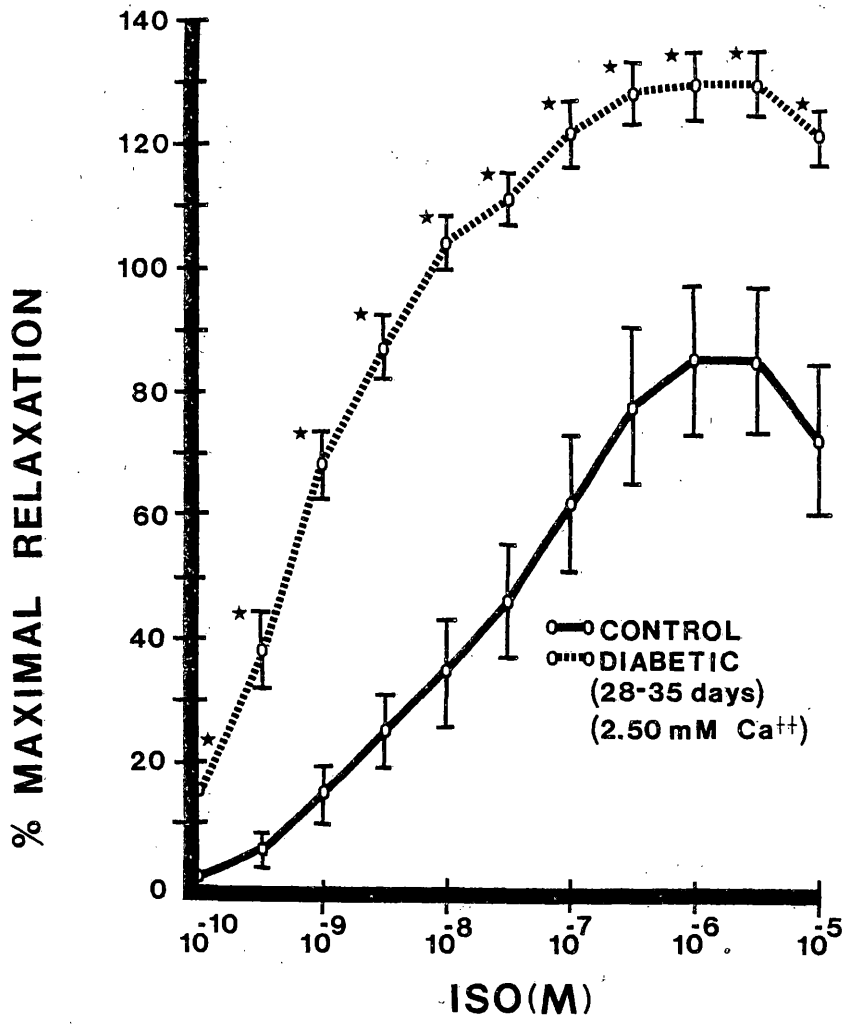
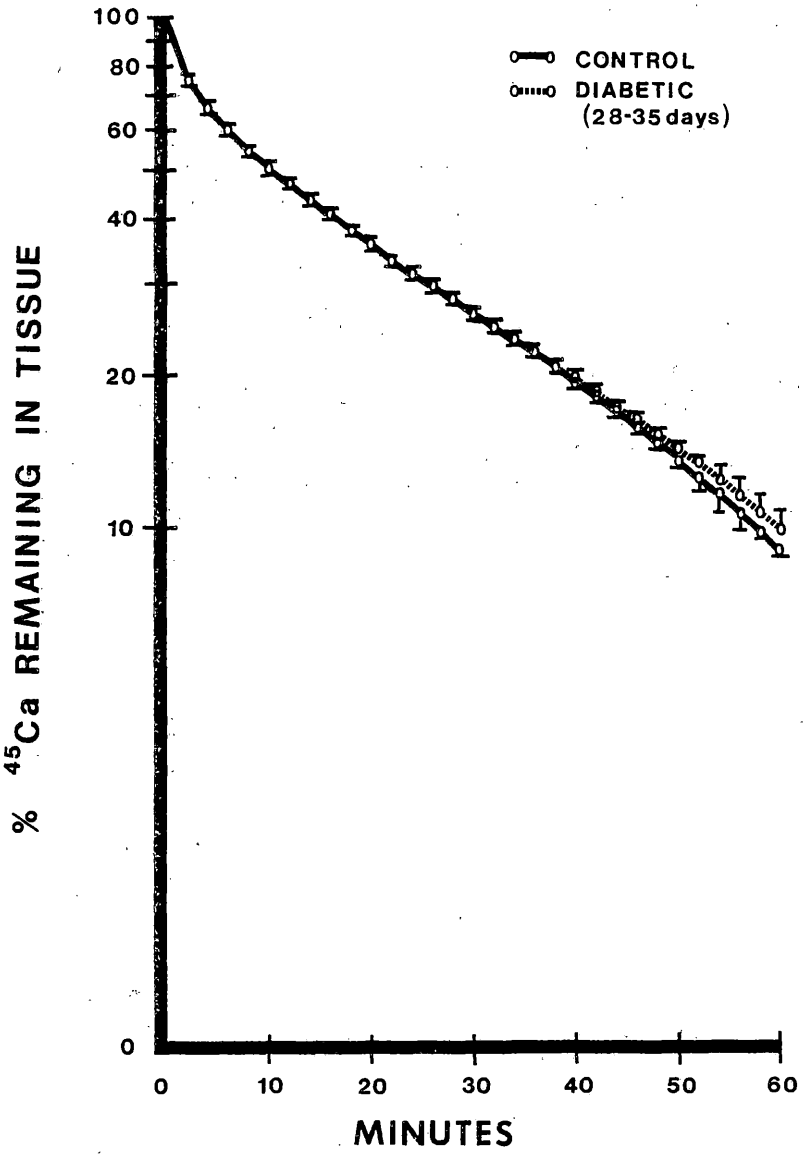

