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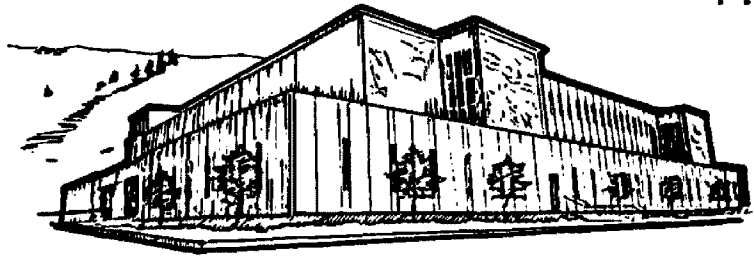
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Date: July 29, 1993



**EFFECTS OF PIOGLITAZONE ON CARDIOVASCULAR FUNCTION IN  
TYPE I AND TYPE II DIABETES MELLITUS.**

**By**

**Mahanandeeshwar Gattu**

**B.A.M.S., Osmania University, India, 1990**

**B.Sc., A.P. Open University, India, 1991**

**Presented in partial fulfillment of the requirements for the**

**Degree of Master of Science**

**University of Montana**

**1993**

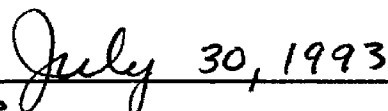
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**Mahanandeeshwar Gattu, M. S., July 1993**  
**Dept. of Pharmaceutical Sciences**

**Effects of Pioglitazone on Cardiovascular performance in both Type I and Type II Diabetes Mellitus (65 pp).**

**Director: Charles L. Eyer, Ph.D.** 

**Effects of pioglitazone on cardiovascular function were evaluated in both Type I and Type II diabetic animal models. Sprague Dawley rats and Zucker Diabetic Fatty rats were used in Type I and Type II diabetes studies respectively. Pioglitazone treatment increased the body weight and decreased the blood glucose levels in Type II diabetic animals, but did not alter either body weight or blood glucose levels in both control and Type I diabetic animals. Pioglitazone treatment did not alter the glycosylated hemoglobin values either in control or Type I diabetic rats.**

**Maximal contractile response to norepinephrine (NE) and potassium chloride (KCl) was decreased by pioglitazone in Type II diabetic and control animals. In Type I diabetic animals, vascular reactivity to KCl was decreased with pioglitazone treatment, but vascular reactivity to NE was unaltered. Combination of insulin and pioglitazone or insulin alone did not show any effects on vascular reactivity to NE. Pioglitazone treatment decreased the endothelial dependent relaxation in control animals, but endothelial independent relaxation was unaltered in both control and diabetic rats.**

**Coronary flow and double product were decreased in Type I diabetic animals. Pioglitazone administered to diabetic rats (DP) showed the highest mortality and the lowest values of heart rate, left ventricular developed pressure (LVDP), coronary flow and double product. Insulin treatment normalized the cardiac function in Type I diabetic animals, and also, the combination of insulin and pioglitazone treatment normalized the LVDP and double product in Type I diabetic animals.**

**The results of the present study suggests that the severity of myocardial ischemia and reperfusion (MI/R) injury is identical in both Type I diabetic and control animals. Neither pioglitazone nor insulin treatment protected against MI/R injury in either control or diabetic rats.**

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## **ACKNOWLEDGEMENTS**

I would like to thank the Chairman, faculty and staff of the Dept. of Pharmaceutical Science for their educational and financial support that enabled me to attain this milestone in my life. I am especially very grateful to my major advisor Dr. Charles L. Eyer and also to Dr. Jerry R. Smith, for their patience, precious directions, friendly guidance and continued encouragement throughout the course of this research. I would also like to thank the other members of the committee, Dr. Vernon R. Grund and Dr. James A. Walsh, for their investment of time and their suggestions.

Also, a special thanks to Dr. Vernon R. Grund and Heather B. Fraser for allowing me to use body weights and blood glucose data from Type II diabetic animals. I wish to thank Dr. Bryan D. Tarr for his assistance with the PC NONLIN computer program. James D. Hutchison and Heather B. Fraser also deserve thanks for the constructive suggestions they made in the preparation of this thesis.

Thanks to my parents for instilling in me the basic principles of life. Special thanks to my wife, Hima Bindu Gattu, for her patience and encouragement in my foul moods. Finally, I wish to express my great appreciation to my family for their love and encouragement.

## INTRODUCTION

### Diabetes Mellitus:

Diabetes mellitus has been recognized from antiquity. Indian and Chinese medical writings mentioned a syndrome of polyphagia, polydipsia, polyuria and glycosuria. As early as 1869, Langerhans, while still a medical student, described the pancreatic islet cells which now bear his name.

Diabetes mellitus is a disease of worldwide distribution. It is more frequent in some countries than others. In the United States there are approximately 14 million people with diabetes mellitus, close to seven percent of the population. As early as 1936, Himsworth suggested the existence of at least two forms of diabetes (Himsworth, 1936). However, the two types were not confirmed until the development of a bioassay for plasma insulin. It is well known that the disease is divided into two major subtypes, Type I (Insulin-Dependent Diabetes Mellitus) and Type II (Noninsulin-Dependent Diabetes Mellitus) (Weir et al, 1986; Lipson, 1986). Both types of diabetes are characterized by fasting hyperglycemia and glucose intolerance to a glucose challenge. However, the pathologies of the two appear to be distinctly different. Type I diabetes generally occurs in younger persons and is due to the loss of insulin production because of autoimmune destruction directed specifically towards the insulin

producing islet beta cells. The bulk of evidence indicates that autoreactive T lymphocytes initiate and oversee this beta cell specific immune destruction (Eisenbarth, 1986). In contrast, Type II diabetes is seen most frequently in older and overweight people and is associated with either dysfunction of insulin secretion, peripheral insulin resistance, or both (Van d'Heim et al, 1990).

#### **Diabetes and Vascular Sensitivity:**

Diabetics of either type are known to develop vascular complications which contribute to retinopathy (Robison et al, 1986), nephropathy, myopathy (Dvornik, 1987) and accelerated atherosclerosis (Christlieb, 1973).

Hyperglycemia is believed to be the major cause of diabetic vascular complications involving both macro and microvessels. It is unclear how hyperglycemia alters the metabolism and functions of vascular cells, although changes in nonenzymatic protein glycosylation, accumulation of intracellular sorbitol, and reduction of myo-inositol levels may be involved (Kennedy and Baynes, 1984; Greene et al, 1987). Weidmann et al (1979) suggested that some diabetic vasculopathies are a consequence of altered sensitivity to neurotransmitters and circulating neurohormones.

Investigators have attempted to assess the reactivity of the cardiovascular system in vivo in experimental diabetic rats. Christlieb (1974) observed an increased blood pressure response to infused angiotensin II and

norepinephrine in alloxan-diabetic rats. Kawashima et al (1978) demonstrated that STZ-diabetic rats were hypertensive. However, Jackson and Carrier (1980) and Kohler et al (1980) have demonstrated that STZ-diabetic rats are hypotensive 4-6 weeks after injection of the diabetogen and the blood pressure responses to norepinephrine (NE) and angiotensin II were blunted. Discrepancies observed in the literature may be related to the differences in the species of diabetic animals studied and the severity of the disease process.

Abnormal vascular reactivity to several vasoactive agents has been reported in chemically-induced in vitro diabetic animal experiments. The results of experiments in which vascular smooth muscle contractility to NE has been examined fall into two categories. First, in aortic preparations from diabetic rodents, diabetes was associated with a decreased maximal response and either no change or a decreased EC 50 value (Sullivan and Sparks, 1978; Pfaffman et al, 1982; Turlapaty et al, 1980). In contrast to the above observations, an increased maximal response to NE in aortic preparations from diabetic rats has been demonstrated by two other groups (Brody and Dixon, 1964; Owen and Carrier, 1985). Enhanced responses to selective alpha 1 and alpha 2 adrenergic agonists were demonstrated in aortas isolated from STZ-diabetic rats (Scarborough and Carrier, 1983 & 1984). MacLeod and McNeill (1985) found no change in

vascular reactivity to norepinephrine seven days after the onset of diabetes. However, 100 days after the onset of diabetes, they found an increased maximal contractile response to NE.

An increased vascular contractile response to the membrane depolarization by KCl has been reported in aortas isolated from STZ-induced diabetic animals (Owen and Carrier, 1979 & 1980). In contrast, other investigators have reported either decreased or unaltered vascular reactivity to KCl in aortas isolated from STZ diabetic rats. No data on vascular reactivity to NE and KCl in non-insulin dependent diabetic (Type II) have been reported.

Many factors play a role in the regulation of vascular muscle reactivity and tone. Calcium is critical for the initiation of contraction in vascular smooth muscle (Bohr, 1964). The contractile response to NE has been resolved into two components. The first component is attributed to the release of intracellular calcium from the sarcoplasmic reticulum and the second component is associated with influx of extracellular calcium (Karaki and Weiss, 1984). Enhanced vascular contraction in diabetes apparently results from an increased responsiveness to extracellular calcium (White and Carrier, 1988 & 1990; Sakai and Honda, 1987).

It is possible that increased responsiveness to norepinephrine in diabetes might be caused, at least in part, by denervation supersensitivity of the vasculature.

Denervation supersensitivity has been demonstrated after surgical or chemical sympathectomy (Winqvist and Bevan, 1979). Although changes in receptors may be involved (Davies et al, 1982), the decrease in neuronal uptake in the denervated tissue may lead to an increased local concentration of the adrenergic transmitters at the smooth muscle alpha-adrenoceptor, giving rise to an augmented contraction (Aprigliano and Hermsmeyer, 1976).

Scarborough and Carrier (1984) reported that enhanced norepinephrine responses of aortas from diabetic rats may be related primarily to changes in alpha-2 receptor function. They found no differences in the responses to selective alpha-1 agonists in aortas of STZ-diabetic rats.

A functional endothelial cell layer is required for the vasodilatory effects of several pharmacological agents. Removal of the endothelium greatly reduces or abolishes vascular smooth muscle relaxation induced by these agents. The vascular endothelium had been recognized as the important site which modulates the vascular smooth muscle tone (Furchgott and Zanadski, 1980). Endothelial derived relaxing factor (EDRF), one of the endothelium derived substances, has been recognized as an important modulator of vascular tone. Recently, endothelin-1 has been identified as a 21-residue peptide and shown to be a very potent vasoconstrictor (Yanagisawa et al, 1988). Ding et al (1991) reported that the relaxation response to an endothelium

dependent vasodilator, like carbachol, was significantly suppressed in diabetic aorta preparations whereas the relaxant effect of sodium nitroprusside, which does not require a functional endothelium, was not significantly changed. This may suggest either disturbances in the synthesis or an accelerated destruction of EDRF in the diabetic state.

#### **Diabetes Mellitus and Myocardial Function:**

Both type of diabetes mellitus are often associated with cardiovascular complications such as coronary artery lesions and diabetic cardiomyopathy, resulting in a higher incidence of myocardial infarction and congestive heart failure (Jaffe, 1989). Left ventricular systolic duration is abnormal in many diabetics showing no overt evidence of coronary heart disease or hypertension (Uusitupa et al, 1985). In addition to systolic dysfunction, reduced diastolic filling rate and a prolonged isovolumic relaxation are also frequently observed in diabetes (Pozzoli et al, 1984). Diabetic cardiac dysfunction may be due to deterioration of the microcirculation caused by abnormalities in vascular sensitivity and reactivity to neurotransmitters (Friedman, 1989). Another theory to explain cardiac dysfunction suggested by Garber and Neely (1983) is that abnormalities in conduction systems alter cardiac performance. A third explanation for cardiac dysfunction is due to abnormalities in the myocardium such



as depressed myocardial metabolism (Fevury et al, 1979), impaired tissue antioxidant status (Wohaieb and Godin, 1987) and decreased sarcolemmal enzyme activity (Pierce et al, 1983).

The precise subcellular mechanisms responsible for the diabetic cardiomyopathy are unknown. However, it has been suggested that elevated myocardial calcium content is directly involved in contractile dysfunction and cellular necrosis (Pierce, 1988). The mechanism of tissue calcium overload is a complex phenomenon, usually involving impairment in the movement of calcium by one or more calcium transporters (Weir, 1990). In the normally functioning myocardium, the sarcoplasmic reticular calcium pump serves as the primary mechanism for removal of calcium from the cytoplasm. Sarcoplasmic reticular function appears to be defective in diabetic myocardium with depressed ATP-dependent calcium transport and calcium stimulated ATPase activity (Ganguly et al, 1983). In addition, the diabetic myocardium exhibits a variety of abnormalities in sarcolemmal ion transport, including depression of  $\text{Na}^+\text{-H}^+$  (Pierce et al, 1990; Lagdic-Gassmann et al, 1988) and  $\text{Na}^+\text{-Ca}^{++}$  (Makino et al, 1987) exchange processes and inhibition of Ca ATPase (Heyliger et al, 1987) and Na-K ATPase activities (Pierce et al, 1990).

