ALPHA$_1$-ADRENOCEPTOR-MEDIATED PHOSPHOINOSITIDE BREAKDOWN

AND INOTROPIC RESPONSES

IN RIGHT VENTRICLES OF STREPTOZOTOCIN-DIABETIC RATS

By

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ABSTRACT

The morbidity of and the mortality from cardiac disease are higher in diabetic patients. Clinical and experimental evidence suggests that diabetes-induced changes at the level of myocardium can, at least partially, contribute to these cardiac problems. The mechanism(s) involved in this diabetic cardiomyopathy is still unclear, but one defect appears to occur in the $alpha_1$-adrenoceptor system. Altered myocardial sensitivity and responsiveness to $alpha_1$-adrenoceptor agonists have been reported in experimental diabetes mellitus. Stimulation of $alpha_1$-adrenoceptors is known to produce a positive inotropic effect and has been recently shown to stimulate the hydrolysis of phosphoinositides. To evaluate the possibility that the changes in the inotropic responsiveness to $alpha_1$-adrenoceptor stimulation in the diabetic heart could be linked to altered $alpha_1$-adrenoceptor-stimulated phosphoinositide turnover and further to the development of diabetic cardiomyopathy, we studied contractility and receptor-stimulated phosphoinositide turnover following norepinephrine (in the presence of propranolol) stimulation in right ventricles from male Wistar rats (200-225 g) which were made diabetic with streptozotocin (55 mg/kg, i.v.). Rats were sacrificed six weeks after the induction of diabetes. Diabetic rats were characterized by decreased body weight gain, hypoinsulinemia, hyperglycemia and hyperlipidemia.

Stimulation of alpha$_1$-adrenoceptors by norepinephrine (in the presence of propranolol) in right ventricles resulted in the formation of inositol monophosphate (measured with a radioisotope method) and inositol 1,4,5-trisphosphate (measured with an inositol 1,4,5-trisphosphate protein binding assay kit) in a time- and concentration-dependent manner in both control and diabetic rats. The increase in inositol 1,4,5-trisphosphate levels preceded the increase in the alpha$_1$-adrenoceptor-mediated positive inotropic effect. Diabetic hearts showed a greater maximum inotropic response to norepinephrine stimulation and also had a higher inositol 1,4,5-trisphosphate levels. However, with the radioisotope method, a decreased inositol monophosphate formation was shown in diabetic hearts compared with controls.

Omega-3 fatty acids supplementation (Promega^R, 0.5 ml/kg/day) had no significant effect on the changes in norepinephrine-stimulated inositol monophosphate formation in diabetic hearts.

In the presence of the cyclooxygenase inhibitor indomethacin or the thromboxane synthetase inhibitor imidazole, the norepinephrine-stimulated positive inotropic effect and inositol 1,4,5-trisphosphate formation were significantly increased in control hearts, but were unaltered in the hearts from diabetics. The addition of the prostacyclin synthetase inhibitor tranylcypromine reduced the norepinephrine-stimulated positive inotropic effect and

inositol 1,4,5-trisphosphate formation only in diabetic hearts and had no effect in the controls.

While inositol 1,4,5-trisphosphate may be able to mediate only transient inotropic effects produced by alpha$_1$-adrenoceptor stimulation, diacylglycerol may provoke a sustained positive inotropic effect by activating slow Ca^{2+} channels through stimulation of protein kinase C. Our results showed that the diabetic hearts had a higher protein kinase C activity in the membrane fraction compared with controls and this was accompanied by a decrease in cytosolic protein kinase C activity.

The present study suggests that the increases in inositol 1,4,5-trisphosphate levels and the membrane fraction protein kinase C activity may be implicated in the increased inotropic responsiveness to alpha$_1$-adrenoceptor stimulation in the hearts of the streptozotocin-diabetic rats. The increases in inositol 1,4,5-trisphosphate level and protein kinase C activity could induce Ca^{2+} overload in the diabetic heart which might be involved in the development of diabetic cardiomyopathy. The results from the omega-3 fatty acid study indicate that the changes in cardiac alpha$_1$-adrenoceptor-mediated inositol phosphates formation cannot contribute to the previously described improved cardiac function of omega-3 fatty acid-treated streptozotocin-diabetic rats. The nature and physiological significance of the enhanced positive inotropic effect and inositol 1,4,5-trisphosphate formation in the control heart

with the addition of indomethacin and imidazole is still unclear. The effect of tranylcypromine may indicate the participation of prostaglandins in mediating the enhanced $alpha_1$-inotropic effect of norepinephrine in the diabetic heart.

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AA	arachidonic acid
AMP	adenosine 5'-monophosphate
ATP	adenosine 5'-triphosphate
CDP-DG	cytidine-5'-diphospho-1,2-diacyl-sn-glycerol
CK solution	Chenoweth-Koelle solution
cpm	counts per minute
DG	diacylglycerol
DM	diabetes mellitus
-dP/dT	rate of left ventricular pressure decline
dpm	disintegrations per minute
GDM	gestational diabetes mellitus
GppNHp	guanosine 5'-[βγ-imido]triphosphate
GTP	guanosine 5'-triphosphate
12-HETE	12-hydroxyeicosatetraenoic acid
HHT	17-carbon hydroxy acid
HLA	human leukocyte antigen
12-HPETE	12-hydroperoxyeicosatetraenoic acid
HPLC	high performance liquid chromatography
IDDM	insulin-dependent diabetes mellitus
IGT	impaired glucose tolerance
Inositol	myo-inositol
Ins(1)P	D-inositol 1-phosphate
Ins(3)P	D-inositol 3-phosphate
Ins(4)P	D-inositol 4-phosphate

$Ins(1,3)P_2$	D-inositol 1,3-bisphosphate
$Ins(1,4)P_2$	D-inositol 1,4-bisphosphate
$Ins(3,4)P_2$	D-inositol 3,4-bisphosphate
$Ins(1,3,4)P_3$	D-inositol 1,3,4-trisphosphate
$Ins(1,4,5)P_3$	D-inositol 1,4,5-trisphosphate
$Ins(1:2cyc)P$	D-inositol 1,2-cyclic phosphate
$Ins(1:2cyc,4)P_2$	D-inositol 1,2-cyclic-4-bisphosphate
$Ins(1:2cyc,4,5)P_3$	D-inositol 1,2-cyclic-4,5-trisphosphate
$Ins(1,3,4,5)P_4$	D-inositol 1,3,4,5-tetrakisphosphate
$Ins(1,3,4,6)P_4$	D-inositol 1,3,4,6-tetrakisphosphate
$Ins(1,3,4,5,6)P_5$	inositol 1,3,4,5,6-pentakisphosphate
$Ins(1,2,3,4,5,6)P_6$	inositol 1,2,3,4,5,6-hexakisphosphate
IP_1	inositol monophosphate
IP_2	inositol bisphosphate
IP_3	inositol trisphosphate
IP_4	inositol tetrakisphosphate
kg	kilogram
min	minute(s)
ml	millilitre
mmol/l	millimoles per litre
MRDM	malnutrition-related diabetes mellitus
MW	molecular weight
NE	norepinephrine
NIDDM	non-insulin-dependent diabetes mellitus
PA	phosphatidic acid
PGD_2	prostaglandin D_2
PGE_2	prostaglandin E_2

6-Keto PGF$_{1\alpha}$	6-keto prostacyclin F$_{1\alpha}$
PGF$_{2\alpha}$	prostaglandin F$_{2\alpha}$
PGH$_2$	prostaglandin H$_2$
PGI$_2$	prostacyclin
PI	phosphatidylinositol
PtdIns(4)P	phosphatidylinositol 4-phosphate
PtdIns(4,5)P$_2$	phosphatidylinositol 4,5-bisphosphate
S.E.M.	standard error of the mean
SR	sarcoplasmic reticulum
STZ	streptozotocin
TXA$_2$	thromboxane A$_2$
TXB$_2$	thromboxane B$_2$
μU/ml	microunits per millilitre
WHO	World Health Organization

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Hong Xiang

DEDICATION

TO MY PARENTS,

TO MY AUNT,

TO MY PROFESSOR WEI-CHIN LIU,

INTRODUCTION

1.1. OVERVIEW.

The pancreas, a flattened elongate organ lying against the posterior wall of the upper abdomen, is both an exocrine and an endocrine gland. The exocrine role of the pancreas is carried out by cells within the tubular and acinar units of the gland. These cells secrete enzymes that catalyze the digestion of proteins, carbohydrates and fats (Luckmann and Sorensen, 1987). The endocrine functions of the pancreas are carried out by several hundred thousand microscopic nests of cells called the islets of Langerhans, which are scattered randomly through the pancreas and account for only 1-2% of its weight. The islets contain several cell types including A cells secreting glucagon, B cells secreting insulin, D cells secreting somatostatin and F cells secreting pancreatic polypeptide. Most of the pancreatic islet cells (60-80%) are of the B type (Steinberg, 1985).

Insulin is a 6000 MW polypeptide composed of an alpha chain and a beta chain linked together by disulfide bridges. The insulin molecule can exist as a monomer, dimer or hexamer (3 dimers). Two molecules of zinc are coordinated in the hexamer which is presumably the form stored in the B cell. The biologically active form of the hormone is thought to be the monomer (Cahill, 1971). Main stimulants to insulin secretion are blood glucose, amino acids, gastrointestinal peptide hormones (especially gastric

inhibitory peptide, GIP), ketone bodies, glucagon, parasympathetic and beta-adrenergic stimulation. Factors which inhibit insulin release include somatostatin, alpha-adrenergic stimulation, as well as pharmacological factors such as B cell poisons alloxan and streptozotocin (Steinberg, 1985). Normal insulin secretion by a pancreas is about 30-60 units/day (Cahill, 1971).

Insulin has important effects on carbohydrate, fat and protein metabolism as well as on ion transport and growth development. With regard to carbohydrate metabolism, insulin reduces the rate of release of glucose from liver and increases the rate of uptake of glucose into all insulin-sensitive tissues, notably muscle and adipose tissue. As for lipid metabolism, insulin reduces the rate of release of free fatty acids from adipose tissue and stimulates *de novo* fatty acid synthesis and also the conversion of fatty acids to triglycerides in liver. In protein metabolism, insulin stimulates transport of free amino acids across the plasma membrane in liver and muscle; as well, it stimulates protein biosynthesis and reduces release of amino acid from muscle (Steinberg, 1985). All of the metabolic effects of insulin are initiated by the interaction of the insulin molecule with a highly specific receptor on the plasma membrane (Stadie et al., 1953; Kohn et al., 1981). The insulin receptor has been partially purified and characterized (reviewed by Czech, 1981). It is a membrane protein of high molecular weight (about 350,000)

made up of four disulfide-linked subunits: two identical alpha chains of about 125,000 daltons and two beta chains of 45,000-90,000 daltons. It is clear that insulin receptor activation provokes increases in tyrosine kinase activity and autophosphorylation of the insulin receptor (Kahn and White, 1988), but subsequent molecular events that couple the insulin-receptor interaction to the regulation of cellular metabolism remain uncertain. Recent work by Saltiel et al.(1986) has indicated that inositol phosphate-glycan, produced by the insulin-sensitive hydrolysis of a glycosyl-phosphatidylinositol in the plasma membrane, may be involved in the signal transduction system of insulin. This hydrolysis reaction, which is catalyzed by a specific phospholipase C, also results in the production of 1,2-diacylglycerol that may regulate protein kinase C. Both inositol phosphate-glycan and protein kinase C may mediate the effects of insulin.

Diabetes mellitus comprises an etiologically and clinically heterogeneous group of disorders characterized by an absolute or relative deficiency of insulin and subsequent disorders in the metabolism of carbohydrate, fat and protein. These abnormalities account for both the acute and chronic complications of the disease.

According to the World Health Organization (WHO) classification adopted in 1985 (WHO Study Group, 1985), diabetes mellitus and allied categories of glucose intolerance are classified and characterized as shown in

Table 1 (Brunner and Suddarth, 1988). As indicated, two common types of diabetes mellitus are insulin-dependent diabetes mellitus (IDDM) (type I) and non-insulin-dependent diabetes mellitus (NIDDM) (type II).

1.1.1. Insulin-Dependent Diabetes Mellitus (IDDM).

Insulin-dependent diabetes mellitus is clearly associated with an absolute deficiency of insulin secretion. This marked decrease in insulin secretory capacity is due to a specific loss of pancreatic B cell mass (Kahn, 1985). The etiology of IDDM is not completely understood. Genetic predisposition, viruses and/or autoimmune factors have been suggested to be involved in the pathogenesis of disease (Craig, 1980). Genetically, a significantly increased frequency of certain human leukocyte antigens (HLA) has been recognized among type I diabetes (Cudworth and Woodrow, 1975; Nerup, 1978). It has been suggested that the antigens are determinants of the molecular nature of some beta cell surface proteins, some of which may have unique defects that render the cell more susceptible to certain insults (Nerup, 1978). The characteristically abrupt appearance of type I diabetes could also be the result of an infection with a diabetogenic virus. A variant of Coxsackie B_4 virus has been identified as being able to damage the pancreatic beta cells (Yoon et al., 1979). A large number of experiments carried out in animal models also seem to implicate viruses

Table 1.   Classification of diabetes mellitus and allied categories of
            glucose intolerance.