% MAXIMAL RELAXATION
140
120
100
80
60
40
20
0
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-5}
ISO(M)
CONTROL
DIABETIC
(28-35 days)
(2.50 mM Ca^{++})

Figure 35. *^{45}Ca efflux from aortae obtained from rats diabetic for 28-35 days and corresponding age-matched control rats*

Each point represents the mean of 7 different aortic rings. The vertical bars represent the S.E.M. There was no significant difference between diabetic and control tissues.


100
80
60
40
20
10
0
% 45Ca REMAINING IN TISSUE
CONTROL
DIABETIC
(28-35 days)
0
10
20
30
40
50
60
MINUTES

tightly bound, intermediate, and labile ^{45}Ca pools. Rate constants (k_s, k_i, k_f) of these "slow," "intermediate," and "fast" components were taken as $0.693/t_{\frac{1}{2}}$ ($t_{\frac{1}{2}}$ = half-time read from plot) (Carrier and Jurevics, 1973). Table 4 illustrates that there was no significant difference between the diabetic aortae (28-35 days) and control aortae with reference to their respective component relative size values, $t_{\frac{1}{2}}$ values, and k values.

G. Body Weights and Heart Weights Obtained from Diabetic (Streptozotocin Injected) and Control (Vehicle Injected) Rats

1. Body Weight and Heart Wet Weights

a. Weights Obtained from Rats 14-20 Days following Injection

The body weight of the diabetic rats (14-20 days) (207 $\pm$ 12 g) was significantly less than the body weight of the control rats (294 $\pm$ 80 g) (Table 5). The heart wet weight, right ventricle wet weight, and left ventricle wet weight also were significantly decreased in the diabetic animal. When the parameters of left ventricle wet weight/body weight, left ventricle wet weight/heart wet weight, and heart wet weight/body weight were compared in the diabetic versus the control rat, the parameters were not significantly different.

Table 4

Relative Sizes, Half-times, and Rate Constants of the Three Linear Components Determined in Aortae from Rats Diabetic for 28-35 Days and Corresponding Age-matched Control Rats

Each group contained 7 different aortic rings. There was no significant difference between diabetic and control aortic rings.

Table 4

Group	Control (10-11 weeks old)	Diabetic (28-35 days diabetic) (10-11 weeks old)
Fast Component		
Relative size (% ± S.E.M.)	16.29 ± 1.12	17.43 ± 1.10
$t_{\frac{1}{2}}$ (min ± S.E.M.)	0.73 ± 0.09	0.75 ± 0.06
k_f (min^{-1} ± S.E.M.)	1.05 ± 0.14	0.96 ± 0.08
Intermediate Component		
Relative size (% ± S.E.M.)	14.93 ± 1.14	16.14 ± 0.61
$t_{\frac{1}{2}}$ (min ± S.E.M.)	2.64 ± 0.29	3.11 ± 0.27
k_i (min^{-1} ± S.E.M.)	0.29 ± 0.05	0.23 ± 0.02
Slow Component		
Relative size (% ± S.E.M.)	68.79 ± 1.81	66.43 ± 1.23
$t_{\frac{1}{2}}$ (min ± S.E.M.)	21.71 ± 1.00	22.68 ± 0.77
k_s (min^{-1} + S.E.M.)	0.032± 0.002	0.031± 0.001

Table 5

Body Weights and Heart Wet Weights Obtained from Rats Diabetic for 14-20 Days and 28-35 Days and Corresponding Age-matched Control Rats

N denotes number of different rats contained in each rat group. Values represent the mean ± S.E.M.

Wt. = Weight
RV = Right Ventricle
LV = Left Ventricle

* Denotes significant difference at least at the $p < 0.05$ level from control rats in the same age group.

** Denotes significant difference at least at the $p < 0.05$ level from the younger group of rats (control group versus control group and diabetic group versus diabetic group).