Diabetes results in several changes in cardiac metabolism. The most notable change is decreased glucose

transport into muscle cells by facilitative glucose transporter proteins (GLUT4). A decreased myocardial glycolytic flux is also observed in diabetics due to inhibition of the glycolytic enzyme phosphofructokinase. The activity of this key enzyme is not directly affected by diabetes; rather reduced flux through the enzyme occurs secondary to the accumulation of citrate, a potent inhibitor of phosphofructokinase (Randle et al, 1966).

The role of reactive oxygen radicals in determining myocardial ischemia and reperfusion injury (MI/R) now seems fairly well established (McCord, 1985; Van der Vusse, 1985). In addition to ischemic heart disease, other complications of diabetes suggested by experimental studies to involve oxygen radical damage include atherosclerosis (Fogelman et al, 1980), retinal damage (Crouch et al, 1978) and renal injury (Paller et al, 1984). Diabetes is known to have several effects on antioxidant status (Wohaieb and Godin, 1987). The levels of free radicals such as superoxide and hydroxyl radicals are controlled by various cellular mechanisms consisting of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX)/ oxidized glutathione reductase (GSSG-RD) systems] and nonenzymatic scavenger components (glutathione, vitamin C and vitamin E) (Simmons, 1984). Diabetes is associated with significantly increased activities of CAT, GSSG-RD and SOD in the pancreas and of CAT and GSSG-RD in the heart. On

the other hand, the liver of diabetic rats showed a generalized decrease in CAT, GSH-PX and SOD as well as in the levels of reduced glutathione (GSH). Diabetic kidney also showed decreases in CAT and SOD, but the activities of GSH-PX were increased. Insulin treatment reversed all of the alterations in tissue antioxidant status in Type I diabetic animals (Wohaieb and Godin, 1987).

Reperfusion of the myocardium after an ischemic period can produce MI/R injury. Reperfusion damage is evidenced by a sudden and marked increase in ultrastructural changes, enzyme release and calcium influx (Bourdillon et al, 1981). Various mechanisms have been suggested as triggers for reperfusion damage including 1) depletion of high energy phosphates (Jennings et al, 1978) and catecholamines (Gaudel et al, 1979), 2) accumulation of calcium (Nayler, 1981) and lysophosphoglycerides (Katz et al, 1983) and 3) activation of phospholipases (Chein et al, 1980). Whether alterations in myocardial metabolism in diabetics have any direct effects on MI/R is not clear. Several isolated heart studies report that diabetic hearts are more sensitive than normal hearts to ischemic injury (Haider et al, 1977; Palik et al, 1982), but this disparity in sensitivity was minimized by increasing the glucose concentration in the perfusate (Ingebretsen, 1980). Vogel and Apstein (1988) suggested that diabetic hearts are comparable to normal hearts with regard to the severity of MI/R injury. Higuchi

et al (1990) have shown that diabetic hearts were more vulnerable to hypoperfusion damage while normal hearts were more susceptible to reperfusion injury. Other reports in the literature have demonstrated that diabetic hearts are more resistant to ischemic injury than normal hearts (Tani and Neely, 1988; Pieper, 1990).

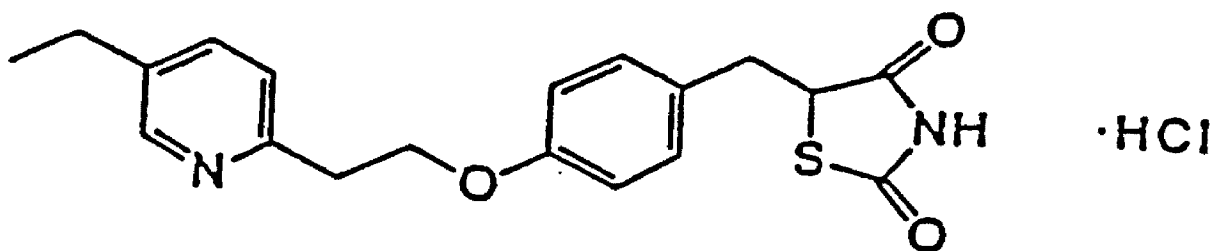
Various mechanisms have been proposed to explain the resistance of diabetic hearts to ischemic injury. Shen and Jennings (1972a & 1972b) have shown that massive amounts of  $\text{Ca}^{2+}$  accumulate in ischemic reperfused myocardium. They, as well as others, have suggested an association between  $\text{Ca}^{2+}$  accumulation and irreversible cell damage. Tani and Neely (1988) suggested that upon reperfusion  $\text{Ca}^{2+}$  uptake was less in diabetics than in control and this low accumulation of  $\text{Ca}^{2+}$  may account for the greater resistance of diabetic hearts to ischemia.

Plasma levels of arachidonic acid and prostacyclin have been reported to be decreased in diabetic patients (Silberbauer et al, 1979) as well as in diabetic animals (Holman et al, 1983; Harrison et al, 1978). Prostacyclin is synthesized in response to MI/R injury and Pieper et al (1989, 1990) have demonstrated that inhibition of prostacyclin synthesis in diabetics increases post-ischemic cardiac function.

**Pioglitazone:**

Thiazolidinediones represent a new structural class of anti-diabetic compounds that were discovered empirically by observing their hypoglycemic effects in animal models of Type II diabetes mellitus (Colca and Morton, 1990).

Pioglitazone, a representative of such agents, appears to lower blood glucose levels in diabetic rodent models through a mechanism that involves increased insulin sensitivity in target tissues (Colca and Morton, 1990). It increases insulin sensitivity by phosphorylation of the tyrosine kinase associated with the beta chain of the insulin receptor (Kobayashi et al, 1992).



**PIOGLITAZONE HYDROCHLORIDE**

Administration of pioglitazone to insulin resistant fatty rats results in a dose-dependent decrease in blood levels of glucose, triglycerides and insulin (Ikeda et al, 1990). Pioglitazone treatment corrects the deficiencies in GLUT 4 glucose transporter expression in insulin-resistant

obese KKA<sup>Y</sup> mice (Hofmann et al, 1991). This increased glucose transporter expression resulted despite an accompanying reduction of circulating insulin levels. Glucose transport in mammalian tissues is mediated by a family of structurally related, but genetically distinct, glucose-transporter proteins. The facilitative glucose-transporter isoforms have distinct tissue distribution as follows: GLUT1 in erythrocyte, GLUT2 in liver, GLUT3 in brain, GLUT4 in muscle & fat and GLUT5 in small intestine. Hofmann et al, (1992) reported that the two-fold elevation in the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK) and its mRNA abundance was normalized after treatment with pioglitazone.

Sugiyama et al (1990 & 1991) reported that pioglitazone treatment reverses all effects in noninsulin dependent diabetes mellitus (Type II), but has no effect on insulin dependent diabetes mellitus (Type I) when administered alone. The combination of pioglitazone and insulin decreased the blood glucose and triglyceride levels in STZ-diabetic rats (Hofmann, 1991).

Pioglitazone lowered cholesterol levels in Type I diabetic rats fed a high cholesterol diet and produced a significant reduction in cholesterol absorption. Drug treatment was ineffective in rats that were not given dietary cholesterol. The mechanism of the effect on cholesterol absorption is unknown. Pioglitazone treatment

alone did not affect the cholesterol absorption in Type I diabetic rats fed a normal diet; however, the combination of insulin and pioglitazone synergized to lower absorption of cholesterol and circulating cholesterol levels (Colca et al, 1991).

The effects of pioglitazone on vascular reactivity or on vascular changes produced by either Type I or Type II diabetes are unknown. Also there are no reports on effects of pioglitazone on cardiac function or MI/R injury in either healthy or diabetic animals. We have investigated the cardiovascular effects of pioglitazone in two models of diabetes with the following objectives:

1. To determine the effects of pioglitazone on vascular reactivity in Type II diabetes mellitus.
2. To investigate the effects of pioglitazone on vascular reactivity in Type I diabetes mellitus.
3. To evaluate the effects of pioglitazone on cardiac function and MI/R injury in Type I diabetic animals.
4. To compare the effects of pioglitazone on vascular reactivity in both Type I and Type II models of diabetes mellitus.

## MATERIALS & METHODS

### Animals & Housing:

Male Zucker diabetic fatty rats (Drt-fa) weighing 250 - 300 g and male Zucker lean rats (nondiabetics) weighing 250 - 300 g and obtained from Genetic Models, Inc., Indianapolis, IN, were used in the Type II diabetes mellitus study. Zucker diabetic fatty rats were housed in individual wire-mesh cages over woodchip bedding. Two Zucker lean rats were housed together in wire mesh cages.

Male Sprague Dawley rats, weighing 250 - 300 g and obtained from Bantin & Kingman Co. Fremont, CA., were used in the Type I diabetes mellitus study. Rats were housed in individual wire mesh hanging cages. Purina lab chow and water were available ad lib during 12 hour light-dark cycles. All animals were allowed a minimum of 3 to 5 days acclimatization before starting the experiments.

### Reagents & Drugs:

Reagents and drugs were obtained from the indicated sources: Streptozotocin, norepinephrine, carbamylcholine chloride, sodium nitroprusside, sodium pyruvate and sodium pentobarbital from Sigma Chemical Co. St Louis, MO; sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ), sodium chloride ( $\text{NaCl}$ ), magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ), sodium bicarbonate ( $\text{NaHCO}_3$ ), potassium phosphate monobasic ( $\text{KH}_2\text{PO}_4$ ) and ethyl ether from Fisher Scientific Co. Fair Lawn, NJ; heparin



from Elkins - Sinn, Inc., Cherry Hill, NJ; calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) from EM Science, Cherry Hill, NJ; potassium chloride (KCl) and glucose from J.T. Baker Chemical Co., Phillipsburg, NJ; pioglitazone from Upjohn Co., Kalamazo, MI; Linplant<sup>TM</sup> sustained release insulin implant from Linshin Canada, Ontario, Canada; One Touch<sup>R</sup> II Glucose analyzer kit from Life Scan Co., Milpitas, CA.

### **EXPERIMENTAL PROTOCOLS**

#### **I. Type II Diabetes Mellitus Study:**

The study focused on vasoreactivity in Type II diabetic animals. Diabetic and non-diabetic rats were divided into the following groups.

1. Control (C) : Non-diabetic control.
2. Control Pioglitazone (CP): Non-diabetic pioglitazone treated.
3. Diabetic (D): Diabetic control or untreated.
4. Diabetic Pioglitazone (DP): Diabetic pioglitazone treated.

A total of nine to eleven rats were used in each treatment group. Treatment began at seven weeks of age and continued for six weeks with daily dosing (10 mg/kg/day) of pioglitazone. Pioglitazone hydrochloride was suspended at the concentration of 10 mg/ml in 0.5 M citrate buffer (pH 4.5) and was administered by oral gavage to CP and DP group rats. Citrate buffer was administered to control and diabetic group rats by the same mechanism. All animals were

weighed daily. Once a week the rats were fasted for five hours and blood samples were collected from the distal part of the tail. The blood glucose concentration was determined with a One Touch<sup>R</sup> II glucose analyzer.

At the end of the treatment period, rats were anesthetized with sodium pentobarbital (100 mg/kg i.p.). The thoracic aortas were removed rapidly and dissected free of excess fat and connective tissue in Krebs-Henseleit solution containing (mM): NaCl 118; KCl 4.7; CaCl<sub>2</sub> 2.5; KH<sub>2</sub>PO<sub>4</sub> 1.2; MgSO<sub>4</sub> 1.2; NaHCO<sub>3</sub> 25; Glucose 11. A part of thoracic aorta was cut into transverse ring of 4 mm in length and opened longitudinally to prepare 8 X 4 mm strip. The aortic strip was attached to an F-60 Myograph (Narco Biosystems) to record isometric contraction on a polygraph (Narco Biosystems). The tissue was placed in 37<sup>0</sup>C KH solution and aerated with 95% O<sub>2</sub> and 5% CO<sub>2</sub> and maintained at 37<sup>0</sup>C. The bath volume was 25 ml. The preparation was allowed to equilibrate for 30 minutes under an optimal resting tension of 1 g. During equilibration the aortic strip was washed with fresh solution every 10 minutes.

After equilibration for 30 minutes, contractile function of the aortic tissue was examined by replacing the K-H solution with a similar solution containing 40 mM KCl. After KCl-induced contraction, the aortic strip was washed with K-H solution. Washings were repeated every five minutes until base line was achieved. Alpha adrenoreceptor-

mediated contractility was determined by a cumulative increase in norepinephrine concentration ( $10^{-9}$  M to  $10^{-6}$  M) in the bath solution. The concentration of the agonist in the bath was increased approximately five-fold every three minutes. The aortic strip was washed several times with fresh K-H solution. A period of 5-10 minutes was allowed for re-equilibration. Voltage dependent contractility was then determined by exposure to isotonic K-H solution containing 40 mM and subsequently 100 mM KCl. The contractile force was expressed in grams.

## **II. Type I Diabetes Mellitus Study:**

This study focused on myocardial and smooth muscle function in Type I diabetic rats. Diabetes was induced under light ether anesthesia by a single 55 mg/kg body weight i.v. injection of streptozotocin (STZ) into the jugular vein of 9 week old male Sprague-Dawley rats. Streptozotocin was dissolved at a concentration of 55 mg/ml in 0.5 M citrate buffer (pH 4.5). Age-matched male rats used as control were injected with citrate buffer (pH 4.5). Forty-eight rats weighing 250-300 g were used for the study. The rats were divided into six groups of eight rats per group as indicated below.