Category	Clinical Characteristics
A. CLINICAL CLASSES	
DIABETES-MELLITUS (DM)	
Insulin-dependent diabetes mellitus (IDDM) (5-10% of all diabetes)	- Any age but usually young - Mostly thin at diagnosis - Causes may be genetic, viral or abnormal immune responses - Often have islet cell antibodies - Little or no endogenous insulin - Need insulin to preserve life - Ketosis-prone
Non-insulin-dependent diabetes mellitus (NIDDM) (90-95% of all diabetes) Non-Obese - 20% of NIDDM Obese - 80% of NIDDM	- Any age, usually over 40 but occasionally under 21 - Mostly obese at diagnosis - Causes may be genetic, obesity or environmental factors - No islet cell antibodies - Varying amounts of endogenous insulin present, often higher than normal levels - May need insulin to avoid hyperglycemia - Rare ketosis, except in stress or infection - Non-ketotic hyperosmolar coma
Malnutrition-related diabetes mellitus (MRDM)	
(a) Fibrocalculous pancreatic diabetes	- Any age but usually below 30 - Mostly thin - Causes may be high cassava intake combined with inadequate protein intake - Require insulin - Absence of ketosis
(b) Protein-deficient pancreatic diabetes	- Any age but usually below 35 - Extreme degrees of wasting and emaciation - Causes may be malnutrition - Partial resistance to insulin - Absence of ketosis
Other types of diabetes associated with certain conditions and syndromes:	
pancreatic disease, disease of hormone etiology, drug or chemical-induced conditions, abnormalities of insulin or its receptors, certain genetic syndromes, etc.	
IMPAIRED GLUCOSE TOLERANCE (IGT) (non-obese, obese, or associated with certain conditions or syndromes)	- Blood glucose levels between normal and that of diabetes - Above normal susceptibility to atherosclerotic disease - Renal and retinal complications usually not significant
GESTATIONAL DIABETES MELLITUS (GDM)	- Begins or is recognized during or after pregnancy - Above normal risk of perinatal complications - Glucose intolerance transitory but frequently recurs - 50% go on to develop overt diabetes within 15 years - 80% go on to develop overt diabetes after 20 years
B. STATISTICAL RISK CLASSES	
(Subjects with normal glucose tolerance but substantially increased risk of developing diabetes.)	
PREVIOUS ABNORMALITY OF GLUCOSE TOLERANCE	
POTENTIAL ABNORMALITY OF GLUCOSE TOLERANCE	

in the pathogenesis of diabetes (Craighead, 1975; Yoon and Notkins, 1983). The viruses may cause direct cytolytic infection of pancreatic beta cells or trigger autoimmune responses (Yoon et al., 1987). Autoimmune responses may also be important in the type I diabetes. Circulating islet cell antibodies capable of destroying beta cells in the pancreatic islets are found in type I diabetics (Lernmark et al., 1978).

In all, type I diabetes is a heterogeneous group of disorders with varying etiologies. Viruses and autoimmunity may be the possible causes of a beta cell destruction in genetically predisposed individuals.

1.1.2. Non-Insulin-Dependent Diabetes Mellitus (NIDDM).

Non-insulin-dependent diabetes mellitus accounts for 90-95% of the diabetic population. A large number (between 60 and 90%) of those having NIDDM are obese and exhibit hyperinsulinism and associated insulin resistance. They usually have considerable preservation of the beta cell mass (Maclean and Ogilvie, 1955; Westermark and Wilander, 1978). It has been suggested that, in type II diabetes, either a "post-receptor defect", a decreased affinity of the receptors for insulin (Bar et al., 1978), or a "down regulation" of the insulin receptors as a consequence of permanantly increased levels of insulin (Kahn et al., 1977) might be possible factors in the pathogenesis of the disease.

1.2. PATHOPHYSIOLOGY OF DIABETES AND ASSOCIATED HEART DISEASE.

1.2.1. Pathophysiology of Diabetes.

Regardless of the etiology of diabetes mellitus, absolute or relative deficiency of insulin can result in abnormal metabolism of carbohydrate, lipid and protein, leading to a series of clinical symptoms (Figure 1). Glucose uptake into muscle and adipose tissue, and glycogenesis in liver and muscle are reduced. At the same time, glycogenolysis and gluconeogenesis increase. All of these changes result in markedly elevated blood glucose levels and glucosuria occurs when the blood glucose levels exceed the renal threshold for glucose. Increased amounts of glucose in the urine cause osmotic diuresis giving rise to the symptom of polyuria, followed by polydipsia and polyphagia due to metabolic imbalance. Lipids are mobilized resulting in hyperlipidemia. Insulin deficiency also causes the net breakdown of muscle protein and weight loss follows.

These disturbances in carbohydrate, lipid and protein metabolism in diabetes are known to result in a large number of acute and chronic complications which affect various systems and organs. Acute complications include ketoacidosis (McGarry and Foster, 1977) and non-ketotic diabetic coma (Arieff and Caroll, 1972) (Figure 1), most of which are reversible with insulin therapy. Much more common now are chronic complications including retinopathy and

Figure 1

Pathophysiological alterations in carbohydrate, lipid and protein metabolism in diabetes (modified from Steinberg, 1985).

INSULIN DEFICIENCY

↓ Glucose Uptake
↓ Glycogenesis
↑ Glycogenolysis
↑ Gluconeogenesis

↑ Activity of Hormone-sensitive Lipase

↓ Rate of Free Fatty Acids (FFA) Re-Esterification

↓ Lipoprotein Lipase

Loss of Stimulus for Protein Synthesis

Decreased Rate of Amino Acid Transport

Hyperglycemia

Glycosuria

Polyuria

Polyphagia and Polydipsia

↑ Plasma FFA

↓ Very Low Density Lipoproteins (VLDL) and Chylomicron Clearance

Net Protein Breakdown → Weight Loss

Dehydration and Electrolyte Depletion

↑ Hepatic FFA Uptake

Muscle Wasting

Aminoacidemia

Hypovolemia

Ketonemia

↑ Triglyceride Synthesis

↑ VLDL Secretion

Loss of K⁺ and H₂O

Gluconeogenesis

Hypotension

Ketoacidosis

Fatty Liver

Lipemia

Urinary K⁺ and Urea

Poor Tissue Perfusion

Anuria   Lacticacidemia

Coma

9

cataract formation which may lead to blindness in patients with long-standing diabetes (Davis, 1974), nephropathy (Balodimos, 1971), neuropathy (Sibley, 1982), an increased incidence of infection (Brunner and Suddarth, 1988), and cardiovascular diseases.

1.2.2. Heart Disease and Diabetes.

Cardiovascular disease represents one of the major complications of the diabetic state. Clinical studies have shown that the incidence of cardiovascular disease is two to three fold greater among diabetics than among non-diabetics (Kannel and McGee, 1979), and it now accounts for approximately 80% of all diabetic deaths (Kannel, 1978).

The pathogenesis of cardiac disease in diabetes is uncertain. It is often associated with an increase in atherosclerosis but it may also be due to a combination of factors including microangiopathy, macroangiopathy and autonomic neuropathy (Ledet et al., 1979). Recently, it has become apparent that cardiac disease associated with diabetes is not always associated with the above factors since they do not occur in a significant number of diabetic patients, and various studies have now suggested that a cardiomyopathy may also be a causal factor in producing the increase in morbidity and mortality of diabetics. For example, clinically and pathologically, Hamby et al. (1974) reported left ventricular dilation and hypertrophy in the

absence of large coronary artery disease or systemic hypertension in 16 diabetic patients. A shorter left ventricular ejection time and a longer pre-ejection period were shown in diabetic subjects without myocardial ischemia or other cardiovascular disease (Ahmed et al., 1975). Post-mortem examination by Regan et al. (1977) showed nine out of eleven diabetics had no significant coronary artery disease, although six had had heart failure at death. In experimental animal studies, a primary myocardial abnormality in diabetes was first shown in dog, a species known to have a low incidence of spontaneous arterial disease (Regan et al., 1974). The end-diastolic volume and the stroke volume response were significantly less in eleven-month alloxan-diabetic dogs, associated with a two-fold greater end-diastolic pressure increment, suggesting a decreased ventricular compliance in the absence of coronary artery disease. Similar abnormalities in ventricular function were reported in 18-month diabetic rhesus monkeys (Haider et al., 1978). Fein et al.(1985) examined papillary muscle function in rabbits made diabetic with alloxan and demonstrated a markedly prolonged duration of isometric and isotonic contraction and relaxation. Studies with rats also showed a slowing of relaxation as well as a depression of shortening velocity in left ventricular papillary muscles from severely diabetic rats (Fein et al., 1980). Using the isolated working heart preparation, a decreased rate of contraction and relaxation at higher filling pressures

(Penpargkul et al., 1980; Vadlamudi et al., 1982) and afterloads (Ingebretsen et al., 1980) have been reported in streptozotocin- or alloxan-diabetic rats.

Although these clinical and experimental data have strongly suggested that diabetes mellitus is associated with the development of a cardiomyopathy--diabetic cardiomyopathy, the mechanism(s) involved are still unclear. However, increasing evidence suggests that the cardiomyopathy associated with diabetes may be due to an alteration of myocardial enzyme systems and subcellular organelles such as sarcolemma, sarcoplasmic reticulum, mitochondria, contractile proteins as well as receptors.

1.2.2.1. Sarcolemma.

The contraction and relaxation cycle of the heart is generally viewed as the consequence of raising and lowering the intracellular concentration of free calcium. Ca^{2+} movements in the cardiac cell can be modified at a number of sites: sarcolemma (Langer, 1984), sarcoplasmic reticulum (Fabiato, 1983), mitochondria (Carafoli, 1985), and at myofilaments (Fabiato and Fabiato, 1975; Lab et al., 1984). The sarcolemma, a surface membrane of the cell, is composed of the glycocalyx (the basement membrane) and the plasmalemma. The glycocalyx is the exterior layer of the sarcolemma and contains polysaccharides which may be associated with lipids (glycolipids) or with proteins (glycoproteins). Sialic acid residues, located at the ends

of short branched carbohydrate chains attached to proteins, are the site for calcium binding at the basement membrane. The plasmalemma, on the other hand, is a bilayered structure and contains phospholipids (Nayler et al., 1984). In addition to their role as major structural constituents of cell membranes, phospholipids bind large quantities of calcium (Philipson et al., 1980). Several enzymatic and nonenzymatic systems are also associated with this lipid bilayer which, together with phospholipids, are involved, either directly or indirectly, in the regulation of myocardial calcium transport and contractility. These include Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase (Ca^{2+}-pump), adenylate cyclase, Na^+-Ca^{2+}-exchanger (Schwartz, 1974; Dhalla et al., 1978; Caroni and Carafoli, 1981; Philipson et al., 1982). The membrane-bound enzymes are also involved in transmembrane methylation of phospholipids (Strittmatter et al., 1981), which alters membrane fluidity and, in turn, influences several enzyme systems (Hirata and Axelrod, 1980). Thus, both glycocalyx and plasmalemma bind large quantities of calcium which are believed to play a strategic role in the beat-to-beat control of the heart. When the cardiac cell is depolarized, there is an influx of calcium through the sarcolemma as well as a release of calcium from sarcolemmal stores, which results in contraction. Relaxation, on the other hand, is partly achieved by calcium efflux through the sarcolemma (Dhalla et al., 1978).

In diabetic myocardium, defects in sarcolemmal enzymatic activities and composition have been observed. A depression of Na^+-K^+-ATPase activity was reported in STZ-diabetic hearts (Ku and Sellers, 1982; Pierce and Dhalla, 1983). Decreased Na^+-Ca^{2+}-exchanger (Makino et al., 1987) and Ca^{2+}-pump activities (Heyliger et al., 1987) have also been shown. It is possible that the changes in enzyme activities as well as ion transport systems associated with cardiac sarcolemma during diabetes might be partially induced by phospholipid alteration. In fact, phospholipid methylation has been reported to be depressed in the diabetic rat heart (Ganguly et al., 1984). Also a decrease in the sialic acid residues of the sarcolemma has been shown (Pierce et al., 1983). Parallel with these changes, a significant loss in the ability of the cardiac sarcolemma to bind calcium was found in chronic STZ-diabetic rats (Pierce et al., 1983). These alterations in the composition and enzyme activities of the sarcolemma could change its ability to transport calcium effectively and the cardiac contraction and relaxation process may thus be altered.

1.2.2.2. Sarcoplasmic reticulum.

Sarcoplasmic reticulum (SR) is an intracellular organelle and occupies about 2% of each ventricular myocyte (David et al., 1979). Ca^{2+} transport by SR is another major mechanism by which intracellular Ca^{2+} levels and thereby contractility of the heart are regulated. This is

accompanied by a K^+-sensitive Ca^{2+}-dependent ATPase on the SR membrane (Maclennan, 1970), which represents up to 90% of the total protein content of the membrane and which transports Ca^{2+} from the cytoplasmic space into the vesicles of SR with a high velocity and affinity (Chamberlain et al., 1983) and thus is involved in the relaxation of the heart. The Ca^{2+}-dependent ATPase appears to be regulated either directly via ionic control (such as K^+) at allosteric sites (Jones et al., 1977) or indirectly through phosphorylation of phospholamban, the protein closely associated with the ATPase protein in the membrane, by calmodulin (Katz, 1980) and/or cAMP-dependent protein kinase (LaRaia and Morkin, 1974).

In the diabetic rat heart, the ability of the SR to transport calcium is impaired (Penpargkul et al., 1981; Lopaschuk et al., 1983a; Ganguly et al., 1983). This may explain the impairment of relaxation in the diabetic heart. As a consequence of this lowered uptake of Ca^{2+}, the amount of calcium available for release during the following beats may be lower than normal which may cause defects in contraction. This calcium-transport defect may be due to biochemical changes in the diabetic heart (Lopaschuk et al., 1983b).