Table 5

Rat Group	Control (8-9 weeks old)	Diabetic (14-20 days diabetic; 8-9 weeks old)	Control (10-11 weeks old)	Diabetic (28-35 days diabetic; 10-11 weeks old)
N	10	10	11	10
Body Wt. (g)	294 ± 8	207 ± 12*	382 ± 8**	247 ± 17*
Heart Wet Wt. (mg)	1130 ± 40	833 ± 39*	1318 ± 40**	957 ± 56*
RV Wet Wt. (mg)	205 ± 11	143 ± 7*	228 ± 7	166 ± 10*
LV Wet Wt. (mg)	761 ± 25	553 ± 26*	915 ± 31**	651 ± 41*
$\frac{\text{LV Wet Wt.}}{\text{Body Wt.}}$ $(\frac{mg}{g})$	2.59 ± 0.05	2.69 ± 0.04	2.39 ± 0.04**	2.65 ± 0.05*
$\frac{\text{LV Wet Wt.}}{\text{Heart Wet Wt.}}$ $(\frac{mg}{mg})$	0.674± 0.006	0.665± 0.006	0.694± 0.008**	0.680± 0.006
$\frac{\text{Heart Wet Wt.}}{\text{Body Wt.}}$ $(\frac{mg}{g})$	3.85 ± 0.07	4.05 ± 0.10	3.44 ± 0.04**	3.91 ± 0.10*

b. Weights Obtained from Rats 28-35 Days following Injection

The diabetic rats (28-35 days) weighed significantly less (247 ± 17 g) than the control rats (382 ± 8 g) (Table 5). In addition the heart wet weight, right ventricle wet weight, and left ventricle wet weight of the diabetic rats were significantly less than the corresponding wet weights of the diabetic rats. The parameter of left ventricle wet weight/body weight was significantly greater in the diabetic rat while the parameters of left ventricle wet weight/heart wet weight and heart wet weight/body weight were not significantly different for diabetic and control rats.

c. Weight as a Function of Age and Duration of Diabetes

The older control rats (10-11 weeks old) (382 ± 8 g) weighed significantly more than the younger control rats (8-9 weeks old) (294 ± 8 g) (Table 5). The same was not true for the corresponding diabetic rats. The younger diabetic rats (14-20 days, 8-9 weeks old) weighed 207 ± 12 g, while the older diabetic rats (28-35 days, 10-11 weeks old) weighed 247 ± 17 g. Heart wet weight and left ventricle wet weight for the

older control animals was significantly greater than for the younger control animals, but right ventricle wet weight was not significantly elevated. There was no significant difference between the younger and older diabetic groups with respect to heart wet weight, right ventricle wet weight, and left ventricle wet weight. The parameters of left ventricle wet weight/body weight and heart wet weight/body weight were significantly less for the older control group than for the younger control group. The parameter of left ventricle wet weight/heart wet weight was significantly greater for the older control rats than for the younger control rats. Left ventricle wet weight/body weight, left ventricle wet weight/heart wet weight, and heart wet weight/body weight were parameters that did not differ significantly between younger and older diabetic rats.

2. Body Weight and Heart Dry Weights

   a. Weights Obtained from Rats 14-20 Days following Injection

      The control rats had significantly greater calculated heart dry weight, right ventricle dry weight, and left ventricle dry weight than the diabetic rats (14-20 days) (Table 6). The

Table 6

Body Weights and Heart Dry Weights Obtained from Rats Diabetic for 14-20 Days and 28-35 Days and Corresponding Age-matched Control Rats

N denotes number of different rats contained in each rat group. Values represent the mean ± S.E.M.

Wt. = Weight
RV = Right Ventricle
LV = Left Ventricle

* Denotes significant difference at least at the $p < 0.05$ level from control rats in the same age group.

** Denotes significant difference at least at the $p < 0.05$ level from the younger group of rats (control group versus control group and diabetic group versus diabetic group).

Table 6

Rat Group	Control (8-9 weeks old)	Diabetic (14-20 days diabetic; 8-9 weeks old)	Control (10-11 weeks old)	Diabetic (28-35 days diabetic; 10-11 weeks old)
N	10	10	11	10
Body Wt. (g)	294 ± 8	207 ± 12*	382 ± 8**	247 ± 17*
Calculated Heart Dry Wt. (mg)	214 ± 6	157 ± 7*	268 ± 8**	191 ± 13*,**
RV Dry Wt. (mg)	39 ± 1	27 ± 1*	47 ± 2**	33 ± 2*,**
LV Dry Wt. (mg)	157 ± 5	115 ± 5*	199 ± 7**	142 ± 9*,**
$\frac{\text{LV Dry Wt.}}{\text{Body Wt.}}$ $(\frac{mg}{g})$	0.54 ± 0.01	0.56 ± 0.01	0.52 ± 0.01	0.58 ± 0.01*
$\frac{\text{LV Dry Wt.}}{\text{Calculated Heart Dry Wt.}}$ $(\frac{mg}{mg})$	0.735± 0.005	0.733± 0.004	0.740± 0.006	0.744± 0.008
$\frac{\text{Calculated Heart Dry Wt.}}{\text{Body Wt.}}$ $(\frac{mg}{g})$	0.73 ± 0.02	0.76 ± 0.02	0.70 ± 0.01	0.78 ± 0.02*

parameters of left ventricle dry weight/body weight, left ventricle dry weight/calculated heart dry weight, and calculated heart dry weight/body weight obtained from the diabetic rats did not differ from the same parameters measured from the control rats.