1. Control (C): Non-diabetic control.
2. Control Pioglitazone (CP): Non-diabetic pioglitazone treated.
3. Diabetic (D): Diabetic control.

4. Diabetic Pioglitazone (DP): Diabetic Pioglitazone treated.
5. Diabetic Insulin (DI): Diabetic insulin treated.
6. Diabetic Insulin Pioglitazone (DIP): Diabetic insulin and pioglitazone treated.

Treatment began seven days after injection of STZ or citrate buffer and continued with daily dosing of pioglitazone (10 mg/kg/day) for six weeks. Pioglitazone hydrochloride was suspended at a concentration of 10 mg/ml in 0.5 M citrate buffer (pH 4.5) and administered by oral gavage to CP, DP and DIP group rats. Citrate buffer was administered to control and diabetic group rats by the same mechanism. Sustained release insulin pellets, releasing approximately 2 Units of insulin/day, were implanted subcutaneously in the back of the neck in the DI and DIP groups. All animals were weighed daily and blood glucose concentrations were determined as described in the Type II study. Whenever the blood glucose levels fell below 30 mg/dl, ten percent glucose was added to drinking water and provided ad lib in replacement of regular drinking water. The animals which survived the entire treatment period were used in the MI/R and vascular reactivity experiments.

At the end of the treatment period, rats were anesthetized with sodium pentobarbital (100 mg/kg i.p). They were intubated, mechanically ventilated and anticoagulated with 200 IU heparin I.V. Hearts were perfused by the aortic

root in situ at 37°C with modified Krebs-Henseleit (K-H) solution (112 mM NaCl, 25 mM NaHCO<sub>3</sub>, 5 mM KCl, 12 mM MgSO<sub>4</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 1.25 mM CaCl<sub>2</sub>, 11.5 mM dextrose, 2 mM sodium pyruvate). The perfusate was aerated continuously with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Hearts were quickly excised and placed in a non-recirculating Langendorff apparatus. Coronary perfusion was maintained at a constant pressure of 80 mm Hg. A water filled latex balloon attached to a metal intubation tube was inserted through the left atrium into the left ventricle and connected to a pressure transducer (P 1000b Narco Biosystem) to record the left ventricular developed pressure and heart rate on a polygraph (MK-III-S, Narco Biosystem). Hearts were allowed to equilibrate for 15 minutes, after which end diastolic pressure was adjusted to 5 mm Hg by inflating the balloon. Values for heart rate (HR), left ventricular developed pressure (LVDP), coronary flow (CF) and double product were determined 20 minutes after inflation of the balloon. Heart rate and left ventricular developed pressure were recorded from the polygraph. Coronary flow values were determined by collecting the coronary perfusate for one minute. Cardiac function, estimated as double product, was calculated as  $(HR \times LVDP)/1000$ .

The hearts were then rendered globally ischemic by shutting off the flow of modified K-H solution for 20 minutes. Subsequently, reperfusion with modified K-H solution was initiated and maintained for 30 minutes. Final

values for HR, LVDP, CF and double product were then obtained. Rats which did not recover their cardiac function after 20 minutes ischemia followed by 30 minutes of reperfusion, were not included in the statistical analysis.

Preparation and isolation of thoracic aortas were as described in the Type II study with two exceptions. In Type I studies aortic rings were used instead of aortic strips and two grams of resting tension was used instead of one gram. In addition, endothelial dependent and independent relaxation was measured using carbachol and sodium nitroprusside. The relaxation caused by carbachol in blood vessels requires a functional endothelial lining, whereas the relaxation produced by sodium nitroprusside is independent of the endothelium. Since endothelial dependent relaxation is known to be impaired in Type I diabetes, this experiment was designed to study the effect of pioglitazone treatment on endothelial dependent and independent relaxation. Relaxation was assessed by inducing prior contraction with norepinephrine ( $10^{-5}$  M). Relaxation values were calculated by using the below formula.

$$\text{Percent relaxation} = \frac{(\text{M.C.} - \text{C.R.R.})}{\text{M.C.}} \times 100$$

M.C. = Maximal contraction in grams

C.R.R = Contraction in grams after relaxation with either carbachol or sodium nitroprusside.

**Statistics:**

The mean and 95% confidence intervals of the EC 50 and maximal response to NE were calculated by using the curve fitting computer program PC NONLIN. The EC 50 value was defined as the concentration of agonist which produced 50% of the maximal response. The rest of the data was analyzed using Analysis of Variance (ANOVA). Tukey's protected t test was used to determine significant differences ( $P < 0.05$ ).

## RESULTS

### **Type II Model:**

The body weights of ZDF rats were significantly greater than the non-diabetics. Pioglitazone treatment did not affect the body weights of control rats, but did significantly increase the body weight of diabetic rats (Fig. 1).

Blood glucose levels in the diabetic rats were significantly elevated. Pioglitazone treatment significantly decreased the blood glucose levels in diabetic rats, but blood glucose levels were unaltered in control rats (Fig. 2).

Maximal contractile response to NE in aortas from diabetic rats was not altered. Pioglitazone treatment significantly decreased the maximal response to NE in both diabetic and control animals (Fig. 3). The maximal contraction and EC 50 values for NE are shown in Table 1. EC 50 values from diabetic animals were also not altered, but EC 50 values were significantly greater in the control than the diabetic pioglitazone animals.

No difference was observed between control and diabetic aortic maximal contractile response to 40 mM KCl. Diabetic rats showed significantly greater maximal contractile response than the control pioglitazone (CP) group rats. Further depolarization with 100 mM KCl did not produce



greater contractile response, but a similar contractile pattern to that seen at 40 mM was observed (Fig. 4).

#### **Type I Model:**

During the treatment period D, DP, and DIP groups had a mortality rate of 12.5%, 50% and 25% respectively. Because of the high mortality rate in DP and DIP groups, the experiment was shortened to 40 days instead of 42 days.

The body weights of diabetic rats seven weeks after the STZ-treatment were significantly decreased. Diabetic rats lost weight initially and never returned to their initial weights. Pioglitazone treatment did not affect the body weight change in either control or diabetic rats. Insulin treated diabetic rats gained weight at approximately the same rate as control animals, but due to initial weight differences they remained lighter throughout the treatment period (Fig. 5).

Blood glucose levels in the diabetic rats were significantly elevated. Pioglitazone treatment did not affect the blood glucose levels in either control or diabetic rats. Insulin normalized the blood glucose levels in diabetic animals. When pioglitazone was administered along with insulin, the blood glucose levels were significantly lower than the diabetic insulin treated group at the second and sixth weeks of the study (DI =  $111 \pm 18$  and DIP =  $29 \pm 1$  mg/dl at week two; DI =  $83 \pm 15$  and DIP =  $44.5 \pm 10$  mg/dl at week ten). During the final week of the

study (week 6) glucose levels in both diabetic groups treated with insulin (DI and DIP) increased slightly (Fig. 6).

Diabetic rats showed elevated glycosylated hemoglobin (Hb) values. Pioglitazone treatment did not affect glycosylated Hb values either in control or diabetic rats. Insulin significantly decreased the glycosylated Hb values in diabetic rats. The combined treatment of insulin and pioglitazone did not decrease the percentage of glycosylated Hb values in diabetic rats (Fig. 7).

The maximal contractile response to NE in aortas from diabetics was significantly increased. Pioglitazone treatment did not alter the maximal response to NE either in control or diabetic rats. Although statistical significance was not observed, pioglitazone appears to decrease the maximal contractile response to NE in CP and DIP groups. Insulin treatment did not show any effect on diabetes-induced maximal contraction. The combination of insulin and pioglitazone treatment decreased the maximal response to NE in diabetic rats, but statistical significance was not observed (Table 2; Fig. 8).

EC 50 values indicate that control rats were significantly more sensitive to NE than the diabetic rats. Neither pioglitazone nor insulin showed any affect on EC 50. The combined treatment of pioglitazone and insulin decreased the EC 50 value in diabetic rats (Table 2).

Maximal contractile response to KCl in aortas from diabetic rats was significantly reduced. Administration of either insulin or pioglitazone to diabetic animals prevented this decrease. However, the combination of insulin and pioglitazone (DIP) produced a decreased maximal contractile response similar to that seen in the diabetic group (Fig. 9).

All concentration response curves for the carbachol-induced relaxation of rat aortas were shifted to the right of the control. There were, however, no significant differences observed in the maximal relaxation between control and the D and DI groups. Pioglitazone treatment significantly decreased the maximal relaxation in the control group. Although significant differences were not observed, pioglitazone appeared to decrease maximal relaxation in the DP and DIP groups (Fig. 10).

No significant differences were observed in the maximal endothelial-independent relaxation of aortic tissue to sodium nitroprusside (Fig. 11).

#### **Pre-ischemic Cardiac Function:**

Although statistical significance was not observed, heart rate tended to be reduced in diabetic rats. Pioglitazone treatment significantly decreased the heart rate in diabetic rats. Insulin treatment normalized the heart rate in diabetic rats. The combination of insulin and pioglitazone treatment demonstrated higher heart rates than

pioglitazone treatment alone, but not as high as insulin treatment (Table 3).

Diabetic rats showed no change in left ventricular developed pressure. However, diabetic groups without insulin treatment showed a tendency for lowered LVDP. Neither pioglitazone nor insulin treatment significantly increased the LVDP, but the combination of insulin and pioglitazone treatment significantly increased the LVDP in diabetic rats (Table 3).

Pre-ischemic double product was significantly lower in D and DP groups compared to all other groups. Pioglitazone significantly increased the double product in the control group. Insulin treatment and the combination of insulin and pioglitazone treatment normalized the double product in diabetic rats (Table 3).

Diabetic rats had significantly reduced coronary flow. Pioglitazone treatment did not alter the coronary flow in either control or diabetic rats. Although statistical significance was not observed, insulin treatment and the combination of insulin and pioglitazone treatment appeared to normalize the coronary flow in diabetic rats (Table 3).

#### **MI/R Injury:**

After 20 minutes of ischemia followed by 30 minutes of reperfusion, all hearts showed measurable cardiac function in D, DP and DI groups. C, CP and DIP group hearts showed measurable cardiac function in only seven out of eight, five

out of eight and four out of six, respectively (Table 4). Only hearts that recovered to some degree were used in the statistical analysis.

Post-ischemic heart rate was not different from pre-ischemic heart rate in all groups. Diabetic rats showed no change in the percentage of post-ischemic recovery of heart rate. Neither pioglitazone nor insulin showed any effect on post-ischemic recovery of heart rate. The combination of insulin and pioglitazone treatment (DIP) significantly reduced the post-ischemic recovery of heart rate in diabetic animals (Table 4).

Post-ischemic left ventricular developed pressure was significantly reduced in all groups except, DP. Diabetic rats showed no change in the percentage of post-ischemic recovery of LVDP. Neither pioglitazone nor insulin treatment showed any effect on post-ischemic recovery of LVDP. The combination of insulin and pioglitazone treatment (DIP) significantly diminished post-ischemic recovery with respect to the DP group (Table 4).

Post-ischemic recovery of double product was significantly reduced in all groups except DP. There is no significant difference between diabetic and control rats. Neither pioglitazone nor insulin treatment showed any effect on post-ischemic recovery of double product. The combination of insulin and pioglitazone treatment (DIP) also did not show any effect on double product (Table 4).

Post-ischemic coronary flow was significantly reduced in all groups except DP and DI. Diabetic rats showed no change in the percentage of post-ischemic recovery of the coronary flow. Neither pioglitazone nor insulin treatment showed any effect on post-ischemic recovery of coronary flow. The combination of insulin and pioglitazone treatment (DIP) significantly diminished post-ischemic recovery with respect to the DI group (Table 4).