1.2.2.3. Mitochondria.

Mitochondria occupy a large portion (33%) of each myocyte (David et al., 1979) and are the chief source of the

myocardial energy (as ATP) that the cells need to survive and function. This energy can be distributed to a number of energy-utilizing systems of the cell, such as Na^+-K^+-ATPase and the Ca^{2+}-pump of sarcolemma and Ca^{2+}-dependent ATPase of sarcoplasmic reticulum. Besides generation of ATP as its main function, cardiac mitochondria have also been reported to actively accumulate calcium (Carafoli and Crompton, 1978). It is generally believed that mitochondrial calcium transport does not play a major role in the regulation of cytoplasmic calcium during the contraction-relaxation cycle. It may, however, help to relax the myocardium when intracellular Ca^{2+} concentrations become extremely high or in certain pathological states such as myocardial ischemia (Katz, 1977). Studies have also revealed a Na^+-induced Ca^{2+}-release as well as spontaneous calcium release from mitochondria (Goring et al., 1977), though the physiological significance of this calcium release has not been established.

A depression in calcium uptake activity was observed in the diabetic mitochondria (Pierce and Dhalla, 1985). A defect in phosphorylation of creatine in the presence of succinate or malate was also shown in alloxan-diabetic heart (Goranson and Erulker, 1949) which may lead to a reduction in ATP levels (Haugaard and Haugaard, 1964; Pieper and Murray, 1987). Both low tissue ATP levels and intracellular calcium accumulation may eventually impair normal cellular

function and may thus be involved in the pathogenesis of cardiomyopathy during diabetes.

1.2.2.4. Contractile proteins.

The contractile apparatus of the cardiac cell consists of myofilaments. The thick filament is composed of myosin molecules. Myosin has the ability to split ATP, i.e., it acts as an ATPase. When myosin combines with actin, it forms an actomyosin complex that is enzymatically even more active in its ability to split ATP. Myosin itself can be separated into three isozyme components--V_1, V_2, V_3, among which the V_1 form is thought to be the fastest of the myosin isoenzymes with regard to ATP hydrolysis as well as cross-bridge formation. The thin filament is composed of actin molecules (Braunwald et al., 1984).

A number of studies have shown a diabetes-induced depression of myosin and actomyosin ATPase activity in rat hearts (Dillman, 1980; Malhotra et al., 1981; Pierce and Dhalla, 1981; Garber et al., 1983). This depression was suggested to be due to altered myosin isoenzyme distribution. In non-diabetic rats, myosin ATPase is present predominantly in the most active V_1 form (about 72% of the total) and about 13% in the V_3 form (slowest form). In diabetic rat hearts, however, the V_3 form predominates while the V_1 content is low (Dillman. 1980). Depression of myosin ATPase activity has also been demonstrated in the alloxan-diabetic rabbit (Bhimji et al., 1985). However,

normal rabbits predominantly have the V_3 form, so the mechanism for depression of myosin ATPase would be different from that seen in rats. Various studies have demonstrated the presence of hypothyroidism in diabetic patients and animals (Dillman, 1980; Pittman et al., 1979). The shift of myosin ATPase isoenzymes and depression of myosin ATPase are thought to occur as result of the diabetes-induced hypothyroidism (Yazaki and Raben, 1975; Flink et al., 1979; Dillmann, 1982). Since activity of myosin ATPase is known to correlate closely with contractility (Scheuer and Bhan, 1979), it can be hypothesized that diabetes induces depression of myosin ATPase in the heart which then leads to altered contractility.

1.2.2.5. Receptors.

Evidence currently available indicates that induction of diabetes has a profound effect on myocardial muscarinic and adrenergic receptor mediated events. With regard to muscarinic receptor responses, clinically, persistent resting tachycardia (Rundles, 1945; Wheeler and Watkins, 1973) and loss of beat-to-beat variation (sinus arrhythmia) during deep breathing (Lloyd-Mostyn and Watkins, 1975; Feldman, 1981) are some of the characteristic symptoms of defective vagal control of the heart in diabetic patients. Partial and occasionally total vagal denervation of the heart has been encountered in chronic diabetic patients (Wheeler and Watkins, 1973). Pathological changes such as

severe loss of myelinated axons and excess deposition of collagen have been demonstrated in the vagus nerve during postmortem examination of diabetic patients (Duchen et al., 1980). The defective parasympathetic (vagal) control is an early detectable feature of cardiac autonomic neuropathy in diabetics, which is, at least partially, responsible for disturbances in the cardiovascular system in diabetics. Experimentally, an initial (up to 100 days of STZ or alloxan-diabetes) reduction in sensitivity to carbachol was noted followed by a supersensitivity to carbachol which lasted up to 360 days of diabetes (Vadlamudi and McNeill, 1983a). This supersensitivity in long-term diabetics has also been reported by Tomlinson and Yusof (1981) who showed that isolated left atria from 7- to 8-month alloxan-diabetic rats were supersensitive to the negative inotropic effect of acetylcholine. Defective parasympathetic innervation of the heart could lead to a lack of the neurotransmitter acetylcholine and to the development of postjunctional supersensitivity to exogeneously administered cholinergic drugs.

The myocardial beta-adrenergic receptor system is also affected by the induction of diabetes. However, reports concerning beta-adrenergic effects in the experimentally-induced diabetic heart are conflicting. Foy and Lucas (1978) have demonstrated that the inotropic responses to isoproterenol were reduced while the chronotropic responses to isoproterenol increased in isolated spontaneously beating

atria from 2-week STZ diabetic rats. Ramanadham and Tenner (1986) also showed a decreased sensitivity of ventricular tissue to the inotropic effects of isoproterenol in chronic diabetic rats. However, Ingebretsen et al. (1980) reported no change in the inotropic response of isolated perfused working hearts from acute alloxan-diabetic rats to isoproterenol. Furthermore, results from Vadlamudi and McNeill (1984) showed that the positive inotropic effect of isoproterenol was not altered in isolated working hearts from alloxan- or STZ-diabetic rats at any time points after induction of the disease. Apart from producing a positive inotropic effect, beta-agonists are known to exert a profound relaxing effect on the heart. In this aspect, maximum changes produced in -dP/dT by isoproterenol were depressed in diabetic rat hearts at various time points after the induction of the disease (Vadlamudi and McNeill, 1984). The changes in the beta-adrenergic system in diabetes also include decreases in beta-adrenoceptor density (Savarese and Berkowitz, 1979) and decreases in isoproterenol stimulated adenylate cyclase activity (Atkins et al., 1985). The responses of the cardiac cyclic AMP system to isoproterenol have been reported to be decreased in acute diabetic rats (Ingebretsen et al., 1981) or to be unaltered in both acute and chronic diabetic rats (Vadlamudi and McNeill, 1983b).

1.3. CARDIAC ALPHA$_1$-ADRENOCEPTORS AND PHOSPHOINOSITIDE METABOLISM.

1.3.1. Receptor-stimulated Phosphoinositides Metabolism.

Over thirty years have now passed since the original report by Hokin and Hokin (1953) of receptor-stimulated turnover of inositol lipids. Since then, the phosphoinositides have enjoyed periods of interest, neglect, controversy and finally acceptance as important intermediaries in biological signalling processes in a wide variety of systems. The pivotal contributions which resulted in this tumultuous history came from a number of laboratories. The hypothesis (Michell, 1975) that the phosphoinositides somehow served to couple receptors to cellular calcium mobilization provoked considerable research and criticism. However, further progress in understanding the exact role of inositol lipid turnover in receptor mechanisms was hindered by an imprecise knowledge of the biochemical pathway involved. Only in the early 1980's did the experimental evidence begin to indicate that the initial reaction in stimulated phosphoinositide turnover was the breakdown of not the major known inositide, phosphatidylinositol, but rather a minor phosphorylated derivative, phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P$_2$] (Abdel-Latif et al., 1977; Kirk et al., 1981). Berridge (1983) realized that the water soluble product of this reaction, D-inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$], was a likely candidate for a second

messenger to activate the release of Ca^{2+} from intracellular stores. Shortly, Berridge and others (Streb et al., 1983) demonstrated that this molecule in fact had the predicted biological activity; $Ins(1,4,5)P_3$ in micromolar concentration rapidly released Ca^{2+} from a non-mitochondrial store in permeabilized pancreatic acinar cells. This result was quickly confirmed in a number of different laboratories (Berridge, 1986). A parallel story evolved from the work in Nishizuka's laboratory (Nishizuka, 1983; Nishizuka, 1984a; Nishizuka, 1984b) which demonstrated that the other product of inositol lipid breakdown, diacylglycerol (DG) was also a cellular messenger; this intermediate of phospholipid metabolism was shown to be a potent and specific activator of a ubiquitous protein kinase which Nishizuka designated as C-kinase.

$PtdIns(4,5)P_2$ is hydrolysed by phospholipase C under the stimulation of receptors to form $Ins(1,4,5)P_3$ and DG. There are indications that receptor coupling to phospholipase C might involve a G-protein mechanism similar to the adenylate cyclase system. In electrically permeabilized platelets, the effects of thrombin on secretion were potentiated by guanine nucleotides (Haslam and Davidson, 1984). More recently, several studies have demonstrated an activation by guanine nucleotides of phospholipase C in membranes or in permeable cells (Cockcroft and Gomperts, 1985; Smith et al., 1986). Further, activation of phospholipase C shows the same

relative sensitivity to guanine nucleotide analogs as for regulation of adenylate cyclase: GTP-gamma-S>GppNHp>GTP (Merritt et al., 1986). In many systems, receptor activation of phospholipase C is potently inhibited by pertussis toxin (Ui, 1986). However, phospholipase C-linked receptors have been shown to be insensitive to pertussis toxin in the exocrine pancreas (Merritt et al., 1986). These data suggest that the coupling of receptors to phospholipase C involves a guanine nucleotide dependent regulatory protein which is similar, but not identical to the proteins which regulate adenylate cyclase. The differential sensitivity to pertussis toxin may indicate that different G-proteins are involved in the regulation of inositol lipid metabolism in different systems.

Only six years ago, the formation of inositol tris- and bisphosphates in response to the activation of receptors was demonstrated (Berridge et al., 1983). At that time a rather simple biochemical pathway was described involving the sequential dephosphorylation of Ins(1,4,5)P$_3$ to D-inositol 1,4-bisphosphate [Ins(1,4)P$_2$], to D-inositol 1-phosphate [Ins(1)P] and finally to free inositol by a lithium-sensitive inositol 1-phosphatase. Today, the picture of inositol phosphate metabolism is quite complex (Figure 2), which is largely due to the power of high performance liquid chromatography (HPLC) analyticl procedures that can separate inositol phosphates with only subtle structural differences. Ins(1,4,5)P$_3$ is dephosphorylated by an extremely active 5-

Figure 2

Inositol phospholipid metabolism. DG, diacylglycerol (1-stearoyl 2-arachidonyl-sn-glycerol); PA, phosphatidic acid; CDP-DG, cytidine-5'-diphospho-1,2-diacyl-sn-glycerol; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PtdIns(4)P, phosphatidylinositol 4-phosphate; PtdIns, phosphatidylinositol; Ins(1:2cyc,4,5)P_3, D-inositol 1,2-cyclic-4,5-trisphosphate; Ins(1,2cyc,4)P_2, D-inositol 1,2-cyclic-4-bisphosphate; Ins(1,2cyc)P, D-inositol 1,2-cyclic phosphate; Ins(1)P, D-inositol 1-phosphate; Ins(1,4)P_2, D-inositol 1,4-bisphosphate; Ins(4)P, D-inositol 4-phosphate; Inositol, myo-inositol; Ins(1,4,5)P_3, D-inositol 1,4,5-trisphosphate; Ins(1,3,4,5)P_4, D-inositol 1,3,4,5-tetrakisphosphate; Ins(1,3,4)P_3, D-inositol 1,3,4-trisphosphate; Ins(3,4)P_2, D-inositol 3,4-bisphosphate; Ins(3)P, D-inositol 3-phosphate; Ins(1,2,3,4,5,6)P_6, inositol 1,2,3,4,5,6-hexakisphosphate; Ins(1,3,4,5,6)P_5, inositol 1,3,4,5,6-pentakisphosphate; Ins(1,3,4,6)P_4, D-inositol 1,3,4,6-tetrakisphosphate; Ins(1,3)P_2, D-inositol 1,3-bisphosphate.