b. Weights Obtained from Rats 28-35 Days following Injection

Calculated heart dry weight, right ventricle dry weight, and left ventricle dry weight were significantly greater when obtained from the control animals than when obtained from the diabetic animals (28-35 days) (Table 6). Left ventricle dry weight/body weight and calculated heart dry weight/body weight were two parameters which were significantly greater when obtained from the diabetic rats than when obtained from the control rats. Control rat left ventricle dry weight/calculated heart dry weight was not significantly different from diabetic rat left ventricle dry weight/calculated heart dry weight.

c. Weight as a Function of Age and Duration of Diabetes

Calculated heart dry weight, right ventricle dry weight, and left ventricle dry weight were significantly greater when obtained from the

older diabetic rat group (28-35 days, 10-11 weeks old) than when obtained from the younger diabetic rat group (14-20 days, 8-9 weeks old) (Table 6). This phenomenon occurred at the same time that the diabetic body weights did not differ significantly. Also calculated heart dry weight, right ventricle dry weight, and left ventricle dry weight were significantly greater when obtained from the older control group (10-11 weeks old) than when obtained from the younger control group (8-9 weeks old). The control rat body weights were significantly greater for the older group than for the younger group. Left ventricle dry weight/body weight, left ventricle dry weight/calcualted heart dry weight, and calculated heart dry weight/body weight were three parameters that did not differ significantly for younger diabetic group versus the older diabetic group or for the younger control group versus the older control group.

DISCUSSION

Evidence for the successful inducement of experimental diabetes in the rats used in the reported studies included elevated serum glucose levels, and no weight gain, or a much slower rate of weight gain with age than control rats. Additional evidence which was observed, but not quantitated, was polyuria, polydipsia, and polyphagia. Histological examination of pancreatic tissue obtained from 37 week old rats which had been diabetic for 31 weeks revealed the appearance of decreased volume of the islets of Langerhans (Figures 1-4). This effect is similar to that reported by Junod *et al.* (1967) as early as 7 hrs after administration of 65 mg/kg streptozotocin. Arison *et al.* (1967) observed a similar effect one month after streptozotocin injection. Differentiation of the cell types of the islet by the staining procedure (hematoxylin and eosin) used in the reported study was not possible. Gross examination of all diabetic rats and histological examination of the pancreatic tissue obtained from the 37 week old rats which had been diabetic for 31 weeks did not reveal any pancreatic tumors present.

In the reported study, evidence was presented which demonstrated that vascular reactivity is differentially altered in response to NE, KCl, and 5-HT, while vascular reactivity is essentially unaltered in response to PE in

experimental diabetes (Owen and Carrier, 1978, 1979a, 1979b, 1979c, 1979d). The increased responsiveness or sensitivity of aortae from streptozotocin-induced diabetic rats to the neurotransmitter NE was related to the duration of the diabetic state and to the extracellular Ca^{++} concentration. The decreased sensitivity of aortae from diabetic rats to 5-HT also was related to the duration of the diabetic state and to the extracellular Ca^{++} concentration, but, the decreased sensitivity of aortae from diabetic rats to KCl was related only to the extracellular Ca^{++} concentration.

Aortae from rats which were diabetic for 14-20 days demonstrated an increase in sensitivity to NE in low levels of external Ca^{++} (Figures 5 and 6). Effector sensitivity expressed as mean log EC50 values was determined from cumulative dose-response relationships. The diabetic aortae were supersensitive to NE as reflected by the significant decrease in the mean log EC50 (Figure 6) when the bathing media contained 0.20, 0.40, and 0.80 mM Ca^{++}. However, in the presence of 2.50 mM Ca^{++}, there was no difference between the control and diabetic aortae.

The phenomenon of supersensitivity to NE in vascular smooth muscle is recognized (Carrier and Shibata, 1977) and such a condition can be elicited in many ways. Interference or impairment of normal adrenergic innervation (surgical or chemical denervation) results in an increase in the sensitivity of the effector cells to NE (Trendelenburg, 1966;

Shibata *et al.*, 1972; Carrier and Shibata, 1977). Postjunctional supersensitivity or a true increase in the sensitivity of the effector is seen after treatment with reserpine (Carrier and Holland, 1965; Hudgins and Fleming, 1966; Carrier and Jurevics, 1973). Prejunctional supersensitivity of a tissue is seen after the application of cocaine, which blocks neuronal uptake of catecholamines, or corticosterone, which blocks extraneuronal uptake of catecholamines. In the case of prejunctional supersensitivity the responses to specific agonists are greater due to an increase in their effective concentration in the vicinity of the receptors (Carrier and Shibata, 1977).