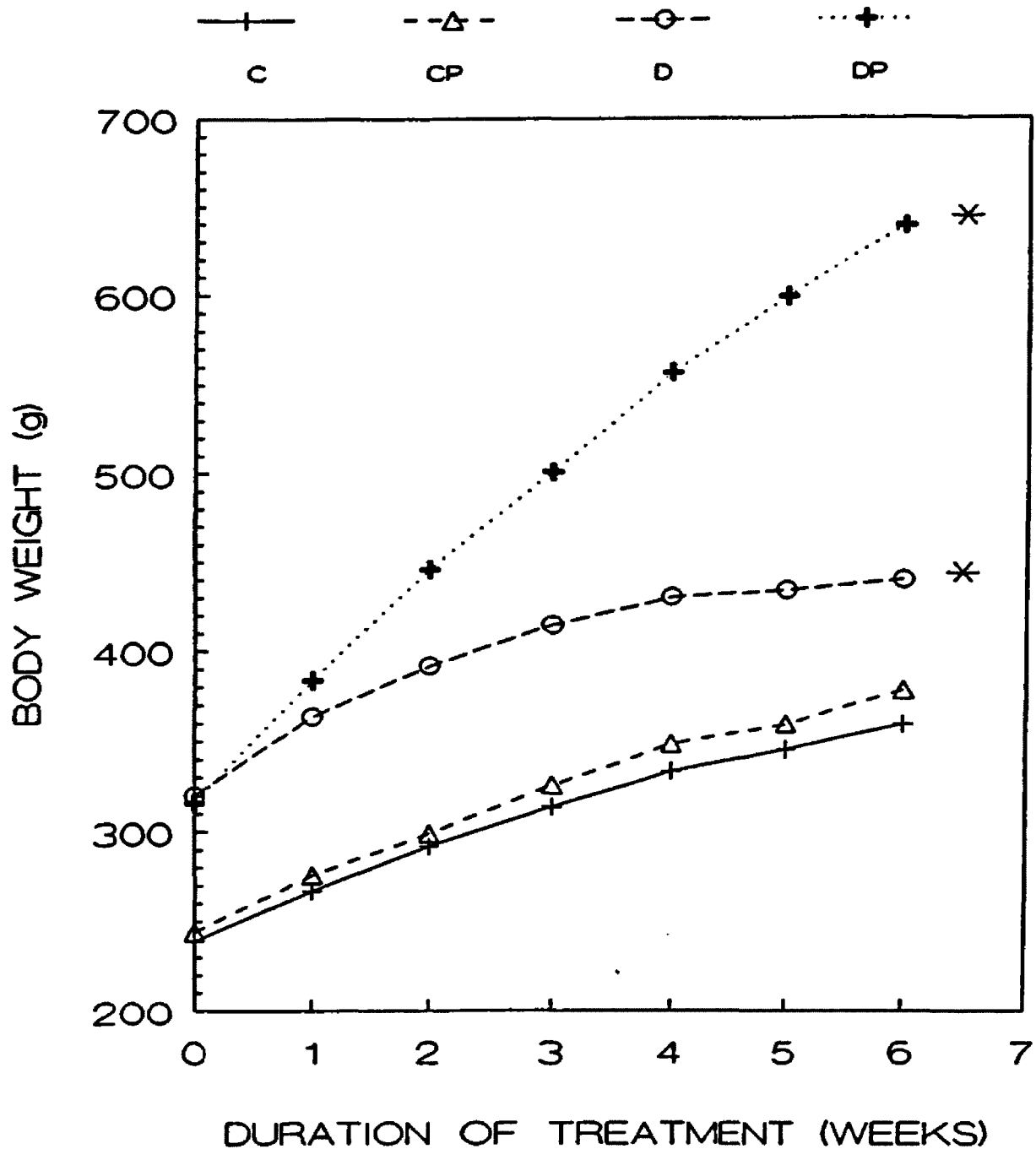


Fig. 1. Effect of pioglitazone on body weight in Type II diabetic rats. C - Control; CP - Control pioglitazone treated; D - Diabetic; DP - Diabetic pioglitazone treated. CP and DP group rats received pioglitazone (10 mg/kg/day) by oral gavage from 0 - 6 weeks. Each point represents means of 9-11 rats. \* Significantly different from all other groups at week 6.

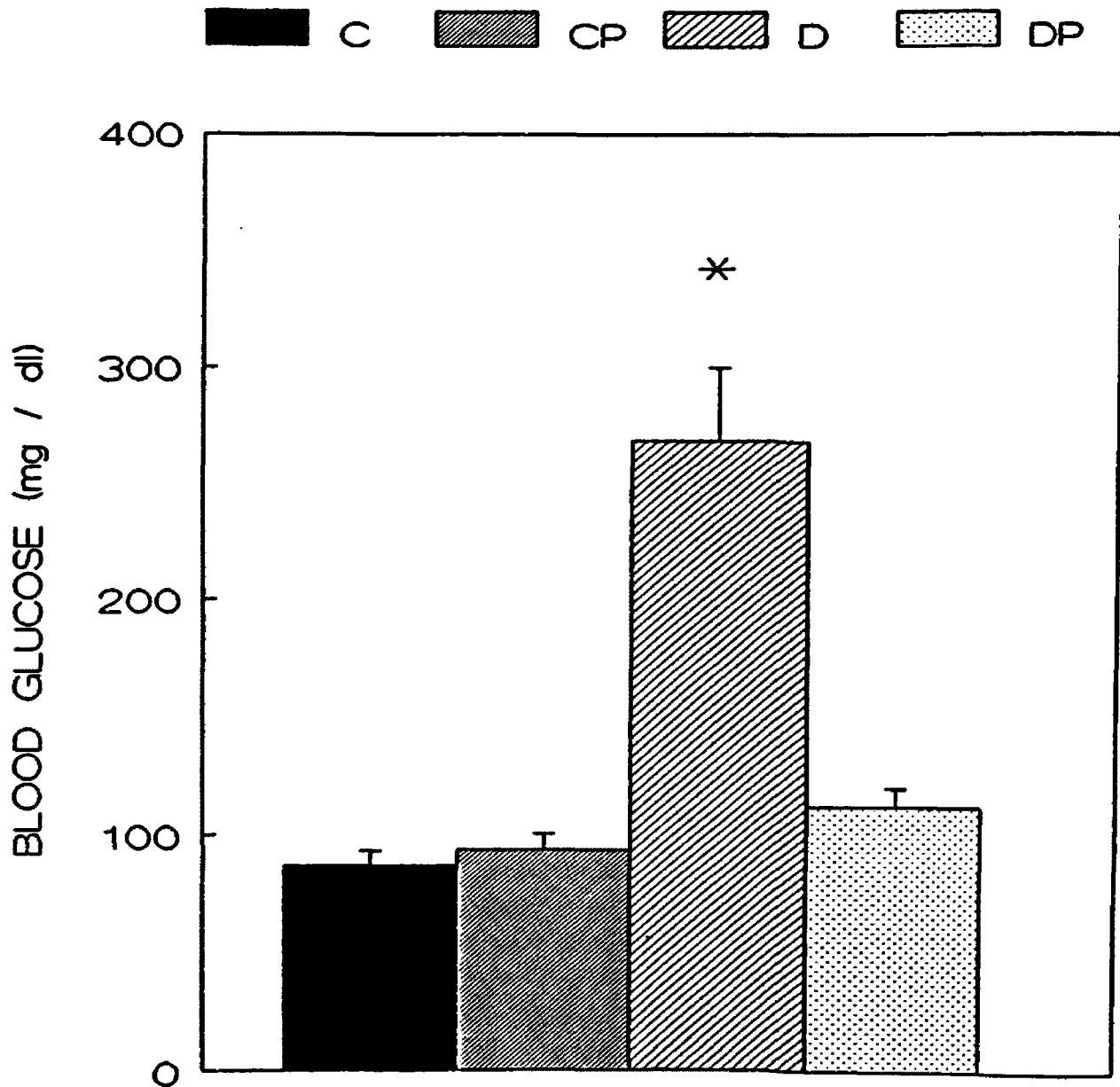
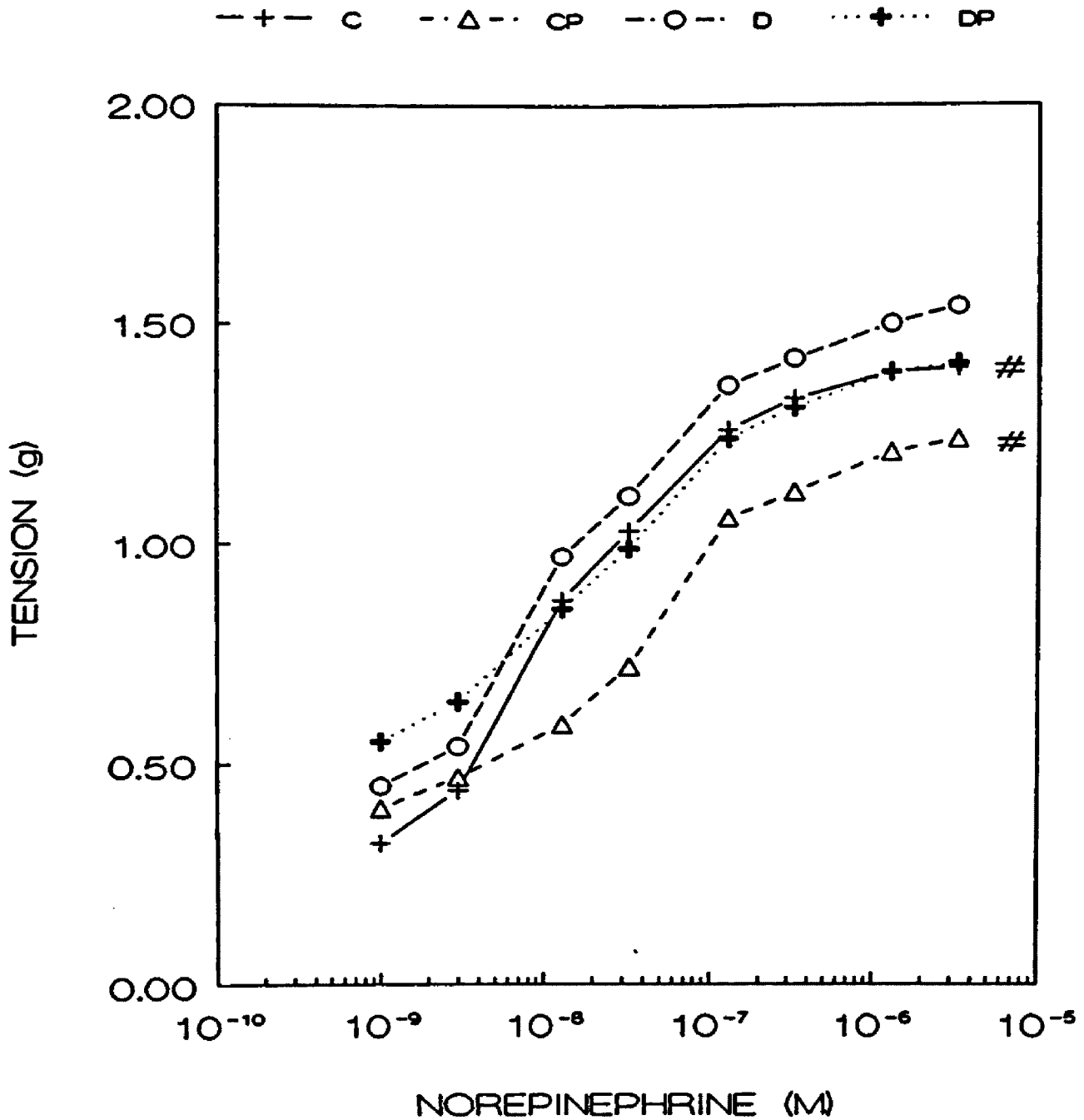


Fig. 2. Final week blood glucose levels in Type II diabetic rats. All values are expressed as Mean  $\pm$  SE. \* Significantly different from all other groups. See Fig. 1 for description of treatment groups.





**Fig. 3. Effect of pioglitazone on vascular reactivity to norepinephrine in Type II diabetic rat aortic strips.**  
 Each point represents mean of 9 - 11 experiments.  
 # Significantly different from respective control group.  
 See Fig. 1 for description of treatment groups.  
 N = 9, 10, 10, 11 for groups C, CP, D, DP respectively.

**Table 1. Maximal contractile response and EC 50 values of NE in aortas from Type II diabetic rats.**

<b>Group</b>	<b>Maximal Response(g) ( 95% C.I.)</b>	<b>EC 50 (nM) (95% C.I.)</b>
<b>C (N = 9)</b>	<b>1.41 ± 0.4 (1.31 - 1.51)</b>	<b>8.31 ± 1.7 (4.77 - 11.84)</b>
<b>CP (N = 10)</b>	<b>1.15 ± 0.4 <sup>1</sup> (1.05 - 1.24)</b>	<b>4.31 ± 1.1 (2.02 - 6.6)</b>
<b>D (N = 10)</b>	<b>1.56 ± 0.06 (1.44 - 1.68)</b>	<b>4.87 ± 1.2 (2.43 - 7.3)</b>
<b>DP (N = 11)</b>	<b>1.27 ± 0.05 <sup>1</sup> (1.17 - 1.37)</b>	<b>2.29 ± 0.6 <sup>2</sup> (1.0 - 3.59)</b>

Data are presented as Mean ± SE with 95% confidence intervals in the parentheses.

Maximal response is expressed in grams of tension.

EC 50 values are expressed as bath concentration (nM) which produced 50% maximal response.

C = Control; CP = Control Pioglitazone; D = Diabetic; DP = Diabetic Pioglitazone.

<sup>1</sup> Significantly lower than respective untreated group.

<sup>2</sup> Significantly lower than control (C).