Arachidonic Acid PtdIns(4,5)P$_2$ $\longrightarrow$ Ins(1,4,5)P$_3$ Ins(1,2,3,4,5,6)P$_6$

Ins(1:2cyc,4,5)P$_3$ Ins(1,3,4,5)P$_4$ $\longrightarrow$ Ins(1,3,4,5,6)P$_5$

DG Ins(1,3,4,6)P$_4$

Ins(1,4)P$_2$

PtdIns(4)P $\longrightarrow$ Ins(1:2cyc,4)P$_2$ Ins(1,3,4)P$_3$

PA Ins(4)P Ins(1,3)P$_2$

Ins(1:2cyc)P Ins(3,4)P$_2$

PtdIns

CDP-DG

Ins(1)P Ins(3)P Ins(1)P
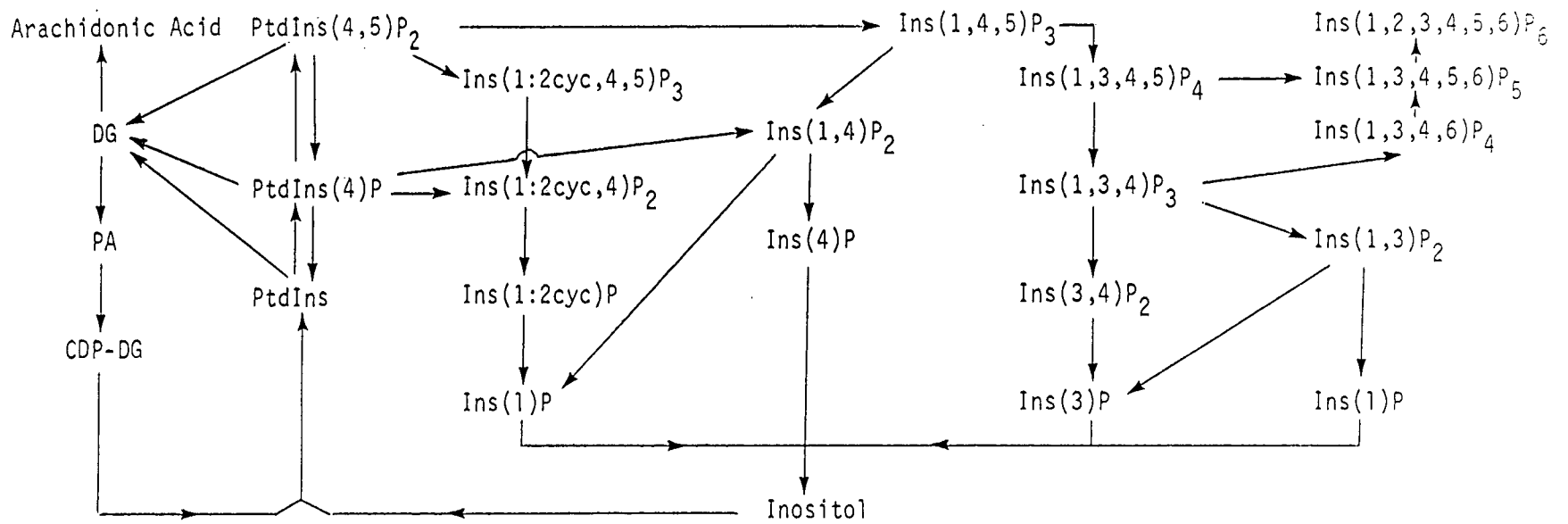
Inositol

phosphatase to Ins(1,4)P_2 (Downes et al., 1982). Ins(1,4)P_2 thus formed is dephosphorylated almost exclusively to D-inositol 4-phosphate [Ins(4)P] by a relatively non-specific inositol polyphosphate 1-phosphatase (Inhorn et al., 1987). In addition to the dephosphorylation of Ins(1,4,5)P_3 by the 5-phosphatase, there exists in most tissues thus far examined a 3-kinase which transfers a phosphate from ATP to the 3 position of Ins(1,4,5)P_3 to form D-inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4] (Irvine et al., 1986). This molecule is then dephosphorylated by, probably, the same 5-phosphatase which degrades Ins(1,4,5)P_3 to form an isomeric inositol trisphosphate, D-inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3]. Ins(1,3,4)P_3 is then dephosphorylated by the inositol polyphosphate 1-phosphatase to D-inositol 3,4-bisphosphate [Ins(3,4)P_2] (Inhorn et al., 1987), and to a lesser extent to D-inositol 1,3-bisphosphate [Ins(1,3)P_2] (Bansal et al., 1987) by an enzyme that has not been well characterized. These bisphosphates are then dephosphorylated primarily to a mixture of Ins(1)P and D-inositol 3-phosphate [Ins(3)P]. The complexity of this metabolic pathway suggests that some biological function may be regulated by one of these metabolites. For example, Ins(1,3,4,5)P_4 has been shown to either potentiate the action of Ins(1,4,5)P_3 or modulate Ca^{2+} entry (Irvine and Moor, 1986; Morris et al., 1987).

The metabolism of the inositol phosphates is further complicated by the demonstration that *in vitro*, the soluble

product of phospholipase C action on PtdIns(4,5)P_2 is a mixture of Ins(1,4,5)P_3 and its cyclic derivative D-inositol 1,2-cyclic-4,5-trisphosphate [Ins(1:2cyc,4,5)P_3] (Connolly et al., 1986). In platelets, Ins(1:2cyc,4,5)P_3 can also mobilize intracellular Ca^{2+} with about the same potency as the non-cyclic variety (Wilson et al., 1985). Ins(1:2cyc,4,5)P_3 is not a substrate for the 3-kinase, and is only slowly dephosphorylated by the 5-phosphatase (Connolly et al., 1987). Thus, if formed *in vivo*, this compound might cause persistent activation of Ca^{2+}-mobilization. Reports on the production of Ins(1:2cyc,4,5)P_3 in cells are somewhat conflicting; it appears that on brief stimulation little of the cyclic derivative is formed (Hawkins et al., 1987), but it accumulates on prolonged stimulation (Sekar et al., 1987) probably due to its slow metabolism. Estimation from kinetics of Ins(1,4,5)P_3 and Ins(1:2cyc,4,5)P_3 turnover in parotid gland suggest that as little as 1% of the PtdIns(4,5)P_2 phospholipase C product may be cyclic (Hughes et al., 1988). Although Ins(1:2cyc,4,5)P_3 can mobilize Ca^{2+} (Wilson et al., 1985), its potency is probably at least an order of magnitude less than Ins(1,4,5)P_3 (Willcocks et al., 1989). In addition, the cellular turnover of Ins(1:2cyc,4,5)P_3 is so slow in the parotid gland that its role in regulating cellular calcium metabolism is doubtful.

Inositol 1,3,4,5,6-pentakisphosphate [Ins(1,3,4,5,6)P_5] and inositol 1,2,3,4,5,6-hexakisphosphate

[Ins(1,2,3,4,5,6)P_6] are also present in most mammalian cells, but their levels do not change noticeably on stimulation (Heslop et al., 1985).

The inositol phospholipid metabolism pathway is stimulated by a variety of receptors. Most of these receptors are Ca^{2+} mobilizing receptors, such as muscarinic, alpha$_1$-adrenergic, histamine (H_1), serotonin (5-H_2), peptidergic [vasopressin(V_1), angiotensin II, bradykinin, substance P and f-methionyl-leucyl-phenylalanine], thrombin, thyrotropin-releasing hormone, glucose, platelet-activating factor, light, etc. Recent findings suggest that certain receptors which do not function through Ca^{2+} mobilization can lead to PtdIns(4,5)P_2 hydrolysis. These receptors are linked to ion channels. Examples of this class of receptors are the nicotinic acetylcholine receptor, a cation channel, and receptors of glutamate which control chloride channels. The receptor-coupled phosphoinositide breakdown occurs in a wide variety of tissues, including: (a) those which are excitable, e.g., smooth muscle, brain, and sympathetic ganglion; (b) those which are nonexcitable, e.g., liver hepatocytes, platelets, and neutrophils; and (c) those which are either endocrine, e.g., adrenal medulla and pineal, or exocrine, e.g., pancreas and parotids.

Most agents that enhance phosphoinositide turnover also increase release of arachidonic acid (AA) and subsequent synthesis of cyclooxygenase and lipoxygenase products-- eicosanoids (prostaglandins, thromboxane, leukotrienes,

etc.). The biosynthesis pathway of the products of AA is summarized in Figure 3.

1.3.2. Myocardial Alpha$_1$-adrenoceptors and Their Positive Inotropic Effect on the Heart.

Both *in vivo* and *in vitro* pharmacologic experiments have demonstrated the presence of alpha-adrenoceptors in the myocardium (Benfey, 1973; Williams and Lefkowitz, 1978; Levy and Martin, 1979). The physiological role of alpha-adrenoceptors in control of cardiac function is, however, unclear. Mammalian cardiac tissues have pre- and post-junctional alpha-adrenoceptors (Benfey, 1980). There is evidence that pre-junctional alpha$_2$-adrenoceptors modulate cardiac noradrenergic transmission (Docherty and McGrath, 1979; Drew, 1980), and that post-junctional alpha$_1$-adrenoceptors may alter the inotropic responses of the heart. In isolated cardiac tissue (atria or ventricle) from various species (guinea pig, rabbit, rat, cat, dog, human, etc.), activation of post-junctional alpha$_1$-adrenoceptors produces positive inotropic responses (Wagner and Brodde, 1978; Endoh, 1982). In general, alpha-adrenoceptor stimulation has no effect on chronotropic response under normal conditions (Wagner and Brodde, 1978).

The alpha$_1$-adrenoceptor-mediated positive inotropic effect is characterized by a slower rate of increase in contraction and relaxation compared with that mediated by beta-adrenoceptors (Osnes et al., 1985). Unlike the beta-

Figure 3

Biosynthesis of the products of arachidonic acid. 12-HPETE, 12-hydroperoxyeicosatetraenoic acid; 12-HETE, 12-hydroxy eicosatetraenoic acid; PGI_2, prostacyclin; 6-Keto $PGF_{1\alpha}$, 6-keto prostacyclin $F_{1\alpha}$; PGH_2, prostaglandin H_2; PGE_2, prostaglandin E_2; PGD_2, prostaglandin D_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; HHT, 17-carbon hydroxy acid; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2.

Arachidonic Acid

Lipoxygenase

Cyclooxygenase

12-HPETE

12-HETE

Prostacyclin
Synthetase

PGH_2

Thromboxane
Synthetase

PGI_2

TXA_2

6-Keto-$PGF_{1\alpha}$

TXB_2

PGE_2

PGD_2

$PGF_{2\alpha}$

HHT
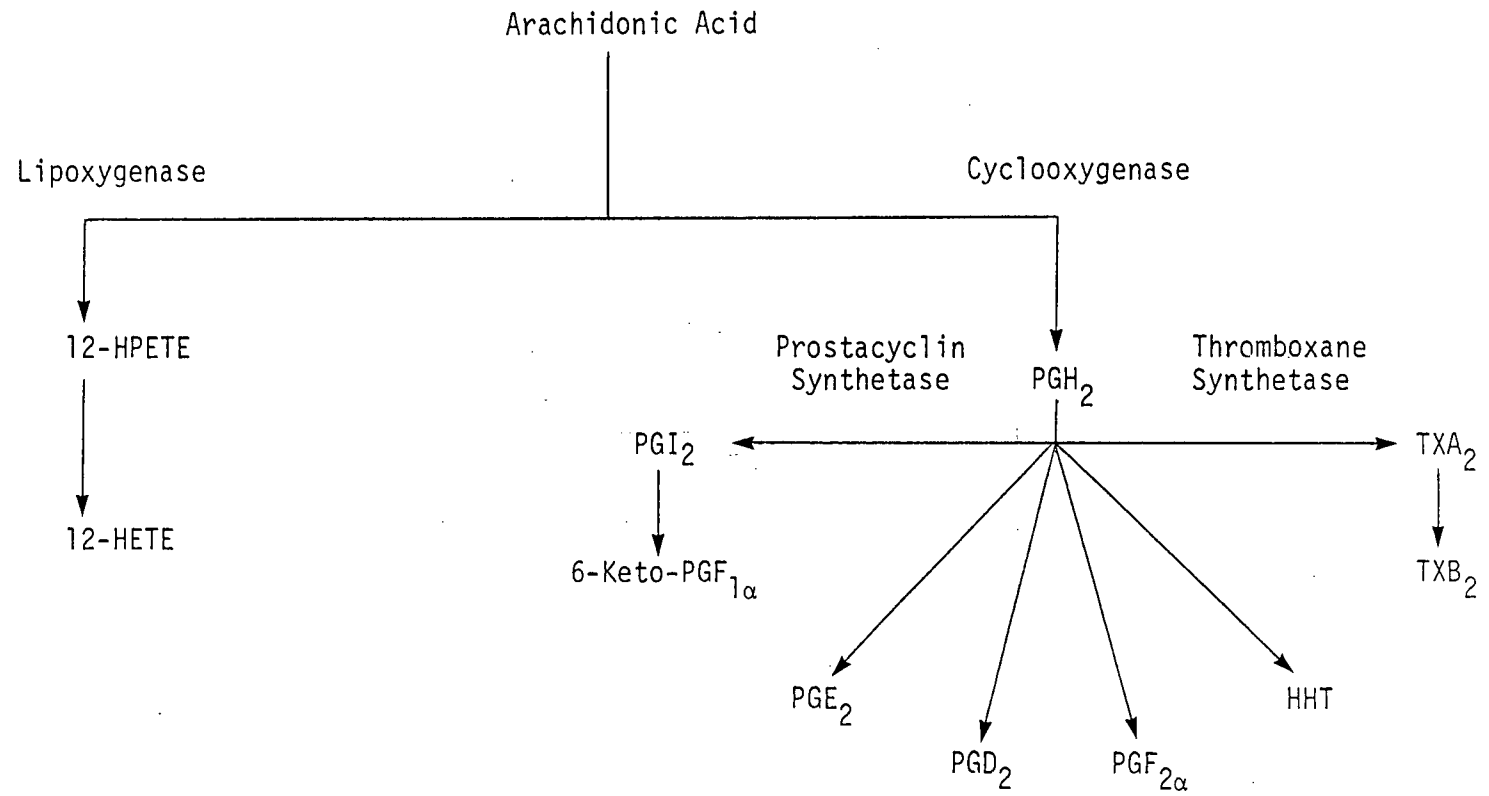
adrenoceptor-mediated effect, the $alpha_1$-adrenoceptor-mediated effect is not associated with shortening of the time-to-peak tension and relaxation time, and the duration of contraction may be prolonged, as seen in heart preparations of rats and guinea pigs (Bruckner et al., 1978; Osnes et al., 1978). In contrast to beta-adrenoceptor stimulation, the $alpha_1$-adrenoceptor-mediated effect occurs only when the rate of beating is relatively slow (Hamakawa et al., 1973). Besides, $alpha_1$-adrenoceptor stimulation does not alter adenylate cyclase activity or cyclic AMP concentration (Osnes and Oye, 1975; Osnes, 1978).