The influence of extraneuronal uptake of NE in this present study has not been determined. The development of supersensitivity could be demonstrated only in the 14-20 day diabetic rats and was related to the external Ca^{++} concentration. Therefore it seems unlikely that the observed phenomenon reflects alterations in the extraneuronal uptake mechanism.

Clinical observations show that diabetes may be accompanied by neuropathy of the sympathetic nervous system which results in an impaired nerve function. Functional abnormalities of the autonomic nervous system in long-term diabetes are well known. They include postural fall in blood pressure (Rundles, 1945), abnormal reflex bradycardia after a Valsalva maneuver (Sharpey-Schafer, 1960), reduced levels

of catecholamines in blood (Christensen, 1972), and cardiovascular tissue (Neubauer and Christensen, 1976), and a reduction in spontaneous movements of the pupil of the eye (Gundersen, 1974). This lends support for the hypothesis that the supersensitivity to NE observed in this study may be analogous to denervation sensitization. On the other hand, Brody and Dixon (1964) concluded that their observation of hyperresponsiveness of perfused hindquarters of diabetic rats (alloxan treated) to NE was not related to a loss of adrenergic function. In addition, the rat aorta which was employed in the present study is essentially devoid of adrenergic nerves (Patil *et al.*, 1972; Fleisch, 1974) and insensitive to cocaine blockage (Maling *et al.*, 1971). In view of the above discussion and the calcium dependency of the supersensitivity phenomenon demonstrated in this study, the increased sensitivity to NE in the diabetic rat is probably not related to impairment of adrenergic nerve fibers.

It is thought that endogenous chemical transmitters have a trophic effect on the postjunctional tissues. When this is lost, the tissue, in a compensatory manner, increases in sensitivity. In skeletal muscle, interruption of transmitter release will induce a spread of receptor area over the entire skeletal muscle cell (Thesleff, 1960) and an increase in sensitivity of the muscle will occur. In vascular smooth muscle, reserpine-induced supersensitivity is not the result of receptor spread (Carrier and Shibata,

1977). It appears to result from some alteration in the physiology of the smooth muscle cell. The present data is compatible with this concept of supersensitivity in vascular muscle. Since our preparation is free of adrenergic innervation, there is no apparent interruption of transmitter release. In addition, the responses of the diabetic (14-20 days) aortic tissues were supersensitive to NE only when the external Ca^{++} concentration was less than 2.50 mM. However contractile responses to PE, another predominately α-adrenergic receptor agonist, of the aortae obtained from the diabetic rats (14-20 days) were not different from the contractile responses to PE of the control aortae in the presence of 0.20 mM external Ca^{++} concentration. This is indicated by the similar pD_2 of phenylephrine and the similar pA_2 of phentolamine for the diabetic and control rats. Therefore, it is unlikely that the demonstrated increase in sensitivity of the effector cells to NE is a result of an increase in α-adrenergic receptor area. Even though both NE and PE are predominately α-adrenergic receptor agonists, differences have been demonstrated in the relative sensitivity of the fast (F-) and slow (S-) components of contraction for these two agonists (Brodie *et al.*, 1959). For example, the F-component appeared to be more sensitive (lower threshold) to NE, whereas the S-component was more sensitive to PE. Therefore, it is not unreasonable to see differences in the contractile response induced by these two agents.

Bohr (1964a) found that substitution of a calcium-free solution for a physiological salt solution resulted in an initial potentiation of the F-component and a marked depression or elimination of the S-component in response to epinephrine. He also observed that increases in calcium concentration above 1.6 mM depressed the F-component, but had little or no effect on the S-component. Since the increase in sensitivity of the diabetic tissues to NE occurred only in low levels of external Ca^{++}, the present data indicate that the diabetic tissues utilize or handle extracellular Ca^{++} in a different manner for NE-induced vascular contractions.

Aortae from rats which were diabetic (28-35 days) for a longer period of time demonstrated a greater maximum force developed than the age-matched controls in response to NE (Figure 10). In contrast to the younger diabetic group, there was no change in the mean log EC50 values (Figures 8 and 9). The greater maximum contractile force of the older diabetic tissue in response to NE occurred at all external Ca^{++} concentrations used. There was a slight tendency, though not significant, for the older diabetic tissues to develop less maximum contractile force in response to PE in the presence of 2.50 mM external Ca^{++} concentration (Figure 29).