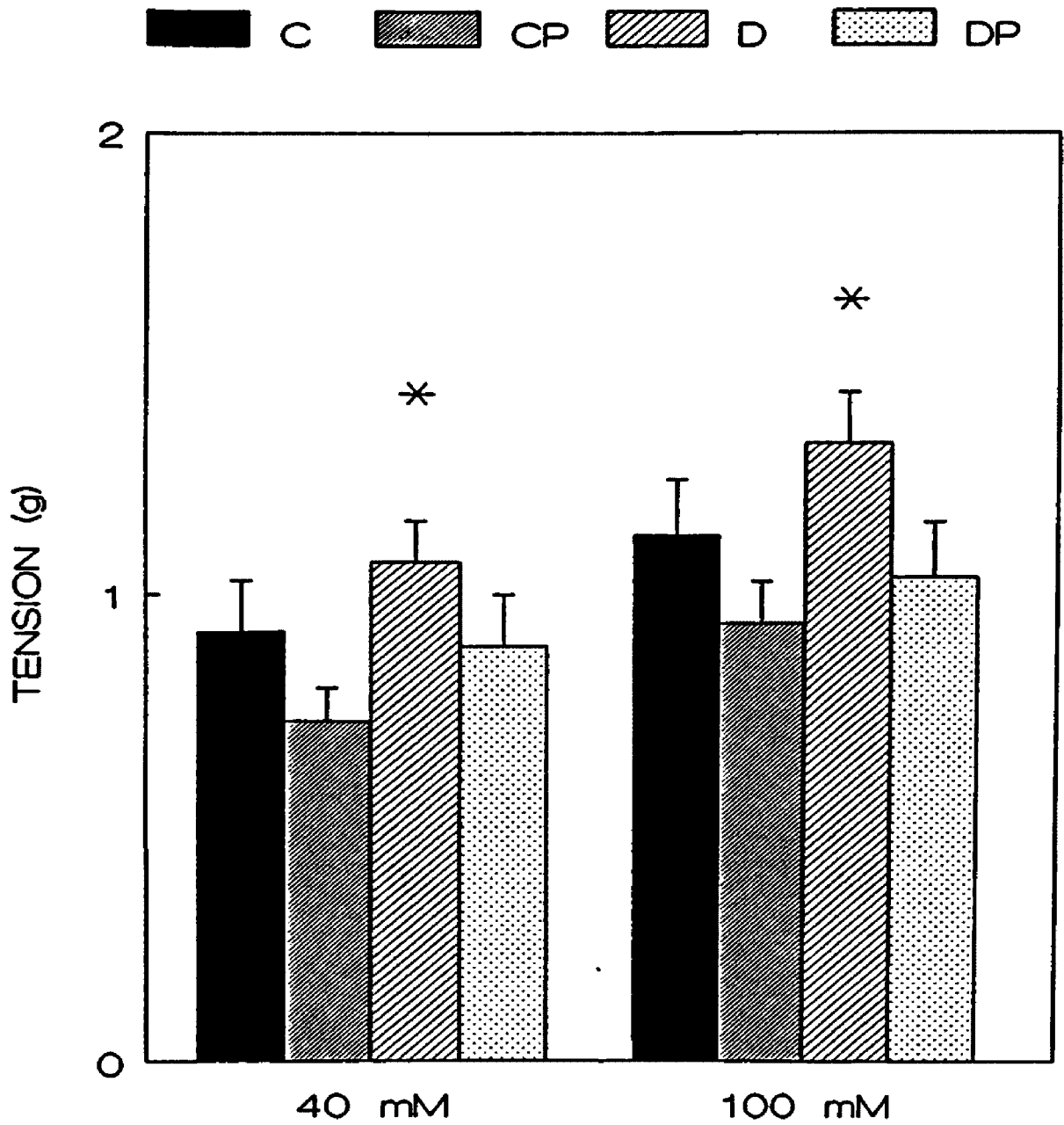


Fig. 4. Maximal contractile response of rat aortic strip to 40 mM and 100 mM KCl.

All values are expressed as Mean  $\pm$  SE.

\* Significantly different from CP group.

See Table 1 for description of treatment groups and N values.

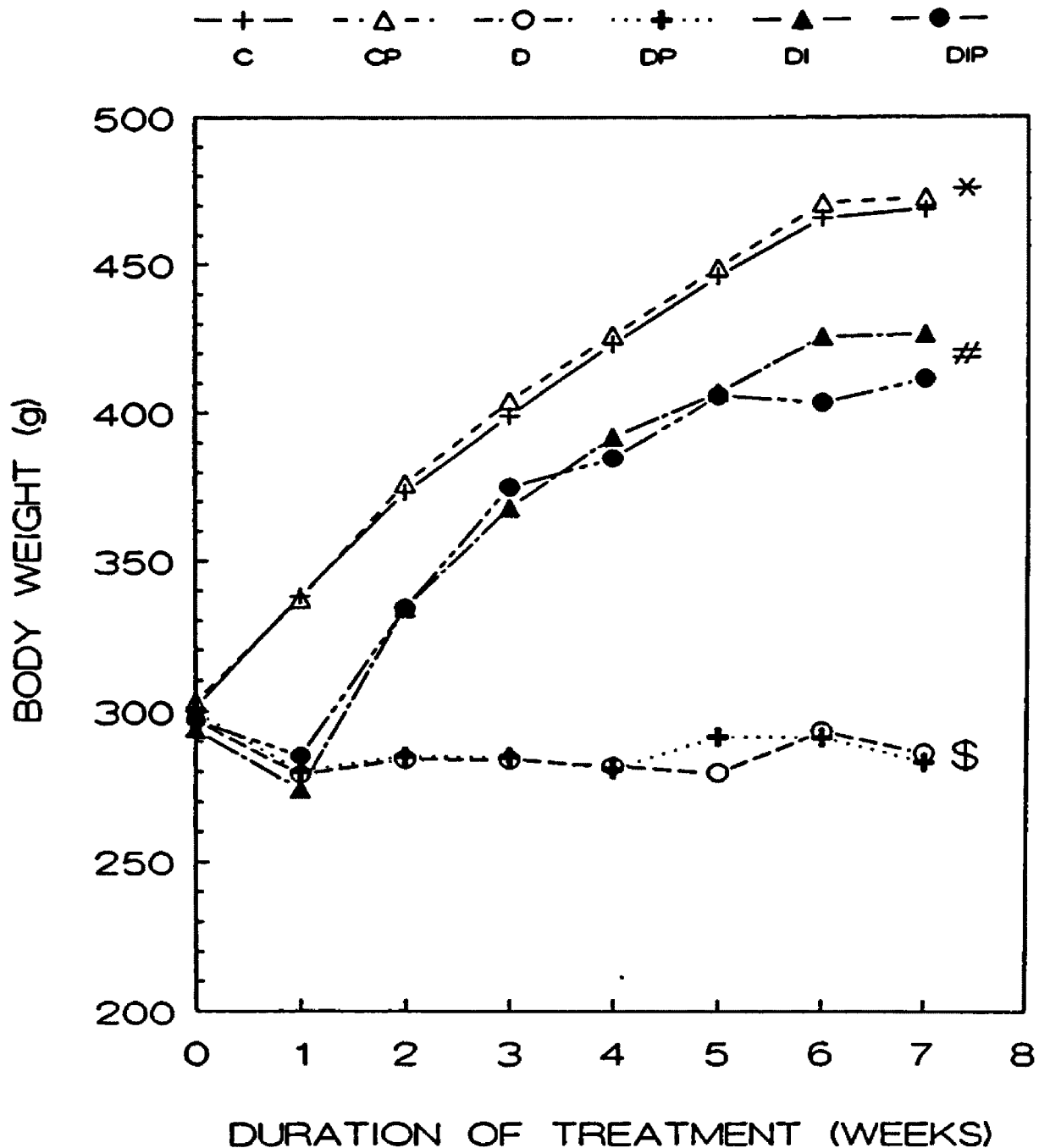


Fig. 5. Mean body weights in Type I diabetic rats. C = Control; CP = Control pioglitazone treated; D = Diabetic; DP = Diabetic pioglitazone treated; DI = Diabetic insulin treated; DIP = Both insulin and pioglitazone treated diabetic group. N values: C = 8; CP = 8; D = 7; DP = 4; DI = 8; DIP = 6. Streptozotocin (55 mg/kg) given intravenously to D, DP, DI and DIP groups at week 0. Pioglitazone (10 mg/kg/day) given by oral gavage to CP, DP and DIP groups from week one to seven. Sustained release insulin pellets, releasing approximately 2 U/day for 40 days, were implanted s.c. at week one to DI and DIP groups. \* C & CP are significantly different from all other groups at week 7. # DI & DIP are significantly different from all other groups at week 7. \$ D & DP are significantly different from all other groups at week 7.

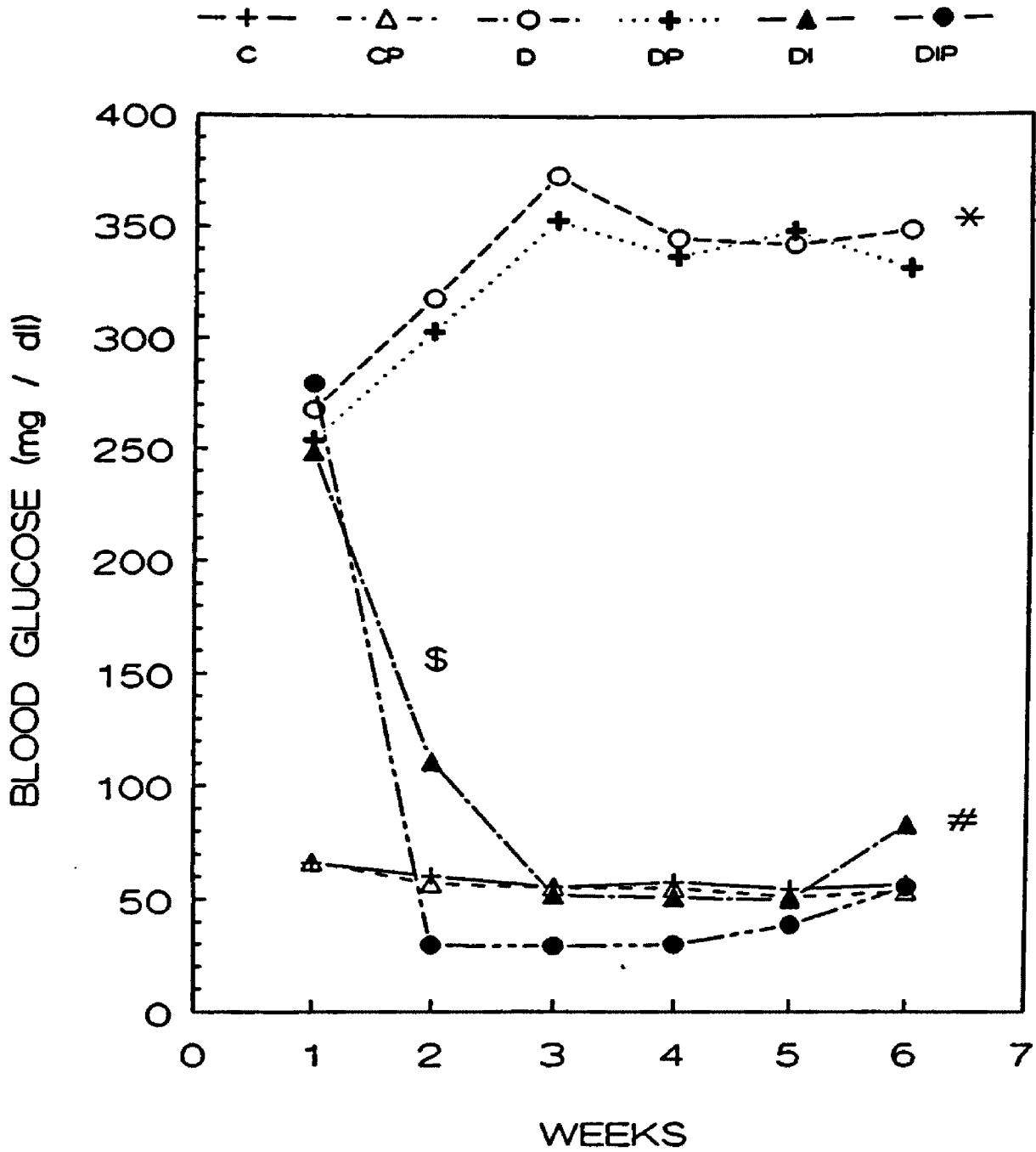
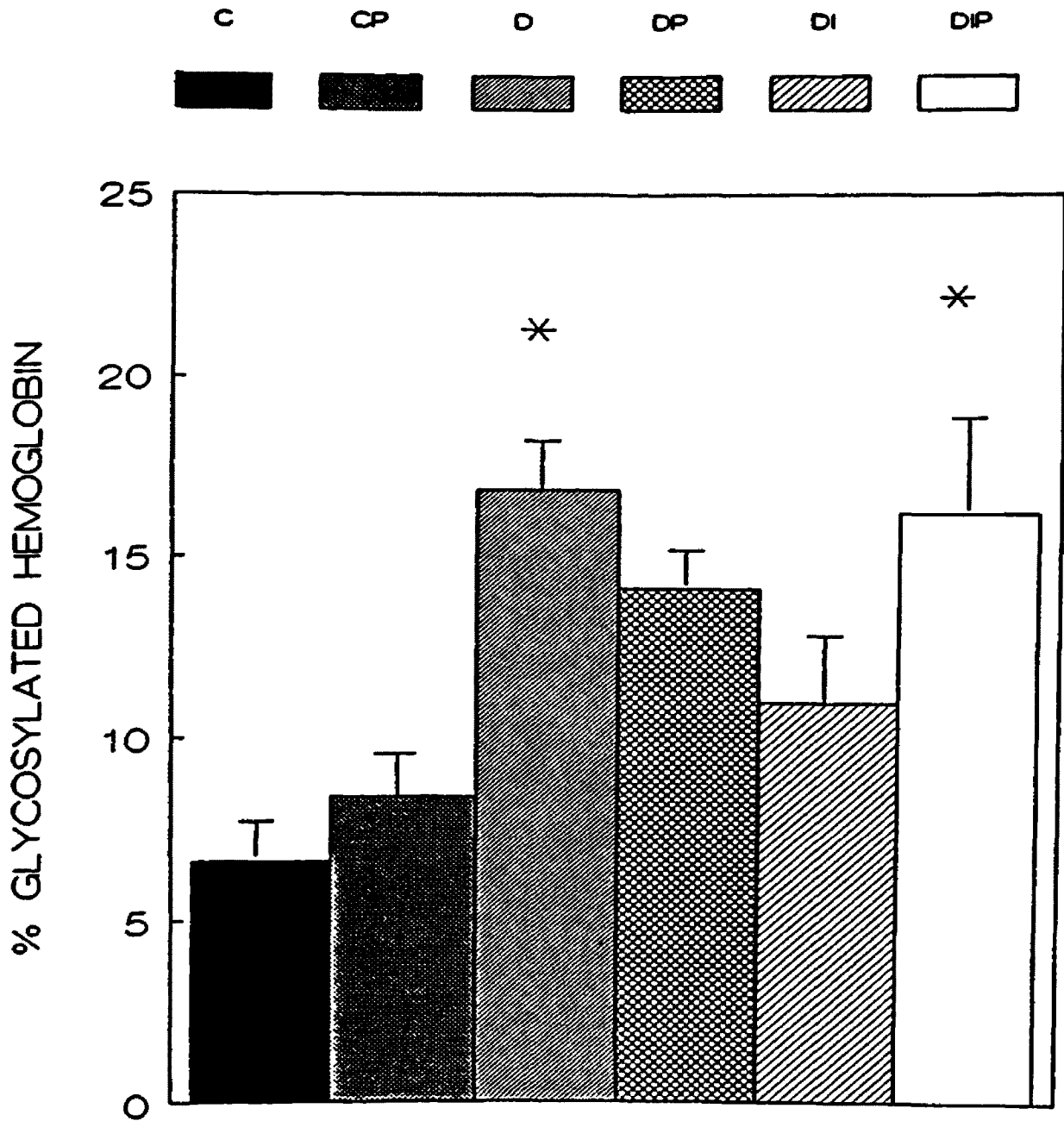