Thus, the $alpha_1$-adrenoceptor-mediated positive inotropic effect observed in isolated heart preparations differs greatly from the beta-adrenoceptor mediated effect. In patients an $alpha_1$-adrenoceptor-mediated inotropic effect has not been described, but may be obtainable particularly in the presence of beta-adrenoceptor blockade.

1.3.3. Myocardial Alpha$_1$-adrenoceptor And Phosphoinositide Hydrolysis.

The primary molecular mechanism involved in $alpha_1$-adrenoceptor-induced positive inotropic effect is largely unknown. It has, at least in part, been attributed to an increase in slow calcium inward current (Bruckner and Scholz, 1984), to an increased calcium sensitivity of the myofibrils (Endoh and Blinks, 1984; Blinks and Endoh, 1986) and to an increased PI turnover (Brown et al., 1985; Otani

et al., 1986; Poggioli et al., 1986; Schmitz et al., 1987a; Scholz et al., 1987; Scholz et al., 1988). Limited evidence further suggests that PIP_2 is a substrate, and that IP_3 is formed by activation of phospholipase C following $alpha_1$-adrenoceptor stimulation. Hydrolysis of PIP_2 produces two presumed intracellular second messengers, IP_3, which releases calcium from intracellular stores and DG, which activates protein kinase C. However, the role of IP_3 as an intracellular calcium mobilizing agent in skeletal muscle, as well as in cardiac muscle is a matter of controversy (Hirata et al., 1984; Movsesian et al., 1985; Scherer and Ferguson, 1985; Vergara et al., 1985; Volpe et al., 1985; Fabiato, 1986; Nosek et al., 1986). Protein kinase C is present in the heart (Kuo et al., 1980; Wise et al, 1982b), and a variety of myofibrillar, SR, and plasma membrane proteins are putative substrates for this enzyme (Wise et al., 1982a; Katoh et al., 1983; Movsesian, 1984; Lim et al., 1985; Lindemann, 1986). It is not yet known whether $alpha_1$-adrenergic stimulation in the heart activates protein kinase C to catalyze relevant protein phosphorylations. Also, there is no direct evidence showing that diacylglycerol levels are increased in cardiac slices or myocytes exposed to $alpha_1$-adrenergic agonists. An active metabolite of diacylglycerol is arachidonic acid, which is a substrate of the enzyme cyclooxygenase to form prostaglandins (Nishizuka, 1984b). The involvement of these metabolites in $alpha_1$-

adrenergic stimulation in the heart is also poorly understood.

1.3.4. Alpha-Adrenoceptor Agonists.

In studies concerning alpha-adrenergic inotropic effects, phenylephrine, methoxamine, epinephrine and norepinephrine have often been used as agonists. Among them, phenylephrine has been mostly used since it has a relatively weak beta-stimulating property compared to its alpha-adrenoceptor stimulating one (Furchgott, 1972; Osnes et al., 1973; Scholz, 1980). The small beta effect of phenylephrine is easily overcome by a beta-receptor blocker like propranolol without interfering either with mechanical or electrophysiological effects of the alpha-stimulation (Osnes et al., 1973; Bruckner et al., 1978). Methoxamine, which is devoid of detectable beta-receptor agonist activity, also evokes alpha-adrenoceptor-mediated inotropic effects (Endoh, 1982). But the alpha-effect of methoxamine seems to be somewhat atypical because it sometimes develops very slowly compared to the alpha-effect of phenylephrine (Skomedal et al., 1982; Siegl and McNeill, 1980). Besides, methoxamine has a marked negative inotropic action at large concentrations (Siegl and McNeill, 1980). Both phenylephrine and methoxamine preferentially stimulate $alpha_1$-adrenoceptors compared to $alpha_2$-adrenoceptors (Starke, 1981).

A prerequisite for implicating an alpha-adrenergic mechanism in heart is that it can be mimicked by the endogenous agonists epinephrine and norepinephrine. Both agonists elicit alpha as well as beta inotropic effects in rat papillary muscles. For epinephrine, the maximal increase in contractility after alpha-stimulation (epinephrine in the presence of propranolol) was about 60% of the corresponding increase after beta-stimulation (epinephrine in the presence of prazosin), while for norepinephrine, they are of the same magnitude (Skomedal and Osnes, 1983). Epinephrine was slightly less potent at the alpha-receptors compared to the beta-receptors while norepinephrine was about 10 times less potent at the alpha-receptors compared to the beta-receptors. At $alpha_1$ receptors, epinephrine is equal to or more potent than norepinephrine (Skomedal et al., 1984). Moreover, norepinephrine is the chemical mediator liberated by mammalian postganglionic adrenergic nerves and therefore mediates physiological and metabolic responses that follow stimulation of sympathetic nerves. For the reasons listed above, norepinephrine was employed in the present study.

1.3.5. Prazosin as a Selective $Alpha_1$-Adrenoceptor Blocker

$Alpha_1$-adrenoceptor blockers are crucial agents in demonstrating an alpha-adrenoceptor-mediated effect. However, obvious difficulties exist when alpha-receptor blockers like phentolamine and phenoxybenzamine are used

since they possess properties in addition to alpha-adrenoceptor blockade (Iversen, 1973; Starke et al., 1971). Both substances were shown to inhibit neuronal and extraneuronal uptake mechanisms, and will probably interfere with the presynaptic alpha$_2$-adrenoceptors regulating neurotransmitter release (Langer, 1974; Starke and Endoh, 1976; Starke, 1981). These effects are most apparent for agonists that are most influenced by these mechanisms (eg. the endogenous catecholamines). As the endogenous catecholamines are very potent stimulators of the beta-adrenoceptors, a blockade of postsynaptic alpha-effects may be masked or even reversed by a 'potentiation' of the beta effects by these alpha-blockers.

Compared with the alpha-blockers mentioned above, prazosin has been shown to have very high affinity and selectivity for the alpha$_1$-subtype of alpha-adrenoceptors (Davey and Massingham, 1976). By using prazosin, it is therefore possible to avoid interference by mechanisms other than the alpha$_1$-receptors. Thus, prazosin was employed in our study.

1.4. CARDIAC ALPHA$_1$-ADRENOCEPTORS AND PHOSPHOINOSITIDE METABOLISM IN DIABETES.

1.4.1. Diabetes and Cardiac Alpha-adrenoceptor Functions.

Cardiac adrenoceptors are affected in both number and function by diabetes. In acute diabetes, an enhanced inotropic response of diabetic atria (Canga and Sterin-

Borda, 1986), ventricle (Wald et al., 1988) and isolated heart (Downing et al., 1983) to methoxamine has been reported accompanied by a reduced number of alpha-adrenoceptor binding sites and an increase in their affinity constants (Wald et al, 1988). Similarly in chronic diabetes, it has been demonstrated that both inotropic and chronotropic responses to phenylephrine (Jackson et al., 1986) in the presence of propranolol, and methoxamine (Goyal et al., 1987) are enhanced in atria. Recently, supersensitivity to phenylephrine in the ventricles of chronic diabetic animals was also shown in our lab (unpublished results). On the contrary, a decrease in the contractile force generation by methoxamine (Heyliger et al., 1982) and norepinephrine (Sunagawa et al., 1987) was observed in isolated papillary muscles and isolated hearts of chronic diabetic animals. The number of alpha-adrenoceptors was shown to be decreased in the ventricles of chronic diabetic rats (Heyliger et al., 1982; Latifpour and McNeill, 1984) without changes in the affinity constants.

1.4.2. Diabetes and Cardiac Phosphoinositide Metabolism

There are very limited data demonstrating that changes in alpha-adrenoceptor-stimulated phospholipid metabolism occur in diabetic heart. Wald et al. (1988) have made some very interesting observations with regard to alpha-adrenoceptor-stimulated phosphoinositide hydrolysis in diabetic ventricles. Alpha-adrenoceptor supersensitivity

was shown in hearts from acute diabetic rats accompanied by an increase in receptor affinity and a decrease in receptor number. Inhibition of phospholipase C by 2-nitro-4-carboxyphenyl-N,N-diphenylcarbamate (NCDC) blocked the ventricular response to methoxamine in non-diabetic as well as in diabetic hearts. Synthetic DG potentiated the inotropic action of the alpha-agonist in normal ventricles and increased the affinity with a decreased number of alpha-adrenoceptor sites in normal ventricles, producing values of K_d and B_{max} similar to those of the acute diabetic heart. Inhibitors of protein kinase C partially reduced the supersensitivity of alpha-agonists in diabetic ventricles and prevented the stimulatory action of DG upon the positive inotropic effect of methoxamine in normal ventricles. These results provide the first evidence that alpha-adrenoceptor-mediated inotropic stimulation is secondary to receptor-mediated hydrolysis of phosphoinositides in diabetic heart as well as in normal heart. In the acute diabetic state, the supersensitivity to alpha-agonists could be due to high activity of phospholipase C (with an increase in DAG production and protein kinase C activation) which induces alterations in the membrane alpha-adrenergic receptors. This is supported by data from Okumura et al. (1988) who showed that 1,2-diacylglycerol content was significantly elevated in diabetic ventricles.

Diacylglycerol is metabolized to arachidonic acid which is the substrate required for cyclooxygenase to produce

prostacyclin. It is therefore important to note that an increased prostacyclin release from perfused hearts of acutely diabetic rats has been reported (Rosen and Schror, 1980). In addition, an increased PI response coupled with $alpha_1$-adrenoceptors has been shown in cardiomyopathic hamsters (Iwakura et al., 1988).

On the contrary, a decrease in the metabolites of the PI pathway has also been demonstrated. Rosen et al. (1983) reported reduced prostacyclin synthesis by the diabetic rat heart. Bydlowski et al. (1985) showed a decrease in the myocardial synthesis of prostacyclin in diabetic offspring. Also, reduced arachidonic acid levels have been observed in heart muscle of the diabetic rat (Holman et al., 1983; Gudbjarnason et al., 1987).

Besides $alpha_1$-adrenoceptor, stimulation of muscarinic receptor in the heart is also associated with an increase in phosphoinositide turnover (Brown and Brown, 1983; Brown et al., 1985). Recently, a few studies have reported the changes in muscarinic receptor-mediated phosphatidylinositol hydrolysis in diabetic animals. It was shown by Bergh et al. (1988) that the incorporation of myo-inositol amounts to about 65% in acute diabetes and 80% in chronic diabetes compared to age-matched controls. They further studied muscarinic receptor-stimulated phosphatidylinositol breakdown in diabetes. It was observed that in the acute form of diabetes with ketosis, the release of IP_1 was significantly reduced whereas in chronic diabetes no defects

in receptor-mediated coupling to the phosphoinositides were apparent.

An important finding by Canga et al (1985) indicated that during diabetes arachidonic acid metabolism in the heart is shifted towards thromboxanes and lipoxygenase metabolites, while in normal atria it is directed towards prostaglandin production. Since these substances affect cardiac function the authors examined the response to methoxamine in the presence and absence of inhibitors of thromboxane and prostacyclin synthesis. First, Canga and Sterin-Borda (1986) showed that inhibitors of the cyclo-oxygenase pathway for arachidonic acid metabolism blocked the atrial response to methoxamine in non-diabetic as well as acute diabetic atria, indicating the involvement of cyclo-oxygenase metabolites in the alpha-adrenoceptor-mediated positive inotropic response. Results further suggest that in acute diabetes, the response to alpha-adrenoceptor stimulation may be mediated by oxidative products generated via thromboxane synthetase and lipoxygenase activities, whereas in normal atria, the action of methoxamine may involve the release of prostacyclin. We therefore conducted a series of experiments to study the effects of inhibitors of cyclooxygenase, thromboxane synthetase and prostacyclin synthetase (indomethacin, imidazole and tranylcypromine) on alpha$_1$-adrenoceptor-mediated inotropic response in chronic diabetic hearts. As well, Ins(1,4,5)P$_3$ formation in these hearts was studied.

1.5. OMEGA-3 FATTY ACID.

Epidemiologic studies suggest that the consumption of fish by humans may offer protection against coronary heart disease (Bang and Dyerberg, 1972; Dyerberg and Bang, 1978; Kromann and Green, 1980; Kagawa et al., 1982; Kromhout et al., 1985). The mechanisms of this protection have not been fully elucidated; however, the omega-3 fatty acid content of the fish may be at least partly responsible. Dietary supplementation with polyunsaturated omega-3 fatty acids has been shown to have potential beneficial effects on platelet function, plasma lipid levels, blood pressure and glycemic control.

It has been demonstrated that dietary fish oil contributes to changes in eicosanoid metabolism leading to decreased platelet aggregation (Croft et al., 1987). Also, omega-3 fatty acid supplementation has been associated with lowering of plasma lipid and blood pressure in normal subjects (Lorenz et al., 1983). In patients with hypertriglyceridemia, omega-3 fatty acid supplementation was associated with decreased serum triglyceride and cholesterol levels (Phillipson et al., 1985), lowering of blood pressure (Singer et al., 1985), and improvement of insulin sensitivity during glucose tolerance testing (Singer et al., 1985).