The maximum force developed in response to NE by the older diabetic tissues (28-35 days) was significantly greater than the response of the younger diabetic tissues

(14-20 days) and the corresponding age-matched controls (Figure 11). These findings occurred at all of the various extracellular Ca^{++} concentrations used. These results cannot be interpreted as an age-dependent increase in vascular tone, since there was no significant difference between the two different age groups of controls. The older control group showed the tendency, though not significant, to develop a greater degree of force than the younger group. However, the age difference between the two control groups is not of sufficient magnitude to detect the age-related increase in responsiveness of NE contractions as reported by Cohen and Berkowitz (1976). The increase in maximum force may reflect changes in β-adrenergic receptor activity. It has been shown that β-receptor mediated relaxation of rat aorta is decreased with increasing age of the animals (Fleisch *et al.*, 1970; Cohen and Berkowitz, 1974). Relaxant responses of aortae obtained from diabetic rats (28-35 days) to ISO in the presence of 2.50 mM external Ca^{++} concentration were increased markedly with the diabetic state when they were plotted as a percent of the assumed maximal possible relaxation, that is, relaxation of the contracted tissue (5-HT-induced contraction) back to the baseline force. Difficulties arise in the interpretation of the results from the relaxant studies due to the influence of initial contractile force and spontaneous tone on vascular relaxation. Diabetic and control tissues were contracted with an EC85

concentration of 5-HT. This resulted in a greater contractile force being developed in the control tissue. Cohen and Berkowitz (1974) have shown that as contractile force is increased, the percent relaxation is decreased. Also arterial responsiveness to ISO would appear to be less in the control tissues if the aortae from the control animals had an intrinsically greater spontaneous tone than aortae from the diabetic animals. A maximum relaxation of 130.6 $\pm$ 5.4% for the diabetic tissue indicates that the diabetic tissue does have a spontaneous tone. A maximum relaxation of 84.9 $\pm$ 12.1% for the control tissue did not answer the question of how much, if any, spontaneous tone the control tissue possesses. Further studies which take into account the influence of initial contractile force and spontaneous tone on vascular relaxation are required to determine whether the increase in maximum force developed to NE reflects change in β-adrenergic receptor activity.

The source of Ca^{++} utilized for vascular contraction has been shown to vary for different agonists (Hiraoka *et al.*, 1968; Hudgins and Weiss, 1968; Greenberg *et al.*, 1973; Cohen and Berkowitz, 1976). The currently known facts support the concept that NE contractions are primarily dependent upon an intracellular source of Ca^{++} and to a less extent on extracellular Ca^{++}, while KCl-induced contraction and 5-HT-induced contraction exhibit a marked dependence on extracellular Ca^{++}.

In the present study NE-induced contractions were unaltered in both age groups of control tissues and the young diabetic group when external Ca^{++} was lowered from 2.50 mM to either 0.80 or 0.40 mM. However, when the extracellular Ca^{++} was reduced to 0.20 mM the NE-contractions were significantly depressed (Figure 11). These findings are in agreement with previous reports (Hudgins and Weiss, 1968; Cohen and Berkowitz, 1976) which indicate that NE contractions are not primarily dependent upon an extracellular source of Ca^{++}. While the NE-induced contractions of the older diabetic tissues also seemed to be primarily dependent upon an intracellular source of Ca^{++}, they were more susceptible to changes in external Ca^{++} than control responses (Figure 11). There was a significant decrease in the maximum force in response to NE when the external Ca^{++} was reduced to both 0.40 mM and 0.20 mM. This relationship may be interpreted to mean that the importance of extracellular Ca^{++} for NE contractions is altered in diabetes.

Even though the maximal contractile force appeared decreased, no diabetic state dependent change in maximal contractile force generated in response to KCl could be demonstrated by a comparison of maximal contractile force generated by either diabetic (14-20 days or 28-35 days) tissue versus its corresponding age-matched control tissue in the presence of 0.20 mM or 2.50 mM external Ca^{++} concentration (Figures 13 and 16). Also no age dependent reduction in maximal contractile force generated in response to

KCl could be demonstrated in either the diabetic or control tissue for the age groups studied (Figure 18). A subsensitivity of the diabetic tissue to KCl was noted when the mean log EC50 of the diabetic tissue and the mean log EC50 of the control tissue were compared in the presence of 2.50 mM Ca^{++} (Figures 12 and 15). This subsensitivity of diabetic tissue to KCl could not be demonstrated in the presence of 0.20 mM Ca^{++} (Figures 14 and 17).