Fig. 6. Mean blood glucose levels in Type I diabetic rats. See Fig. 5 for description of treatment groups and N values.  
 § DI is significantly different from all groups at week 2.  
 \* D and DP are significantly different from all other groups at week 6.  
 # DI is significantly different from D, CP, DP and DIP groups at week 6.



**Fig. 7.** Glycosylated hemoglobin values after 6 weeks in Type I diabetic rats. All values expressed as Mean  $\pm$  SE.  
 \* Significantly different from C, CP and DI groups.  
 See Fig. 5 for description of treatment groups and N values.

**Table 2. Maximal contractile response and EC 50 values of NE in aortas from Type I diabetic rats.**

<b>Group</b>	<b>Maximal Response (g) ( 95% C.I.)</b>	<b>EC 50 (nM) (95% C.I.)</b>
<b>C (N = 7)</b>	<b>1.15 ± 0.07 (1.00 - 1.31)</b>	<b>* 0.83 ± 0.44 (-0.06 - 1.72)</b>
<b>CP (N = 7)</b>	<b>1.08 ± 0.08 (0.91 - 1.25)</b>	<b>* 4.15 ± 2.24 (-0.4 - 8.72)</b>
<b>D (N = 6)</b>	<b>1.54 ± 0.06<sup>1</sup> (1.41 - 1.61)</b>	<b>8.56 ± 2.25<sup>3</sup> (3.93 - 13.18)</b>
<b>DP (N = 4)</b>	<b>1.65 ± 0.15<sup>1</sup> (1.32 - 1.98)</b>	<b>* 2.23 ± 1.58 (-1.1 - 5.55)</b>
<b>DI (N = 8)</b>	<b>1.53 ± 0.13<sup>2</sup> (1.27 - 1.80)</b>	<b>* 6.56 ± 3.73 (-0.9 - 14)</b>
<b>DIP (N = 5)</b>	<b>1.34 ± 0.06 (1.20 - 1.47)</b>	<b>1.42 ± 0.5 (0.37 - 2.47)</b>

Data are presented as Mean ± SE with 95% confidence intervals in the parentheses.

Maximal response is expressed in grams of tension.

EC 50 values are expressed as bath concentration (nM) which produced 50% maximal response.

\* 95% confidence intervals overlap zero.

See Fig. 5 for explanation of treatment groups.

- 1 Significantly different from C and CP groups.
- 2 Significantly different from CP group.
- 3 Significantly different from C and DIP.

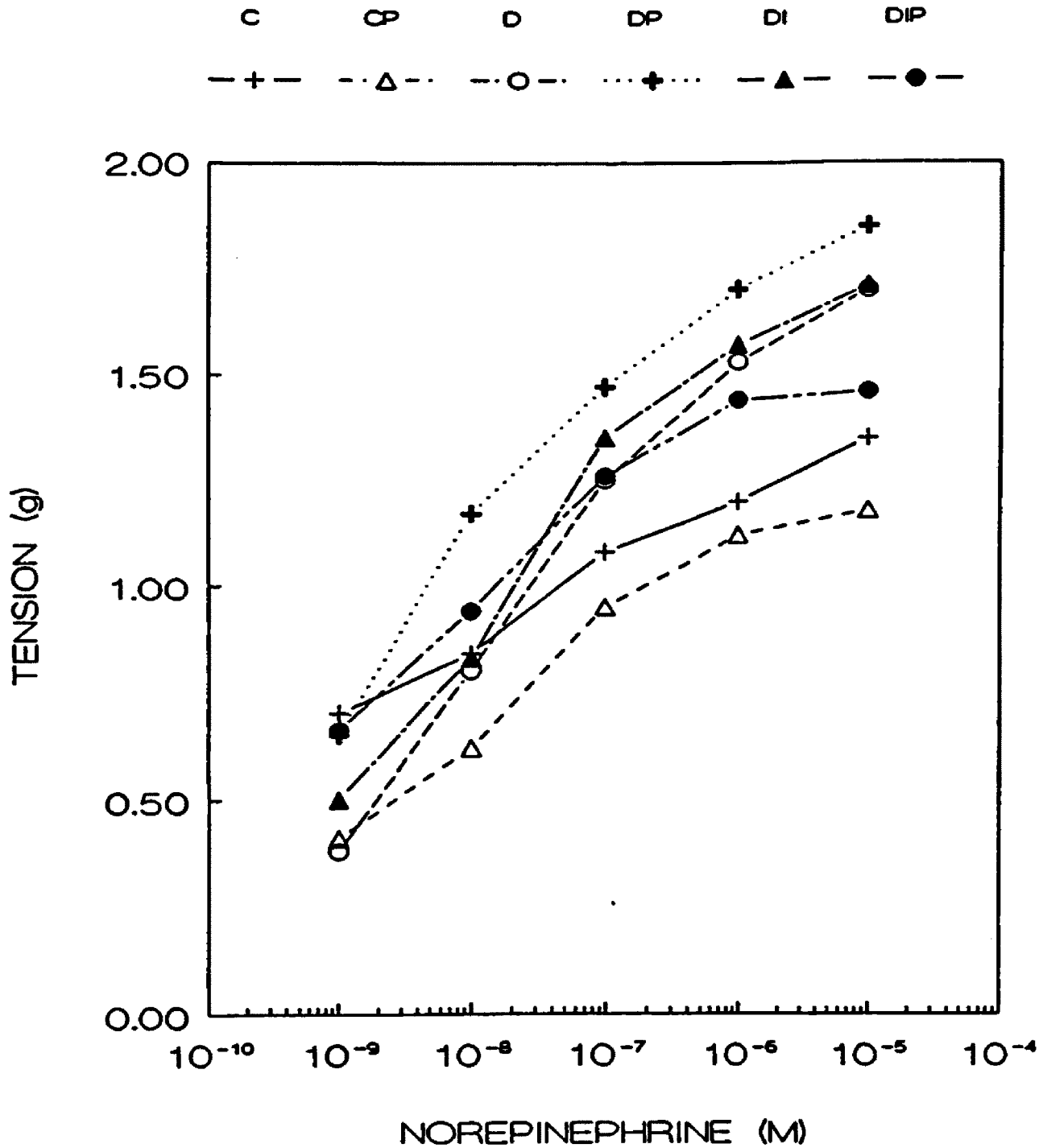
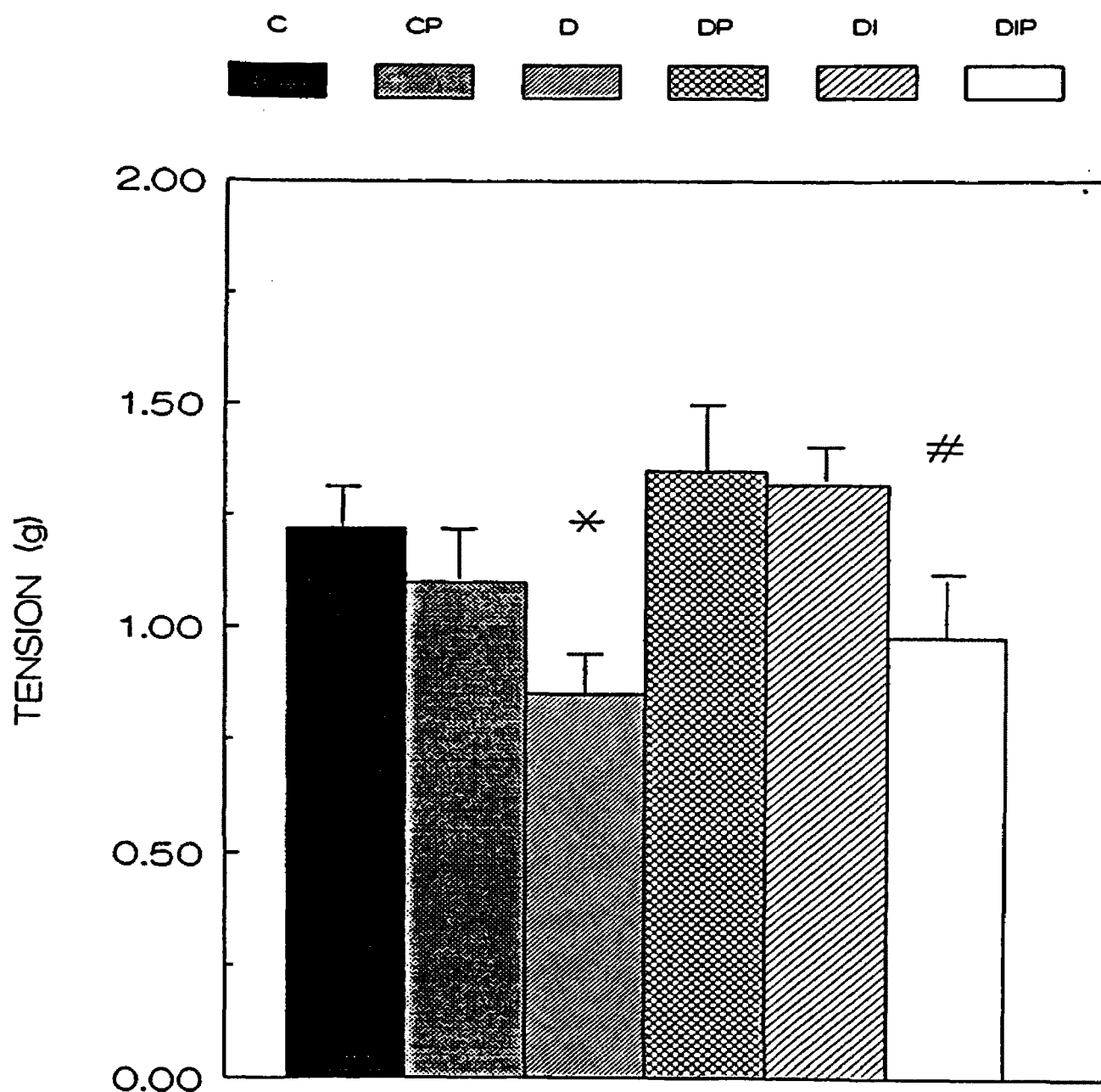


Fig. 8. Effect of pioglitazone on vascular reactivity to norepinephrine in Type I diabetic rat aortic rings. See Fig. 5 for explanation of treatment groups. N values and significant differences are shown in Table 2.





**Fig. 9.** Maximal contractile response of Type I diabetic rat aortic ring to 40 mM KCl.

All values are expressed as Mean  $\pm$  SE.

See Fig. 5 for description of treatment groups.

N values are shown in Table 2.

\* Significantly different from C, DP and DI groups.