Besides the above beneficial effects of fish oil, it has been reported that fish oil supplementation may also

have some effects on lipid composition of myocardial membrane, as well as cardiac function and response to adrenoceptor stimulation. It was shown that dietary fats can greatly influence the lipid composition of cellular membranes in the myocardium (Charnock et al., 1984). For example, omega-3 fatty acid enriched diets have been shown to change the acyl composition of myocardial membrane phospholipids, such that there is a replacement of omega-6 by omega-3 fatty acids (Hock et al., 1987). Additionally, dietary supplementation with an omega-3 fatty acid enriched diet may have a protective effect on the heart during an ischemic episode, as myocardial creatine kinase loss following coronary artery ligation is reduced following omega-3 fatty acid supplementation (Hock et al., 1987). DeDeckere and Ten Hoor (1980) reported increased coronary flow and left ventricular work after 1 and 4 weeks, respectively, in isolated hearts of rats fed sunflower seed oil vs. lard (saturated). Further, it was shown that omega-3 fatty acid feeding results in reduced cardiac responsiveness to alpha-adrenoceptor stimulation (Reibel et al., 1988). In other studies the contractile response to isoprenaline was found to be diminished in isolated hearts of rats fed sunflower seed oil vs. saturated diets (Dryden et al., 1982; Hoffman et al., 1982).

Diabetes mellitus is associated with a high incidence of coronary artery disease and heart failure. Diabetic cardiomyopathy frequently coexists with other known risk

factors, such as abnormalities of lipoprotein metabolism and hypertension. Experimentally, the cardiomyopathy is present after six weeks of streptozotocin-induced diabetes in the rat and is manifest as impaired left ventricular function. In type I diabetes, omega-3 fatty acid consumption has been associated with an improvement of insulin sensitivity (Popp-Snijders et al., 1987). Omega-3 fatty acid treatment in type I diabetes also has variable effects on plasma triglyceride concentrations as these lipids have been reported to increase (Illman et al., 1986), decrease (Miller et al., 1987; Mori et al., 1988) or remained unchanged (Haines et al., 1986; Black et al., 1989) after omega-3 fatty acid treatment/supplementation, and to have similarly variable effects on plasma cholesterol. Recently, Black et al (1989) have shown omega-3 fatty acid treatment attenuates the development of diabetes-induced cardiomyopathy in streptozotocin-induced model of type I diabetes in the rat. The improved isolated working heart function (systolic and diastolic function) of the omega-3 fatty acid-treated diabetic rats was associated with improved sarcoplasmic reticulum transport activity. Cardiovascular effects of omega-3 fatty acids in human type II diabetes include reductions in both systolic and diastolic blood pressures (Kasim et al., 1988).

Experimental diabetes mellitus is also associated with altered responsiveness to alpha$_1$-adrenergic receptor stimulation in the heart (Heyliger et al., 1982; Jackson et

al., 1986). Alpha$_1$-adrenergic receptor stimulation in the heart has been shown to increase phosphoinositide metabolism (Brown et al., 1985; Scholz et al., 1988). Further, we have recently shown that alpha$_1$-adrenergic receptor-mediated phosphoinositide metabolism is altered in the diabetic heart (unpublished results). However, it is not known whether omega-3 fatty acid treatment has any beneficial effect on the cardiac response to alpha$_1$-adrenoceptor stimulation with respect to phosphoinositide metabolism in diabetic rats. Therefore, to further define possible mechanisms for the improved cardiac performance of omega-3 fatty acid-treated STZ-induced diabetic rats, we investigated the effects of omega-3 fatty acid supplementation on alpha$_1$-adrenoceptor-stimulated inositol phosphates formation in STZ-diabetic rats.

1.6. CHEMICALLY-INDUCED ANIMAL MODELS OF DIABETES MELLITUS.

It has long been known that chemicals can produce diabetes in laboratory animals. Among these agents are alloxan, streptozotocin, cyproheptadine, hexamethylmelamine, etc. Cyproheptadine and hexamethylmelamine can produce diabetes only in rat and mouse (Fischer, 1985), which has limited their widespread use. Alloxan was first reported to have diabetogenic activity by Dunn and McLetchie in 1943. Alloxan, a cyclic urea analog, is taken up by pancreatic cells and rapidly reduced to form dialuric acid. This product undergoes auto-oxidation to yield detectable amounts

of H_2O_2, superoxide anion (O_2^-) and hydroxyl free-radicals (·OH). These reduced species of oxygen, particularly the extremely reactive ·OH radical, are believed to initiate the alloxan-based attack on beta cells (Malaisse, 1982). The basis for the specificity of the attack on beta cells by alloxan is not clear. At present, a speculative explanation includes relatively high concentrations of the agent with relatively low levels of the protective substances that deactivate reduced forms of oxygen (Fischer, 1985).

Streptozotocin has now, however, replaced alloxan as the primary compound used to produce experimental, insulin-dependent diabetes in laboratory animals. This is due to the lower mortality rate seen in streptozotocin-induced diabetic animals (Hoftiezer and Carpenter, 1973), the greater selectivity of streptozotocin for beta cells (Junod et al., 1969) and the longer half life of streptozotocin in the body (Agarwal, 1980). Streptozotocin contains a N-methylnitrosourea moiety and is an alkylating agent. This property is capable of initiating its cytotoxic action. The glucose moiety (2-deoxy D-glucose) present in the streptozotocin molecule may direct the alkylating agent to the pancreatic beta cell which may have a special affinity for glucose. Further, streptozotocin has been shown to concentrate in pancreatic islets (Johnson and Tjalve, 1978), which may also account for the cell-selective action of streptozotocin. Both alloxan and streptozotocin share the

ability to produce beta-cell necrosis after single doses to laboratory animals.

1.7. PURPOSE OF THE PRESENT INVESTIGATION.

The purpose of this thesis was to investigate the changes in $alpha_1$-adrenoceptor-stimulated phosphoinositide metabolism in the diabetic heart and its involvement in the diabetes-induced alterations in the $alpha_1$-adrenoceptor-mediated positive inotropic effect. The following are the major objectives of the investigation:

(1) To determine the time course and concentration-responsiveness of $alpha_1$-adrenoceptor-stimulated inositol monophosphate (IP_1) formation (using a radioisotope method) in right ventricles of control and diabetic rats.

(2) To determine the effect of omega-3 fatty acid supplementation on $alpha_1$-adrenoceptor-mediated inositol phosphates formation in right ventricles of control and diabetic rats.

(3) To study the time course and concentration responsiveness of $alpha_1$-adrenoceptor-mediated positive inotropic effect and $Ins(1,4,5)P_3$ formation [using an $Ins(1,4,5)P_3$ protein binding assay kit] in right ventricles of control and diabetic rats.

(4) To study the influence of indomethacin on $alpha_1$-adrenoceptor-mediated positive inotropic effect and $Ins(1,4,5)P_3$ formation in right ventricles of control and diabetic rats.

(5) To study the influence of imidazole on $alpha_1$-adrenoceptor-mediated positive inotropic effect and $Ins(1,4,5)P_3$ formation in right ventricles of control and diabetic rats.

(6) To study the influence of tranylcypromine on $alpha_1$-adrenoceptor-mediated positive inotropic effect and $Ins(1,4,5)P_3$ formation in right ventricles of control and diabetic rats.

(7) To study protein kinase C activity in right ventricles of control and diabetic rats.

MATERIALS AND METHODS

2.1. MATERIALS.

2.1.1. Animals.

Male Wistar rats weighing between 200-225 g were used throughout the study. The rats were obtained from Charles River Canada, Montreal, Canada.

2.1.2. Chemicals.

The following chemicals were purchased from Sigma Chemical Co.: adenosine 5'-triphosphate(disodium salt) (Na_2ATP), adenosine 5'-triphosphate(tris salt) (tris ATP), ammonium formate, ascorbic acid, DEAE (diethylamino ethyl) cellulose anion exchanger, dithiothreitol, DMSO (dimethyl sulfoxide), EDTA (ethylenediaminetetraacetic acid), EGTA [ethyleneglycol-bis-(ß-aminoethyl ether) N,N,N',N'-tetraacetic acid], HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), histone (type III-S, lysine-rich), imidazole, indomethacin, lithium chloride (LiCl), norepinephrine (arterenol), phenylmethylsulfonyl fluoride (PMSF), potassium phosphate (KH_2PO_4), prazosin, propranolol, sodium azide, sodium formate, sodium orthovanadate (Na_3VO_4), sodium tetraborate, streptozotocin, sucrose, tranylcypromine, Triton X-100 and Trizma base.

The following chemicals were purchased from BDH: calcium chloride ($CaCl_2$), chloroform ($CHCl_3$), diethyl ether, glucose, hydrochloric acid (HCl), magnesium chloride

(MgCl$_2$), methanol, potassium chloride (KCl), sodium bicarbonate (NaHCO$_3$), sodium chloride (NaCl), sodium hydroxide (NaOH) and trichloroacetic acid.

Leupeptin was obtained from Calbiochem Co. Phosphatidylserine (PS) (beef brain) and D-1-stearoyl-2-arachidonyl glycerol (DG) were obtained from Serdary Co.

Analytical grade anion exchange resin (AG 1-X8, 100-200 mesh, formate form) was purchased from Bio-Rad Laboratories. Aquasol and myo-[2-^{3}H]inositol (15.2 Ci/mmol) were purchased from New England Nuclear Research Products. [γ-^{32}P]ATP (30 Ci/mmol) was purchased from Amersham.

2.1.3. Assay Kits.

Insulin radioimmunoassay kits and Ins(1,4,5)P$_3$ [^{3}H] assay kits were purchased from Amersham.

Glucose assay kits, cholesterol assay kits and triglycerol assay kits were purchased from Boehringer-Mannheim.

2.2. INDUCTION OF STREPTOZOTOCIN DIABETES IN RATS.

Male Wistar rats (175-200 g) were used. All animals were anesthetized transiently with diethyl ether to allow injection of either STZ or its vehicle into the tail vein. Diabetes was induced by a single intravenous injection of STZ (55 mg/kg) dissolved in citrate buffer (pH 4.5). The citrate buffer was made by mixing 0.1 M citric acid and 0.1 M sodium citrate until a pH of 4.5 was obtained. Control

rats were injected with citrate buffer alone. All rats injected with STZ survived and were housed two to three per cage with Lobound (R) grade corncob bedding (Paxton Processing, Paxton, IL.). All rats had access to Purina rat chow and water *ad libitum*. The lights were left on from 6 a.m. to 6 p.m., keeping in mind that the rats are nocturnal animals. The room temperature was maintained at 25°C.

Diabetes was detected three days later by estimating the extent of glucosuria with the aid of enzymatic test strips (Tes-Tape, Eli Lilly, Toronto, Canada). Those rats displaying glucosuria greater than 2% were used as diabetic rats. The animals were sacrificed six weeks after diabetes induction. The duration of 6-week diabetes was chosen based on a report from our laboratory which showed that alterations in cardiac performance occur six weeks after the onset of diabetes (Tahiliani et al., 1983). At the time of death, whole blood (arterial and venous) samples were collected in heparinized tubes from non-fasting animals and centrifuged at 1300 x g for 20 min to separate cells from plasma. The plasma obtained was stored at -20°C until assayed for glucose, insulin, triglyceride and cholesterol. Hearts were excised and used for inositol phosphates radioisotope assay or for function studies in the isolated tissue bath.

2.3. MEASUREMENT OF INOSITOL PHOSPHATES WITH THE RADIOISOTOPE METHOD.

2.3.1. Right Ventricle Preparation and Phosphoinositide Labelling.

Inositol phosphates formation was measured using a modified method of Brown and Brown (1983) and Jones et al. (1988). Control and diabetic animals were sacrificed by decapitation. The hearts were excised quickly and rinsed in a beaker containing oxygenated Chenoweth-Koelle (CK) solution consisting of (mM): NaCl (120); KCl (5.6); $CaCl_2$ (2.18); $MgCl_2$ (2.1); $NaHCO_3$ (19), and glucose (10). After expressing the blood from the intact heart, the atria and connective tissues were removed and the right ventricle was obtained. The right ventricle was dissected into two pieces (approximately 0.1 g wet weight per piece) and transferred to continuously oxygenated tubes containing CK solution in a shaking waterbath (37°C). Following an equilibration period of 30 min, the solution was removed and replaced with fresh solution containing 4 μCi/mmol myo-[2-^{3}H]inositol in 4.7 ml CK buffer. After 60 min of incubation with myo-[2-^{3}H]inositol, LiCl (10 mM) (an inhibitor of inositol 1-phosphatase) and the beta-adrenoceptor antagonist propranolol (10 μM) were added and tissues were incubated for another 15 min. In the time course study, the $alpha_1$-adrenoceptor agonist norepinephrine (10 μM) was then added and tissues were incubated for time periods ranging from 5 to 30 min. In the concentration-response study, norepinephrine at concentrations ranging from 10^{-7} to 10^{-4} M was added and tissues were incubated for another 15

minutes. In the omega-3 fatty acid treatment study, norepinephrine (10 μM) was added and tissues were incubated for 15 min. In studies involving prazosin (10 μM) (a concentration determined in a preliminary experiment to not affect the basal tension) and DMSO, these were added at the same time as LiCl.

2.3.2. Determination of [^{3}H]inositol Monophosphate (IP$_1$).

At the end of the incubation period, tissues were rinsed in 3 ml of ice-cold 12% trichloroacetic acid (TCA), blotted, weighed, and then transferred to 1.3 ml of chloroform (CHCl$_3$): methanol (MeOH): water (H$_2$O) (5:10:4). Tissues were homogenized for 1 min in a glass homogenizer. The homogenate was transferred to a glass vial, and the homogenizer was rinsed with a CHCl$_3$ (0.342 ml)/water (0.069 ml) mixture. A two-phase system was obtained with the addition of the mixture to the glass vial, making a solution of CHCl$_3$: MeOH: H$_2$O (10:10:5). Samples were centrifuged at 1600 x g at 4°C for 20 min. A 0.65 ml aliquot of the aqueous upper phase was transferred to a column containing approximately 175 mg of anion exchange resin (AG 1-X8, 100-200 mesh, formate form). Columns were washed with a total of 9.6 ml water to remove myo-[2-^{3}H]inositol. Labelled myo-inositol monophosphate [^{3}H-IP$_1$] was eluted with 8 ml of 5 mM sodium tetraborate/150mM sodium formate.