While the calcium dependency of the maximum contractile response to 5-HT in the control aortae appears to increase with age (Cohen and Berkowitz, 1976), this is not true for the calcium dependency of the maximum contractile response in the diabetic aortae (Figure 25). The maximum contractile response to 5-HT in the older diabetic group (28-35 days) in the presence of either 0.20 or 2.50 mM Ca^{++} is significantly less than the maximum contractile response to 5-HT in the corresponding age-matched control group (Figure 23). A subsensitivity of both age groups of diabetic tissue to 5-HT was noted when the log EC50 of the diabetic tissue and the log EC50 of the age-matched control tissue was compared in the presence of 2.50 mM external Ca^{++} concentration (Figures 19 and 22). In 0.20 mM external Ca^{++} this subsensitivity was noted in the younger diabetic tissue group (14-20 days) but not in the older diabetic tissue group (28-35 days) (Figures 21 and 24). Determination of the pA_2 value for a 5-HT antagonist would be necessary to test if there has been any alteration of the interaction of

the 5-HT receptor agonist with the receptor-calcium complex or if there has been an alteration in the receptor-calcium complex itself with the diabetic state.

Another factor one must consider is the structural components of vascular muscle. Bevan (1976) has demonstrated in hypertensive animals the existence of a similar time course of increased arterial pressure and proliferation of smooth muscle cells. Since smooth muscle cell proliferation is one of the earliest structural observations in atherosclerosis (Morrison and Scott, 1977), it is possible that an increased maximum force produced in the diabetic tissue might be due to proliferation of smooth muscle cells. However, an increased maximum contractile force was only developed in response to NE in the diabetic tissue. 5-HT actually caused a decreased contractile force to be developed in the diabetic aortae, while maximum contractile force in response to KCl and PE was not altered. This indicates that smooth muscle proliferation does not account for the contractile force generated by the different agonists in diabetic aortae.

^{45}Ca efflux from aortae obtained from diabetic (28-35 days) rats plotted as desaturation curves did not differ from ^{45}Ca efflux from aortae obtained from control rats (Figure 35). The diabetic state did not affect the component relative size value, $t_{\frac{1}{2}}$ value, or k value (Table 4). Studies examining Ca^{++} uptake and Ca^{++} efflux with the

various contractile agonists (NE, KCl, 5-HT, and PE) would be required to further define Ca^{++} related contractions in diabetes.

When the work requirements of the heart are increased by pressure or volume overload, an increase in heart mass occurs (Fanburg, 1970; Rabinowitz, 1972). Since the maximum contractile force developed in response to the neurotransmitter NE by the diabetic aortae (28-35 days) was elevated and also since insulin is known to decrease the plasma volume of diabetics without complications (Mackay *et al.*, 1978) it was believed that heart mass might be increased in the diabetic rats.

Procedures to normalize body weight were resorted to in order to assess cardiac enlargement since both groups of diabetic rats (14-20 days and 28-35 days) weighed significantly less than the corresponding age-matched groups of control rats (Table 5). Left ventricle wet weight/body weight, heart wet weight/body weight, left ventricle dry weight/body weight, and calculated heart dry weight/body weight were body weight normalized parameters which indicated that the diabetic (28-35 days) heart mass was greater than the control heart mass (Tables 5 and 6). A further analysis of the wet weight data led to questions concerning the validity of using the wet weight/body weight normalized parameters as evidence that the diabetic heart mass was greater than the control heart mass. When the wet

weight/body weight normalized parameters of the younger control group were compared to the wet weight/body weight normalized parameters of the older control group and the wet weight/body weight normalized parameters of the younger diabetic group (14-20 days) were compared to the wet weight/body weight normalized parameters of the older diabetic group (28-35 days), the older groups weighed less than the younger groups. The older control group weighed significantly less than the younger control group, while the decreased weight of the older diabetic group was not significant. However, an analysis of the dry weight data seemed to confirm that the diabetic (28-35 days) heart mass was greater than the control heart mass. The dry weight/body weight normalized parameters of the younger control group were greater, but not significantly greater than the dry weight/body weight normalized parameters of the older group. The dry weight/body weight normalized parameters of the younger diabetic group were less, but not significantly less than the dry weight/body weight normalized parameters of the older group. Also the right ventricle dry weight, left ventricle dry weight, and calculated heart dry weight of the older diabetic rats were significantly greater than the right ventricle dry weight, left ventricle dry weight, and calculated heart dry weight of the younger diabetic rats while the body weight of the older diabetic rats was greater, but not significantly greater, than the body weight

of the younger diabetic rats. Determination of blood pressure, protein and DNA content, and a study of the incorporation of tritiated thymidine into cardiac tissue would be of value in confirming and expanding the conclusions about increased heart mass derived from this study.

In conclusion, the present study provided evidence that vascular responses to NE, KCl, and 5-HT are differentially altered, while vascular responses to PE are essentially unaltered in experimental diabetes. This study also indicates that there is a change in the relative importance of extracellular Ca^{++} for NE, KCl, and 5-HT vascular contractions in experimental diabetes. In the earlier stages of the diabetic state the aortae were supersensitive to NE in the presence of lowered Ca^{++} concentrations. Later the aortae demonstrated a generalized increased responsiveness in maximum force developed to NE. Heart weight mass appeared to be increased in the older diabetic rats (28-35 days). Both age groups (14-20 days diabetic, 8-9 weeks old and 28-35 days diabetic, 10-11 weeks old) of the diabetic aortae were subsensitive to KCl in the presence of 2.50 mM Ca^{++} concentration. Both age groups of diabetic aortae were subsensitive to 5-HT in the presence of 2.50 mM Ca^{++}, while only the younger age group was subsensitive to 5-HT in the presence of 0.20 mM external Ca^{++}. The older group of diabetic aortae demonstrated a generalized decreased responsiveness in maximum contractile force developed to

5-HT. The relationship between the differential alterations of vascular responses in the diabetic rats to contractile agonists cannot be defined at the present time. However, it may reflect progressive cellular changes associated with the disease state. Nevertheless, all alterations exhibited a dependency upon extracellular Ca^{++}.