# Significantly different from DI group.

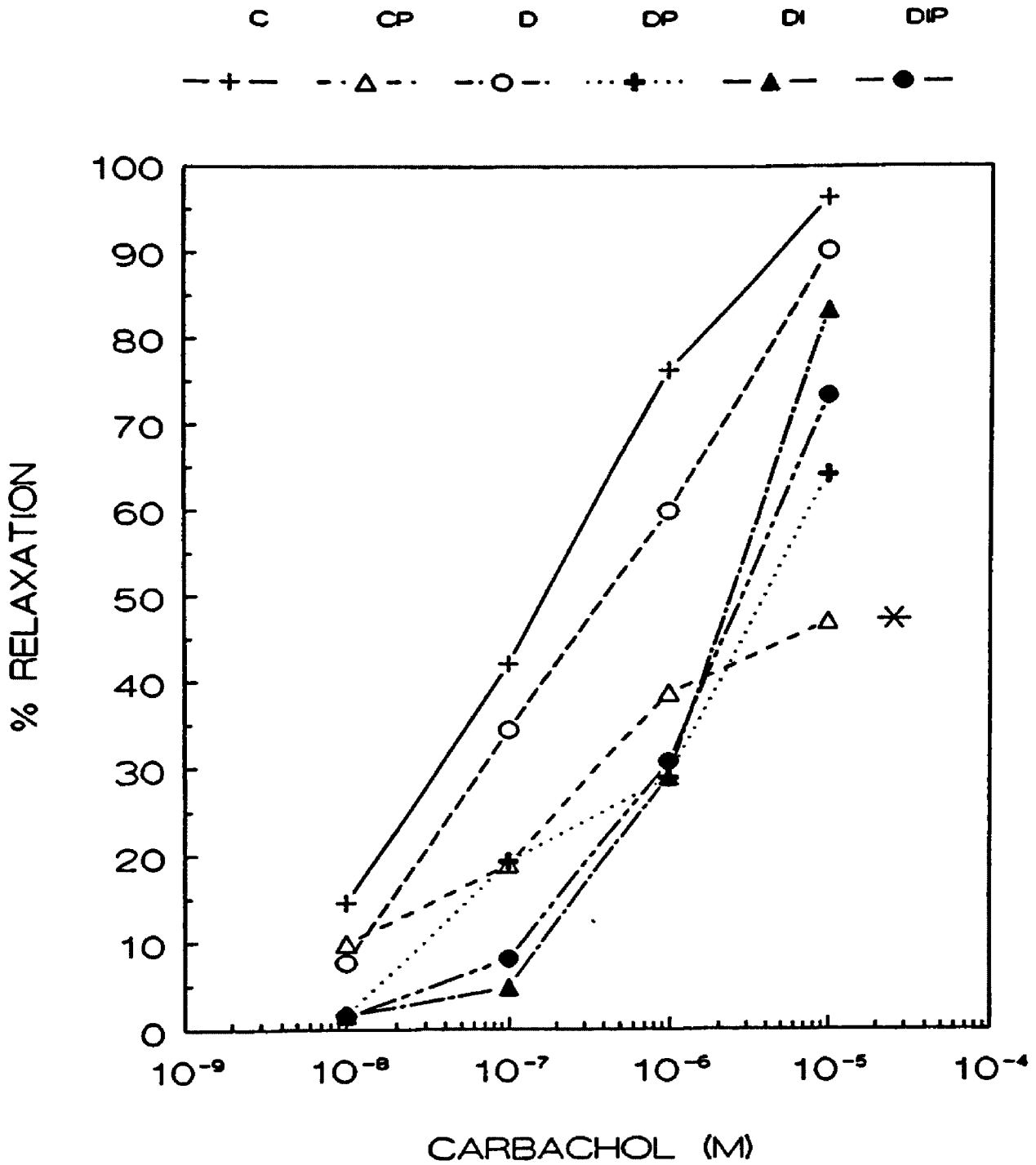


Fig. 10. Relaxant response of Type I diabetic rat aortic ring to carbachol.

\* Significantly different from C, D and DI at maximal dose.

N values are shown in Table 2.

See Fig. 5 for description of treatment groups.

$$\text{Percent relaxation} = \frac{(\text{M.C.} - \text{C.R.R.})}{\text{M.C.}} \times 100$$

M.C.= Maximal contraction in grams.

C.R.R.= Contraction in grams after relaxation with either carbachol or sodium nitroprusside.

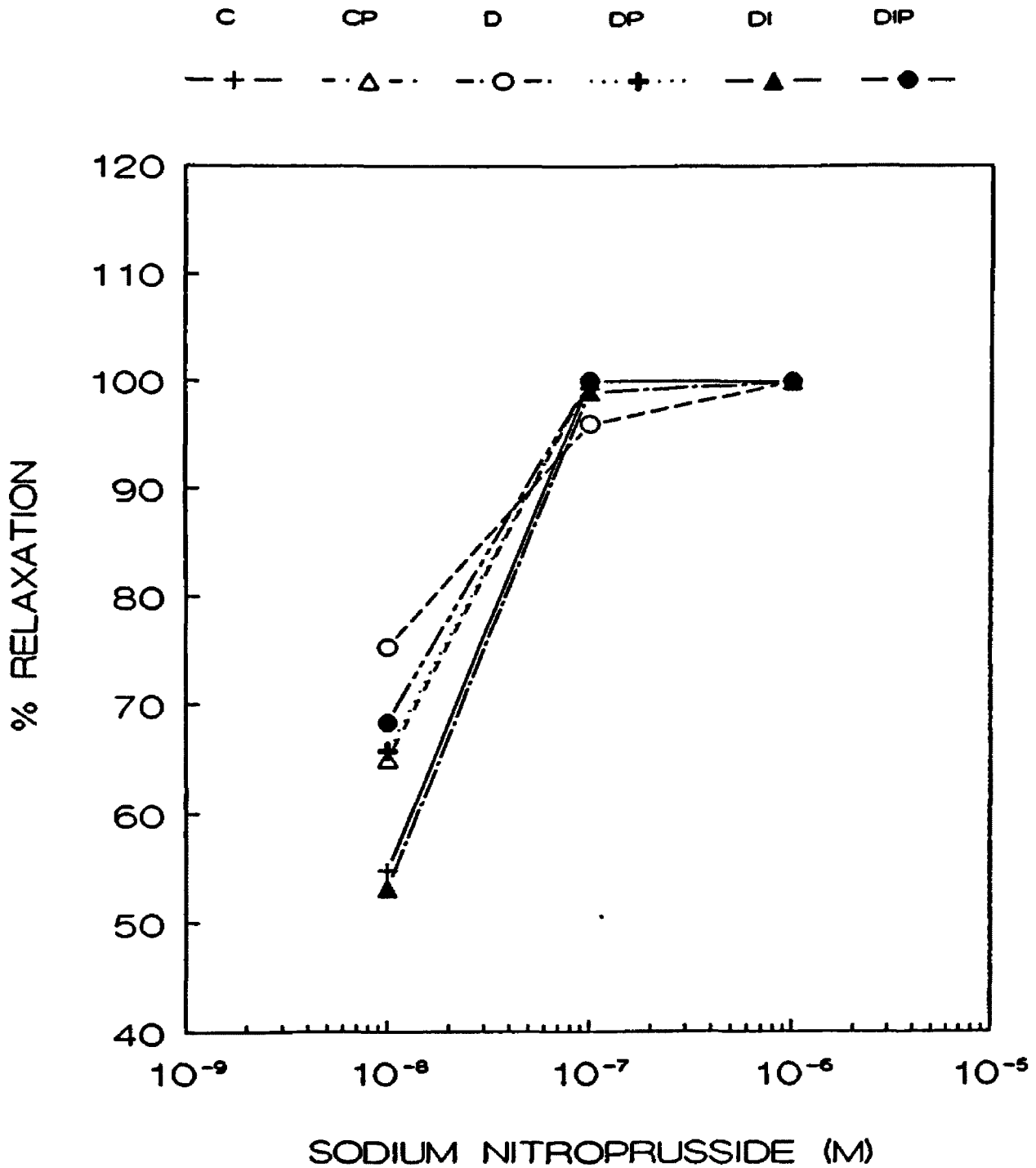


Fig. 11. Relaxant response of Type I diabetic rat aortic ring to sodium nitroprusside. Terms and abbreviations are same as Fig. 10.

**Table 3. Pre-ischemic cardiac function in Type I diabetic rats.**

	HR (beats/Min)	LVDP (mm Hg)	Double Product	CF (ml/Min)
C (N=8)	240 ± 10	94 ± 4	22.3 ± 0.8	15.6 ± 0.5
CP (N=8)	258 ± 9	108 ± 10	27.8 <sup>7</sup> ± 2.6	17 ± 0.5
D (N=7)	210 <sup>1</sup> ± 10	79 <sup>6</sup> ± 8	16.5 <sup>8</sup> ± 1.4	12 <sup>3</sup> ± 1.7
DP (N=4)	150 <sup>2</sup> ± 34	82 ± 12	11.2 <sup>8</sup> ± 1.2	11 <sup>4</sup> ± 2.4
DI (N=7)	250 ± 7	91 ± 5	22.9 ± 1.7	14 <sup>5</sup> ± 0.5
DIP (N=6)	224 ± 8	106 ± 10	23.3 ± 1.6	14.6 ± 0.8

Data are presented as Mean ± SE.

HR = Heart Rate, LVDP = Left Ventricular Developed Pressure,

CF = Coronary Flow, Double Product = (HR X LVDP)/1000.

See Fig. 5 for description of treatment groups.

1 = Significantly different from DI and CP groups.

2 = Significantly different from all other groups.

3 = Significantly different from C and CP groups.

4 = Significantly different from C, CP and DIP groups.

5 = Significantly different from CP group.

6 = Significantly different from CP and DIP groups.

7 = Significantly different from Control.

8 = Significantly different from C, CP, DI and DIP groups.

**Table 4. Post-ischemic cardiac function in Type I diabetic rats.**

	FR	HR	LVDP	Double Product	CF
C (N=7)	7/8	92 ± 4	53 <sup>1</sup> ± 8	49 <sup>1</sup> ± 6	68 <sup>1</sup> ± 3
CP (N=5)	5/8	90 ± 3	65 <sup>1,4</sup> ± 4	59 <sup>1</sup> ± 4	76 <sup>1</sup> ± 5
D (N=7)	7/7	98 ± 2	56 <sup>1</sup> ± 6	56 <sup>1</sup> ± 7	81 <sup>1</sup> ± 6
DP (N=4)	4/4	87 ± 5	72 <sup>3</sup> ± 10	64 <sup>4</sup> ± 12	79 ± 2
DI (N=7)	7/7	92 ± 4	44 <sup>1</sup> ± 3	40 <sup>1</sup> ± 4	92 ± 18
DIP (N=4)	4/6	86 <sup>2</sup> ± 3	48 <sup>1</sup> ± 8	42 <sup>1</sup> ± 8	56 <sup>1,4</sup> ± 5

All values are expressed as percent of mean ± SE of pre-ischemic values except FR.

FR = Fraction of hearts demonstrating measurable postischemic cardiac function; HR= Heart Rate; CF= Coronary Flow; LVDP = Left Ventricular Developed Pressure; See Fig. 5 for description of treatment groups.

1 = Significantly different from respective pre-ischemic value.

2 = Significantly different from Diabetic (D) group.

3 = Significantly different from DI and DIP groups.

4 = Significantly different from DI

## DISCUSSION

### Type II diabetes mellitus:

Pioglitazone increased the body weight in Type II diabetic animals, but did not alter the body weight in non-diabetic controls (Fig 1). Hofmann et al, (1991) provided evidence that after four days of pioglitazone treatment, body weights were increased, although insignificantly, in a dose dependent manner in diabetic KKA<sup>Y</sup> mice. In a similar study with Wistar diabetic fatty rats, after seven days of pioglitazone treatment, the body weight was increased concomitant with an increase in food intake (Sugiyama et al, 1990). These effects of pioglitazone were very prominent in hyperinsulinemic (Type II) animals, but not observed in either normoinsulinemic (control) or hypoinsulinemic (Type I) animals (Ikeda, 1990; Hofmann et al, 1991). This phenomenon seems to be caused by an insulin potentiating effect of pioglitazone.

Peterson et al (1990) reported that the average blood glucose levels of ZDF rats were above 500 mg% by 12 weeks of age and remained high. Although our experiments with ZDF rats were conducted for 14 weeks, the average blood glucose levels were much lower (268 ± 31 mg%). It is unclear why there was a difference. Pioglitazone treatment decreased the blood glucose levels in insulin resistant Type II diabetic animals, but did not alter the blood glucose levels in control rats (Fig. 2). This work is consistent with the

previous report in which pioglitazone decreased the blood glucose levels in Type II diabetic animals, but had no effect in control animals (Colca and Morton 1990).

The results of the present study indicate that the maximal contractile response to NE and KCl in aortas from Type II diabetic rats was not significantly different from nondiabetics (Fig. 3 & 4; Table 1). Although statistical significance was not observed, maximal contractile response to NE and KCl appeared to be somewhat higher in diabetics (D). Our diabetic animals had blood glucose values in the moderately diabetic range ( $268 \pm 31$  mg%), rather than being highly diabetic range (500 mg%). This reduced diabetic state, with respect to blood glucose, could explain why no significant difference was observed between control and diabetic animals. No reports have been found in the literature on the maximal contractile response to NE and KCl in Type II diabetic animals. Pioglitazone treatment significantly decreased the maximal response to NE in both control and Type II diabetic animals. This implies that pioglitazone decreased the maximal contractile response to NE independent of diabetic state.