2.3.3. Determination of [^{3}H]Inositol Bisphosphate (IP$_2$), [^{3}H]Inositol Trisphosphate (IP$_3$) and [^{3}H]Inositol Tetrakisphosphate (IP$_4$).

In experiments where inositol bisphosphate (IP$_2$) and inositol trisphosphate (IP$_3$) and inositol tetrakisphosphate (IP$_4$) were measured, the column was further washed with an additional 32 ml of the same sodium tetraborate/sodium formate solution. IP$_2$ was eluted with 8 ml of 300 mM ammonium formate (AF)/100 mM sodium formate (SF) followed by an 8 ml wash with this same buffer. IP$_3$ was eluted with 8 ml 750 mM AF/100 mM SF followed by an 8 ml wash with this same buffer. IP$_4$ was eluted with 8 ml of 1 M AF/100 mM SF. Radioactivity in all of the eluted fractions was quantified by liquid scintillation counting.

2.4. OMEGA-3 FATTY ACID TREATMENT.

The treatment protocol chosen to study effects of omega-3 fatty acid supplementation was conducted with Promega (Warner-Lambert, Morris Plains, NJ), a refined fish-oil product concentrated in long-chain polyunsaturated omega-3 fatty acids. The dose-response study of the effect of Promega on isolated heart function in control and diabetic rats demonstrated an ameliorative effect of Promega at 0.5 ml/kg/day and 1.0 ml/kg/day (Black et al., 1988). Therefore, Promega was administered to control and diabetic rats at a dose of 0.5 ml/kg/day. Promega was obtained in bulk form; therefore, to prevent oxidation of the highly

unsaturated lipids, aliquots of the stock was drawn and stored in glass vials under nitrogen at -80°C until they were used. Control and diabetic rats were given Promega between 0830 and 0930 daily for the duration of the treatment. Promega was administered to the rats via oral gavage with a 75-mm stainless-steel tube. Promega treatment was initiated 2 week after STZ or citrate buffer injection and was continued for 4 week, after which the animals were sacrificed. Hearts were removed and blood samples collected for further assays.

2.5. MEASUREMENT OF CONTRACTILE FORCE IN ISOLATED TISSUE BATH.

2.5.1. Time Course and Concentration-Response Curves of Inotropic Responses to Norepinephrine.

(1) Tissue Preparations

Isolation of cardiac tissues and measurement of the time course and concentration-response curves were done according to the method of Ramanadham and Tenner (1983). At the time of killing, the heart was excised and immediately placed in a beaker containing ice-cold oxygenated CK solution (pH 7.4). After expressing blood from the heart, atrial tissue, fat and connective tissue were trimmed off. The right ventricle was then carefully dissected and transferred to another beaker containing cold fresh CK solution. Each right ventricle tissue was further dissected into three to four triangular shaped pieces. One end of

each right ventricular piece was attached to a tissue-electrode assembly with two platinum electrodes. The other end was mounted to a Grass FT.03 force-displacement transducer that was connected to a Grass Model 79D polygraph four-channel recorder (Quincy, Mass., USA). The tissue preparations were equilibrated at 37°C for 60 min in 20-ml tissue baths of CK solution under constant oxygenation (95% O_2 and 5% CO_2). During this period, CK solution in the tissue bath was replaced every 15 min. Resting tensions in right ventricular pieces of 1 g were maintained throughout the experiment. After 30 min of equilibration, the right ventricular pieces were stimulated electrically via the punctate platinum electrodes (at a frequency of 1.0 Hz, 2 to 8 V, and a duration of 5 ms). At the end of the equilibration period, basal developed force in right ventricular tissues was recorded.

(2) Time Course and Concentration-Response Curves of Inotropic Responses to Norepinephrine

After the recording of basal developed force in right ventricular tissues, propranolol (10 μM) was added to the solution and the tissues were allowed to equilibrate an additional 15 min. Propranolol was added to block the beta inotropic effect of norepinephrine.

Norepinephrine was prepared as a stock solution in ascorbic acid (0.1 g/dl). The appropriate dilutions (10^{-6} to 10^{-2} mol/l) were made in CK solution. Positive inotropic responses to increasing concentrations of norepinephrine

were obtained by a cumulative increase in the total concentration in the tissue bath. The recordings were made when the responses had reached a steady-state level at each concentration. In general, stable responses were achieved within 3 min after addition of the drug. Tissues were immediately freeze-clamped when the whole concentration-response curves were finished. The tissues were stored at -80°C until assayed for $Ins(1,4,5)P_3$ levels.

In the study where the time course of $Ins(1,4,5)P_3$ formation was measured, inotropic responses to 10^{-5} mol/l of norepinephrine on isolated right ventricles were recorded at each chosen time point (0, 15, 30, 60 and 180 sec) and tissues were freeze-clamped at these time points for the measurement of $Ins(1,4,5)P_3$.

In the study where the $Ins(1,4,5)P_3$ level at each concentration of norepinephrine was measured, inotropic responses to cumulative additions of norepinephrine in isolated right ventricular tissues were recorded when the responses had reached a steady-state level. Tissues were freeze-clamped when the steady-state level was obtained at each concentration of norepinephrine.

Sensitivity of the right ventricular tissues towards the positive inotropic effect of norepinephrine was determined by estimated PD_2 values. The PD_2 value is defined as the negative log concentration of the drug required to produce 50% of the maximum change in tension.

2.5.2. Concentration-Response Curves of Inotropic Responses to Norepinephrine in the Presence of Indomethacin.

Indomethacin was dissolved in 70% ethanol. The drug was diluted in the tissue bath to achieve the final concentration of 10 μM, a concentration determined in a preliminary experiment to not affect the basal tension. After a 60 min equilibration period, isolated right ventricular pieces were incubated for 15 min with propranolol and the cyclooxygenase inhibitor indomethacin before increasing concentrations of norepinephrine were added. Following the determination of the concentration-responses curves, tissues were freeze-clamped in liquid nitrogen and stored at -80°C prior to the measurement of Ins(1,4,5)P$_3$.

2.5.3. Concentration-Response Curves of Inotropic Responses to Norepinephrine in the Presence of Imidazole.

Imidazole was dissolved in distilled water. The drug was diluted in the tissue bath to achieve the final concentration of 1 mM (Canga and Sterin-Borda, 1986). At this dilution, the drug itself had no effect on inotropic responses. After a 60 min equilibration period, isolated right ventricular pieces were incubated for 15 min with propranolol and the thromboxane synthetase inhibitor imidazole before increasing concentrations of norepinephrine were added. Following the determination of the concentration-responses curves, tissues were freeze-clamped

in liquid nitrogen and stored at -80°C prior to the measurement of Ins(1,4,5)P$_3$.

2.5.4. Concentration-Response Curves of Inotropic Responses to Norepinephrine in the Presence of Tranylcypromine.

Tranylcypromine was dissolved in distilled water. The drug was diluted in the tissue bath to achieve the final concentration of 1.25×10^{-4} M, a concentration determined in a preliminary experiment to not affect the basal tension. After a 60 min equilibration period, isolated right ventricular pieces were incubated for 15 min with propranolol and the prostacyclin synthetase inhibitor tranylcypromine before increasing concentrations of norepinephrine were added. Following the determination of the concentration-responses curves, tissues were freeze-clamped in liquid nitrogen and stored at -80°C prior to the measurement of Ins(1,4,5)P$_3$.

2.6. DETERMINATION OF INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P$_3$] LEVELS WITH THE INS(1,4,5)P$_3$ PROTEIN BINDING ASSAY KIT.

2.6.1. Extraction of Ins(1,4,5)P$_3$.

The frozen right ventricular strips were weighed and put in 0.8 ml 6% of trichloroacetic acid (TCA) on ice immediately. The tissues were then homogenized with a polytron PT-10 homogenizer for 10 sec at setting 5. The homogenates were centrifuged at 6000 x g for 15 min at 4°C.

The supernatants were transferred into glass tubes, to which 2 ml of H_2O-saturated diethyl ether was added. The tubes were shaken well and the top layers were removed. The diethyl ether extraction procedure was repeated 5 times. The tubes were then put in a rack which was placed in warm tap water for a few minutes to evaporate diethyl ether. A 100 μl portion of the ether-extracted supernatant was used for subsequent analysis.

2.6.2. Measurement of Ins(1,4,5)P$_3$.

Ins(1,4,5)P$_3$ levels were measured using a protein binding assay kit (Amersham). The assay is based on the competition between unlabelled Ins(1,4,5)P$_3$ and a fixed quantity of tritium labelled Ins(1,4,5)P$_3$ for a limited number of binding sites on a bovine adrenal binding protein preparation. With fixed amounts of binding protein and radioactive ligand, the amount of radioactive ligand bound to the binding protein will be inversely proportional to the concentration of added non-radioactive ligand. The bound Ins(1,4,5)P$_3$ is then separated from free ligand by centrifugation, which brings the binding protein preparation to the bottom of the tube. The free fraction in the supernatant can then be discarded by decantation leaving the bound fraction adhering to the tube. Measurement of the radioactivity in the tube enables the amount of unlabelled Ins(1,4,5)P$_3$ in the sample to be determined by interpolation from the standard curve.

All reagents were allowed to equilibrate to 4°C. Once prepared, all reagents were kept on ice prior to performing the assay.

Reagent preparation: Assay buffer was allowed to thaw and was mixed thoroughly. Standard solution was also allowed to thaw and was mixed thoroughly. A 100 μl aliquot of tracer was removed from the vial and added to 11 ml of distilled water in a glass vessel. The contents were thoroughly mixed. The binding protein solution was allowed to thaw and was mixed well by inversion and swirling.

Preparation of working standards: Eight glass tubes (containing 0.19, 0.38, 0.76, 1.5, 3.1, 6.2, 12.5, 25 pmol) were labelled. Distilled H_2O, 1.5 ml, was added into the tube marked 25 pmol. Then 500 μl of distilled H_2O was added into the remaining marked standard tubes. Exactly 100 μl of standard solution was transferred into the tube marked 25 pmol and vortexed thoroughly. A 500 μl aliquot from the 25 pmol tube was transferred to the 12.5 pmol tube and vortexed thoroughly. This dilution was repeated successively with the remaining tubes. One hundred aliquots from each serial dilution gave 8 standard levels of Ins(1,4,5)P_3 ranging from 0.19-25 pmol per tube.

Assay procedure: Polypropylene tubes in duplicate for total counts (TC), non-specific binding tubes (NSB), zero standard tubes (Bo), standards and samples were labelled and placed in an ice-bath. A 100 μl aliquot of assay buffer was pipetted into all tubes. Distilled H_2O, 100 μl, was put

into the Bo tubes and 200 μl of H_2O into TC tubes. A 100 μl aliquot of each standard solution was put into the appropriately labelled tubes and 100 μl of the stock standard was put into the NSB tubes. A 100 μl aliquot of sample was put into the appropriate tubes. A 100 μl aliquot of tracer was put into all tubes and 100 μl of binding protein was put into all tubes except TC. All tubes were vortexed thoroughly and incubated for 15 min on ice. All tubes except TC were centrifuged at 1000 x g for 1.5 min at 4°C. After centrifugation, the tubes were placed carefully into racks and the supernatants were discarded. A 200 μl aliquot of H_2O was added to each tube except for TC tubes. The tubes were vortex mixed thoroughly to resuspend the pellet. A 180 μl aliquot of the mixture was transferred into the scintillation tubes and 2 ml of scintillant was added to each tube. Radioactivity was measured in a liquid scintillation counter.

2.7. PROTEIN KINASE C ANALYSIS

The preparation of membrane and cytosolic fractions, preparation of DEAE cellulose column and assay of protein kinase C activity were performed by a modification of the method of Kikkawa et al. (1982).

2.7.1. Preparation of Membrane and Cytosolic Fractions

Experiments were carried out on male Wistar rats (200-225 g). Diabetes was induced as described in section 2.2. The animals were sacrificed by decapitation six weeks after

induction of diabetes. The hearts were excised immediately and rinsed in a beaker containing oxygenated CK solution. After expressing the blood from the intact heart, the atria and connective tissues were removed and the right ventricle was obtained. The right ventricle was dissected into four pieces (approximately 0.05-0.08 g wet weight per piece). Where norepinephrine was used, each right ventricular piece was suspended in a tissue bath and stimulated with 10 mM norepinephrine (in the presence of 10 μM propranolol). Tissues were frozen in liquid nitrogen when the inotropic responses reached a steady level. Otherwise, each right ventricular piece was quickly frozen right after dissecting with Wollenberger clamps precooled in liquid nitrogen, weighed, and pulverized to a fine powder with a precooled mortar and pestle. The procedures for the isolation of membrane and cytosolic fractions were done at 4°C. The powdered tissue from each right ventricular piece was thawed and homogenized in 5 ml of buffer A [20 mM HEPES, 0.25 M sucrose, 2 mM EGTA, 0.005% leupeptin, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.4] with a hand homogenizer. Homogenates were centrifuged at 100,000g for 60 min. The resulting supernatants were saved as cytosolic fraction and put on ice until use. The pellets were rehomogenized with a hand homogenizer in 3 ml of 0.075% (v/v) Triton X-100 in buffer A. Homogenates were centrifuged at 100,000g for 60 min. The resulting

supernatants were saved as membrane fraction and put on ice until use.