SUMMARY

1. Aortae obtained from rats diabetic for 14-20 days were supersensitive in contractile response to NE in the presence of 0.20, 0.40, and 0.80 mM external Ca^{++}, but not in the presence of 2.50 mM external Ca^{++} concentration.
2. Aortae obtained from rats diabetic for 28-35 days showed a marked increase in maximum contractile force in response to NE in the presence of 0.20, 0.40, 0.80, and 2.50 mM external Ca^{++} concentration.
3. The maximum contractile force developed in response to NE was greater at all external Ca^{++} concentrations in the older diabetic group (28-35 days diabetic, 10-11 weeks old) as compared to the younger diabetic group (14-20 days diabetic, 8-9 weeks old). There was no significant difference when the two control groups (10-11 weeks old versus 8-9 weeks old) were compared.
4. Maximum contractile force developed in response to NE in 2.50 mM external Ca^{++} was compared to the maximum contractile force obtained from the same tissue in 0.20, 0.40, and 0.80 mM external Ca^{++}. A significant decrease was detected for the control rats (8-9 weeks old and 10-11 weeks old) and for the younger diabetic rats (14-20 days diabetic, 8-9 weeks old) in the

presence of 0.20 mM external Ca^{++}. The older diabetic rats exhibited a greater dependency on extracellular Ca^{++} as demonstrated by the significant decrease in maximum contractile force development both in 0.40 and 0.20 mM external Ca^{++}.

5. Aortae obtained from diabetic rats (14-20 days and 28-35 days) were subsensitive in contractile response to KCl in the presence of 2.50 mM external Ca^{++}, but not in the presence of 0.20 mM external Ca^{++} concentration.
6. Aortae obtained from diabetic rats (14-20 days) were subsensitive in contractile response to 5-HT in the presence of 0.20 and 2.50 mM external Ca^{++} concentration.
7. Aortae obtained from diabetic rats (28-35 days) were subsensitive in contractile response to 5-HT in the presence of 2.50 mM Ca^{++}, but not in the presence of 0.20 mM external Ca^{++} concentration.
8. Aortae obtained from rats diabetic for 28-35 days showed a marked decrease in maximum contractile force in response to 5-HT in the presence of 0.20 and 2.50 mM external Ca^{++} concentration.
9. The contractile force developed in response to 5-HT was significantly greater in the presence of 2.50 mM external Ca^{++} for the older control group (10-11 weeks old) than for the younger control group (8-9 weeks old). There was no significant difference when the older

control group was compared to the younger control group in the presence of 0.20 mM external Ca^{++}. Neither was there a significant difference when the two diabetic groups (14-20 days diabetic, 8-9 weeks old and 28-35 days diabetic, 10-11 weeks old) were compared in the presence of 0.20 mM external Ca^{++} or in the presence of 2.50 mM external Ca^{++} concentration.

10. Aortae obtained from diabetic rats (14-20 days) contracted similar to aortae obtained from control rats in response to PE in the presence of 0.20 mM external Ca^{++}. The pD_2 of phenylephrine and the pA_2 of phentolamine were not significantly different for the diabetic and control aortae.
11. Aortae obtained from diabetic rats (28-35 days) contracted similar to aortae obtained from control rats in response to PE in the presence of 2.50 mM external Ca^{++}. The pD_2 of phenylephrine and the pA_2 of phentolamine were not significantly different for the diabetic and control aortae.
12. ^{45}Ca efflux from aortae plotted as desaturation curves showed no significant difference between diabetic (28-35 days) and control tissue. Component relative size values, $t_{1/2}$ values, and k values were the same for diabetic and control aortae.
13. Left ventricle dry weight/body weight and calculated heart dry weight/body weight were two parameters which

were greater for the diabetic rats (28-35 days) than for the control rats. Also the right ventricle dry weight, left ventricle dry weight, and calculated heart dry weight of the older diabetic rats (28-35 days diabetic, 10-11 weeks old) were significantly greater than the right ventricle dry weight, left ventricle dry weight, and calculated heart dry weight of the younger diabetic rats (14-20 days diabetic, 8-9 weeks old), while the body weight of the older diabetic rats was greater, but not significantly greater, than the body weight of the younger diabetic rats.

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