#### **Type I Diabetes Mellitus:**

When pioglitazone, at a dose of 10 mg/kg/day, was given orally to Type I diabetic animals (DP) for 40 days, an overall rate of 50% mortality was observed in this experiment. The remaining rats were also lethargic and

developed severe diarrhea. Pioglitazone treatment did not alter either body weight or blood glucose levels in Type I diabetic animals. DP group glycosylated hemoglobin values also showed no change when compared with diabetic (D) group. Pioglitazone administered to diabetic rats (DP) showed the highest mortality and the lowest values of heart rate, LVDP, coronary flow and double product. In addition, DP group showed the highest maximal contractile response to NE among the treatment groups. These results suggests that long term treatment of pioglitazone may be highly deleterious to hypoinsulinemic (Type I) diabetic animals.

Both untreated and pioglitazone treated control animals increased body weight significantly over the six weeks of the experiment (Fig. 5). The induction of diabetes with STZ appeared to inhibit the normal increase in body weight observed in control animals. Those diabetic animals treated with either insulin or pioglitazone and insulin also increased body weight. These DI and DIP group rats, however, had a significantly lower body weight than either of the control groups. The D and DP groups showed no increase in body weight throughout the experimental period. Our observations are similar to previous work with STZ-induced diabetic rats (Hofmann et al, 1991). In the present study, coronary flow and double product were depressed in hearts of Type I diabetic animals. The possibility must be considered that this difference may have resulted from the



smaller body weight of diabetic rats. Penpargkul et al (1980) conducted an experiment to determine the effect of body weight and heart size on cardiac performance. This experiment involved two sets of small hearts designed to be similar in weight to hearts of diabetic rats, food restricted rats and younger rats. Unlike hearts of diabetic animals, these two sets of hearts from nondiabetic rats did not exhibit depressed cardiac performance. Although we did not measure the heart weights, taking into consideration the above results, it would appear that the potentially smaller size of hearts in STZ-treated animals can not account for the depressed cardiac performance.

Pioglitazone did not decrease the blood glucose levels in Type I diabetic animals. When pioglitazone was administered along with the insulin, blood glucose levels were significantly lower than insulin treated diabetic animals only at the second and sixth weeks of the study (Fig. 6). At the second week, insulin treatment alone decreased the blood glucose levels to  $111 \pm 18$  mg% whereas the combination of insulin and pioglitazone treatment decreased the blood glucose levels to  $29 \pm 1$  mg% in Type I diabetic animals. These results clearly demonstrate the synergistic effect of insulin and pioglitazone in Type I diabetic animals. Because of hypoglycemia in DIP group, starting from the second week to fifth week, ten percent glucose water was supplemented ad lib instead of normal

drinking water. Due to glucose supplementation to DIP group animals, significant difference was not observed from third to fifth week. After the fifth week, the effectiveness of insulin pellets appears to be decreased. Blood glucose levels were increased in two groups that received insulin. This phenomenon again showed the additive effect of insulin and pioglitazone by showing the significant difference between DI and DIP groups. These results are in agreement with those of Hofmann et al (1991), who demonstrated that the combination of insulin and pioglitazone decreased the blood glucose levels in Type I diabetic animals. These findings again indicated that pioglitazone has antidiabetic effects, but only when administered along with insulin.

Type I diabetic rats showed elevated glycosylated Hb values. Insulin treatment normalized the glycosylated Hb values in diabetic animals. Pioglitazone treatment did not alter the glycosylated Hb values either in control or diabetic animals. Combination of insulin and pioglitazone also did not decrease the glycosylated Hb values in the DIP group (Fig.7). If the blood glucose is chronically elevated (>115 mg%), a variety of body proteins, including HbA, can be nonenzymatically glycosylated by means of glucose interacting with a free amine in a protein resulting in a Schiff-Base aldimine with subsequent formation of a stable product in an Amadori rearrangement. A possible explanation for the elevated glycosylated Hb levels seen in the DIP

group could be that the synergistic effect of insulin and pioglitazone might not reverse the Amadori rearrangement of glycosylated proteins.

Reports in the literature describe increased (White et al 1990) and decreased (Fulton et al 1991; Ramanadhan et al 1984) vascular reactivity to NE in Type I diabetic animal models. Our results are in agreement with those of White et al (1990), which demonstrated increased vascular reactivity to NE in Type I diabetic animals (Table 2; Fig. 8).

Pioglitazone did not alter the maximal contractile response to NE either in control or diabetic animals. The combination of pioglitazone and insulin treatment appears to decrease the maximal response to NE in Type I diabetic rats, but statistical significance was not achieved. Insulin treatment did not show any effect on Type I diabetes-induced increased maximal contraction. In contrast, Pfaffman et al (1981), reported that insulin treatment completely reversed the diabetic-induced decrease in vascular contractility to NE.

The KCl-induced maximal contractile response was decreased in Type I diabetic animals. These results are consistent with a previous report in which KCl-induced maximal contractile response was decreased in Type I diabetic animals (Fulton et al, 1991). Insulin treatment normalized the vascular reactivity to KCl in Type I diabetic animals. Our results are in agreement with Pfaffman et al

1982, who demonstrated that diabetes-induced decrease of KCl contraction is completely reversible by insulin treatment. Pioglitazone treatment also normalized the vascular reactivity to KCl in Type I diabetic animals. This effect however was not observed when insulin and pioglitazone were used in combination (Fig. 9).

Ding et al (1991) reported that the endothelial dependent relaxation produced by carbachol in aortic rings pre-contracted with NE was reduced in Type I diabetic rats. Although statistical significance was not observed, our results show decreased maximal relaxation to carbachol in Type I diabetic animals (Fig. 10). These results suggest that deterioration of the endothelium might exist in diabetic rats. Pioglitazone treatment decreased the maximal relaxation to carbachol in all pioglitazone treatment groups, but only the CP group was significantly lower than the C, D and DI groups. These results indicate that pioglitazone may alter the endothelial function independently of diabetes or insulin. On the other hand, endothelial independent relaxation was not altered either with the diabetic state or with pioglitazone treatment. Endothelial independent relaxation results are in agreement with Ding et al (1991), who demonstrated no significant differences due to diabetes (Fig. 11). No previous reports are available on the effect of pioglitazone on endothelial independent relaxation.

Type I diabetic hearts showed no change in heart rate (Table 3). This result is consistent with the previous reports in which heart rate was unaffected in diabetic animals (Pieper 1990; Vogel and Apstein 1988). LVDP also was not altered in diabetic hearts, but there was a tendency for reduced LVDP in diabetic hearts (Table 3). Vogel and Apstein (1988) and Tani and Neely (1988) found no change in LVDP; whereas, Ingebretsen et al (1980) found significantly decreased LVDP in Type I diabetic hearts. Although diabetic heart rate and LVDP were not significantly lower when compared with control, double product which is the best estimate of cardiac function, showed a significant decrease. In agreement with our results, Tani and Neely (1988) also found decreased double product in Type I diabetic rats. Coronary flow was significantly decreased in diabetic animals (Table 3). In contrast, Ingebretsen et al (1980) found no change in coronary flow.

Pioglitazone treatment significantly decreased the heart rate, coronary flow and double product in Type I diabetic animals, while pioglitazone increased the double product in control animals (Table 3). Moreover, pioglitazone treatment did not show any deleterious effects on heart rate, LVDP and coronary flow in control animals. These results suggests that pioglitazone treatment may be harmful only in the absence of insulin. There are no previous reports available in the literature on the effects

of pioglitazone on cardiac performance in either Type I or Type II diabetic animals. Insulin treatment normalized the heart rate, LVDP, coronary flow and double product in diabetic hearts. In agreement with our results, several authors have shown in animal studies and in isolated preparations that insulin increased the cardiac performance in Type I diabetic animals (Lucchesi et al 1972; Lee and Downing 1976). The combination of insulin and pioglitazone treatment showed the synergistic effect by normalizing the LVDP and double product in diabetic rats.

The results of the present study show that STZ-induced diabetic rats did not show any increased resistance to MI/R injury (Table 4). Tani and Neely (1988) demonstrated that when whole heart ischemia was maintained for 30 minutes followed by 30 minutes reperfusion at 37<sup>0</sup>C, diabetic hearts completely (101 ± 12%) recovered their cardiac function whereas hearts from the control animals recovered only 40 ± 6% of their pre-ischemic cardiac function. In contrast, our results shows that when hearts were rendered ischemic for 20 minutes followed by 30 minutes reperfusion, diabetic hearts recovered 56 ± 7% of their cardiac function whereas hearts from control animals recovered 49 ± 6% of their pre-ischemic function (Table 4). Our results are in agreement with those of Vogel and Apstein (1988), who demonstrated that diabetes did not alter the severity of MI/R injury in isolated heart preparations. On the other hand, previous studies of the

isolated heart suggest that diabetic hearts are more sensitive to ischemic or anoxic injury (Fevury et al 1980; Ingebretsen 1980). In these studies, ischemic or anoxic injury was not followed by reperfusion.

Pioglitazone increased the post-ischemic recovery of LVDP and double product when compared to the DI group. However, pioglitazone demonstrated deleterious effects on pre-ischemic cardiac function. Pioglitazone treatment made animals very sick and caused severe diarrhea. Insulin treatment did not show any effects on post-ischemic recovery of heart rate, LVDP, double product or coronary flow in Type I diabetic animals. In contrast, Tani and Neely (1988) demonstrated that insulin treatment decreased the resistance to MI/R injury in Type I diabetic animals. Our results demonstrated that the combination of insulin and pioglitazone treatment significantly reduced the post-ischemic recovery of heart rate, but did not show any effects on post-ischemic recovery of LVDP, double product or coronary flow in Type I diabetic animals. These results suggest that the combination of insulin and pioglitazone treatment may be deleterious to postischemic heart rate.

**Comparison of Type I and Type II Diabetes Mellitus:**

Type I diabetic rats show decreased body weight, but body weight was increased in Type II diabetic rats. Pioglitazone treatment did not alter the body weight in either Type I diabetic or control rats, but Type II diabetic

rat body weights were significantly increased.

Pioglitazone treatment alone did not show any effect on blood glucose levels in either Type I diabetic or control rats. When administered along with insulin, pioglitazone showed a synergistic effect by decreasing the blood glucose levels in Type I diabetic animals. In Type II diabetes, pioglitazone significantly decreased blood glucose levels. Hofmann et al (1991) reported that GLUT4 transporter proteins and their mRNA expression were decreased in both Type I and Type II diabetic animal models. Treatment of both types of diabetic animals with pioglitazone corrects the deficits in glucose transport, GLUT4 mRNA and protein levels. Treatment with pioglitazone alone is sufficient for correction of glucose transport in Type II diabetic animals, but hypoinsulinemic animals require insulin therapy along with pioglitazone treatment for similar correction. Since insulin and pioglitazone seem to work synergistically, it is likely that pioglitazone acts to amplify cellular responses to insulin (Fig. 6).

Type I diabetic rats showed increased contractile response to NE, but Type II diabetic rats did not show any change in maximal contractile response to NE. Pioglitazone decreased the maximal response to NE in both Type II diabetic and Zucker nondiabetic rats, but did not show any effect in either Type I diabetic or nondiabetic Sprague Dawley rats. It is possible that the decrease in maximal



contractile response to NE with pioglitazone treatment may be species related.

KCl-induced maximal contractile response was decreased in Type I diabetic rats, but not altered in Type II diabetic rats. Pioglitazone increased the maximal response to KCl in Type I diabetic rats, but did not alter the maximal response to KCl in Type II diabetic rats.

**Conclusion:**

Pioglitazone treatment increased the body weight and decreased the blood glucose levels in Type II diabetic animals, but did not alter either body weight or blood glucose levels in control and Type I diabetic animals. Pioglitazone treatment did not alter the glycosylated Hb values either in control or Type I diabetic rats. Maximal contractile response to NE and KCl was decreased by pioglitazone in Type II diabetic and control animals.

In Type I diabetic animals, maximal contractile response to KCl was decreased with pioglitazone treatment, but maximal contractile response to NE was unaltered. The combination of insulin and pioglitazone or insulin alone did not show any effects on maximal contractile response to NE. Pioglitazone treatment decreased the endothelial dependent relaxation in control animals, but endothelial independent relaxation was unaltered in both control and diabetic rats. Coronary flow and double product were decreased in Type I diabetic animals. Pioglitazone administered to diabetic

rats (DP) showed the highest mortality and the lowest values of heart rate, LVDP, coronary flow and double product. Insulin treatment normalized the cardiac function in Type I diabetic animals, and also, the combination of insulin and pioglitazone treatment normalized the LVDP and double product in Type I diabetic animals. The results of the present study suggests that the severity of MI/R injury is identical in both Type I diabetic and control animals. Neither pioglitazone nor insulin treatment protected against MI/R injury in either control or diabetic rats.

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