2.7.2. DEAE Cellulose Column

Two grams of DEAE cellulose anion exchange resin was added to 100 ml of 0.1 N HCl and the mixture swirled. The slurry was allowed to settle for 10 min and the top layer was decanted. The procedure was repeated another four times until the top layer was clear. The mixture was then applied to Whatman No. 1 filter paper (Whatman International Ltd., England). The filter paper was then washed in order with 140 ml each of H_2O, 0.1 N NaOH, H_2O and 0.1 N HCl. The powder was scratched into a beaker containing 100 ml of 0.1 N HCl, which was neutralized with Trizma base to pH 7.4. The mixture was allowed to settle and 50 ml of the top layer was decanted. The rest was swirled and 4.5 ml of the slurry was applied to the column preequilibrated with 0.6 ml buffer B (20 mM Trizma base, 0.5 mM EGTA and 0.5 mM EDTA). A total of 20 ml of buffer B was further applied to the column. The column was stored at 4°C with 3 ml of buffer B in it.

On the day of use, another 6 ml of buffer B containing 2 mM dithiothreitol was run through the column. Cytosolic and membrane preparations were then applied to the columns. The column was washed with another 4 ml of buffer B containing 2 mM dithiothreitol, and the enzyme was eluted by 2 ml buffer B containing 2 mM dithiothreitol and 0.15 M

NaCl. A 25-μl aliquot was analyzed for protein kinase activity.

The column was regenerated by running through the column 20 ml of buffer B containing 1 M NaCl, and stored in 1 ml of buffer B containing 0.02% sodium azide.

2.7.3. Assay of Protein Kinase C Activity

Protein kinase C activity was assayed by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into lysine-rich histone. A 25 μl of the sample was preincubated with buffer C (20 mM HEPES, 2.4 mg histone, 10 mM dithiothreitol, 20 mM MgCl$_2$, 2 mM EGTA, 1 mM Na$_3$VO$_4$, and 0.004% leupeptin) with or without 1.75 mM CaCl$_2$, 0.14 mg phosphatidyl serine (PS) or 14.4 μg D-1-stearoyl-2-arachidonyl glycerol (DG) for 2.5 min at 30°C. PS and DG were first dried under nitrogen and resuspended in 20 mM HEPES by sonication for 20 min at room temperature. The reaction was initiated by the addition of [γ-^{32}P]ATP (5 x 10^5 dpm) and allowed to proceed at 30°C for 1 min. The reaction was terminated by the addition of 1 ml of 5% trichloroacetic acid containing 2.5 mM Na$_2$ATP and 5 mM KH$_2$PO$_4$. The incubation solution was filtered through Whatman GF/C filter paper (Whatman International Ltd., England). The test tubes were washed twice each with 1 ml of 5% trichloroacetic acid solution. The filter papers were then washed three times each with 1 ml of 5% trichloroacetic acid. The filter papers were put into scintillation vials containing 5 ml aquasol, and were counted in a liquid

scintillation counter. Protein kinase activity was expressed as pmol Pi/mg tissue/min. Specific protein kinase C activity was calculated by subtracting the protein kinase activity in the presence of 1.75 mM $CaCl_2$ alone from the protein kinase activity in the presence of 1.75 mM $CaCl_2$, 0.14 mg phosphatidylserine (PS) and 14.4 μg D-1-stearoyl-2-arachidonyl glycerol (DG).

2.8. BLOOD ANALYSIS.

2.8.1. Plasma Glucose Determination.

Plasma glucose levels were determined by the glucose oxidase method using a Sigma glucose kit.

2.8.2. Plasma Insulin Determination.

Plasma immunoreactive insulin was assayed by the radioimmunoassay method using the Amersham insulin radioimmunoassay kit.

2.8.3. Plasma Triglyceride Determination.

Plasma triglyceride was determined using a Boehringer-Mannheim diagnostic reagent kit.

2.8.4. Plasma Cholesterol Determination.

Plasma cholesterol was determined using a Boehringer-Mannheim diagnostic reagent kit.

2.9. STATISTICAL ANALYSIS.

Results are expressed as mean ± S.E.M. (standard error of the mean). The statistical differences between mean values of the various groups were evaluated by two way analysis of variance followed by Newman-Keul's test. For isolated tissue function curves, repeated measures of analysis of variance was used. All other analyses were carried out using one way analysis of variance where appropriate. The level of statistical significance was set at a probability of less than 0.05 (p<0.05).

RESULTS

3.1. THE BASAL LEVEL OF INOSITOL MONOPHOSPHATE (IP$_1$) FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

Rat right ventricular muscles were labelled with [^{3}H] inositol and incubated in CK solution containing 10 μM LiCl and 10 μM propranolol in the absence of norepinephrine for 30 minutes. The basal levels of IP$_1$ formation in the right ventricles of control and diabetic animals were not significantly different from each other (Figure 4).

3.2. TIME COURSE OF ALPHA$_1$-ADRENOCEPTOR-MEDIATED INOSITOL MONOPHOSPHATE (IP$_1$) FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

(1) General features of the experimental rats.

The general features of the experimental rats are presented in Table 2. As shown, the gain in body weight by diabetic rats was significantly less than that of controls. The time course of weight changes over the 6-week period shows that the significant difference in body weight gain started as early as 3 days after the STZ injection and became greater with the increased duration of diabetes (Figure 5). An elevated urinary glucose (>2%) was found in diabetic rats throughout the study period. No detectable glucose was present in the urine of control animals. Other symptoms usually associated with the diabetic state such as polyuria and polyphagia were seen in the diabetic rats.

Plasma glucose levels were elevated markedly in the diabetic rats accompanied by a depression in plasma insulin levels (Table 2).

A plasma lipid profile of the rats used in this study is shown in Table 3. Plasma triglyceride and cholesterol were markedly elevated in the STZ-diabetic rats compared with controls.

In addition, right ventricular weights were reduced in diabetic animals compared with controls (Figure 6).

(2) Time course of $alpha_1$-adrenoceptor-mediated IP_1 formation.

The time course for production of IP_1 in response to norepinephrine stimulation in the presence of LiCl and 10 uM propranolol is shown in Figure 7. In both control and diabetic rats, stimulation of $alpha_1$-adrenoceptors by norepinephrine in right ventricles resulted in the production of IP_1 in a time dependent manner. Right ventricles from diabetic rats, however, showed decreased IP_1 production in response to norepinephrine compared to controls at time periods longer than 10 minutes. At the 5 minute period, there was no significant difference in norepinephrine stimulated IP_1 production between the hearts of control and diabetic animals.

3.3. CONCENTRATION-RESPONSE CURVES FOR ALPHA$_1$-ADRENOCEPTOR-MEDIATED INOSITOL MONOPHOSPHATE (IP$_1$) FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

(1) General features of the experimental rats

The general features of the diabetic rats were quite characteristic of the disease and are summarized in Table 4. Diabetic rats exhibited severe hyperglycemia and hypoinsulinemia. Body weight gain was significantly less in the diabetic group compared with control rats. The body weight difference progressed with the duration of the disease (Figure 8). Plasma glucose levels were elevated significantly in the diabetic rats accompanied by a depression in plasma insulin levels compared with controls (Table 4).

Plasma triglyceride and cholesterol were significantly increased in the diabetic group compared with controls (Table 5).

Right ventricular weights were significantly reduced in the diabetic rats compared with controls (Figure 9).

(2) Concentration-response curves for alpha$_1$-adrenoceptor-mediated IP$_1$ formation.

The concentration-response curves for the effect of alpha$_1$-adrenoceptor stimulation on the formation of IP$_1$ in control and diabetic animals are shown in Figure 10. Addition of norepinephrine (in the presence of propranolol) to right ventricles of the control rats markedly increased

the accumulation of $[^3H]IP_1$ in a concentration-dependent manner.

In contrast, hearts from diabetic rats showed decreased IP_1 formation compared with controls at norepinephrine concentrations higher than 10^{-5} M. Therefore, the maximum IP_1 formation in response to norepinephrine stimulation in the diabetic heart was reduced.

3.4. EFFECT OF PRAZOSIN AND PROPRANOLOL ON NOREPINEPHRINE-STIMULATED INOSITOL MONOPHPOSPHATE (IP_1) FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

Norepinephrine stimulates beta- as well as alpha-adrenoceptors on the heart. Therefore additional pharmacological studies were performed to define the nature of the receptor through which catecholamines stimulate phosphoinositide hydrolysis. Propranolol did not antagonize the stimulatory effect of norepinephrine on IP_1 formation in either control or diabetic animals (Figure 11), indicating that the adrenoceptor coupled to phosphoinositide metabolism is not the beta-adrenoceptor.

If phosphinositide hydrolysis is mediated by the cardiac $alpha_1$-adrenoceptor, the hydrolysis should be blocked by antagonizing the receptors. To test this hypothesis, we used prazosin as an $alpha_1$-adrenoceptor antagonist. When the right ventricles labelled with $[^3H]$inositol were exposed to 10 μM prazosin, the formation

of IP_1 induced by 10 μM norepinephrine was significantly inhibited (Figure 11).

3.5. EFFECT OF PRAZOSIN, PROPRANOLOL AND DMSO ALONE ON INOSITOL MONOPHOSPHATE (IP_1) FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

As mentioned above, propranolol and prazosin were used in the experiments to explore the nature of the receptor through which norepinephrine stimulates phosphinositide hydrolysis. DMSO was used to dissolve prazosin. In order to examine the effect of these agents alone on basal IP_1 formation (unstimulated IP_1 formation), prazosin, propranolol and DMSO were employed separately. Results show that these agents did not affect basal IP_1 production in either control and diabetic hearts (Figure 12).

3.6. EFFECT OF OMEGA-3 FATTY ACID ON CARDIAC $ALPHA_1$-ADRENOCEPTOR-MEDIATED INOSITOL PHOSPHATES FORMATION IN CONTROL AND DIABETIC RATS.

(1) General features of the experimental rats.

Figures 13, 14 and 15 show the general characteristics of animals used in this study. The diabetic rats exhibited reduced body weights compared with all controls (Figure 13). Plasma insulin levels of the diabetic rats were about 50% of the controls (Figure 15) and their glucose values increased about fourfold relative to controls (Figure 14). Omega-3 fatty acid treatment did not seem to have any significant

effects on either control or diabetic rats with respect to body weights, plasma glucose or insulin levels.

Plasma triglyceride (Figure 16) and cholesterol (Figure 17) were significantly higher in the untreated diabetic rats compared with all controls. In contrast, the hypertriglyceridemia of diabetes was significantly reduced in the diabetic treated group. However, the elevated plasma cholesterol of diabetic rats was not affected by the treatment.

Wet right ventricular weights were decreased in the diabetic animals (Figure 18). Omega-3 fatty acid treatment had no effect on the right ventricular weights of either control or diabetic rats.

(2) Effect of omega-3 fatty acid treatment on cardiac alpha$_1$-adrenoceptor-mediated inositol phosphates metabolism.

The effect of omega-3 fatty acid on alpha$_1$-adrenoceptor-stimulated IP$_1$ formation is presented in Figure 19. IP$_1$ formation was measured under different concentrations of norepinephrine stimulation (10^{-6}, 10^{-5} and 10^{-4} M). As shown, stimulation of IP$_1$ production due to norepinephrine was concentration-dependent in all groups. However, the increase in IP$_1$ formation was significantly lower in both untreated and treated diabetic groups compared with controls when the hearts were stimulated with higher concentrations of norepinephrine (10^{-5} and 10^{-4} M).

Norepinephrine stimulated IP_1 formation did not appear to be affected by omega-3 fatty acid in the ventricles from control or diabetic animals.

When $[^3H]IP_2$ was measured, it was noted that IP_2 formation in response to 10^{-4} M of norepinephrine was significantly lower in the diabetic heart compared with controls (Fig. 20). Omega-3 fatty acid had no effect on IP_2 production in response to 10^{-4} M of norepinephrine in diabetic rats. At norepinephrine concentrations lower than 10^{-4} M, there were no significant differences in norepinephrine-stimulated IP_2 formation in any of the groups.

In this experiment, we also showed that $[^3H]IP_3$ and $[^3H]IP_4$ were formed in response to alpha$_1$-adrenoceptor stimulation (Figure 21 and 22). Concentration-dependent increases in IP_3 and IP_4 in response to norepinephrine were evident in most cases. However, differences in IP_3 or IP_4 formation between untreated and treated, or control and diabetic rat hearts were not evident. This is probably due to the large standard error and the low specific activity of the myo$[2-^3H]$inositol (4 μCi/mmol) employed.

3.7. EFFECT OF ALPHA$_1$-ADRENOCEPTOR STIMULATION ON POSITIVE INOTROPIC EFFECT AND INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P$_3$] FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

(1) General features of the experimental rats.

Rats injected with STZ exhibited symptoms characteristic of the diabetic state. Diabetic rats did not gain as much weight as age-matched controls and had significantly lower body weights at the time of sacrifice (Table 6). This occurred despite the fact that diabetic animals had dramatically elevated fluid (polydipsia) and food (hyperphagia) intake. Diabetic rats also had increased fecal and urine output which were not measured quantitatively.

Urine glucose was qualitatively assessed three days after STZ injection using Tes-Tape. On a scale of 1+ (0.1%) to 4+ (2.0%), it was found to be greater than 2% for diabetic rats. No detectable glucose was present in the urine of control animals. Plasma glucose levels of diabetic animals were found to be elevated by three-fold (Table 6). On the other hand, plasma insulin levels measured at the time of sacrifice were significantly depressed in diabetic animals (Table 6).

(2) Effect of alpha$_1$-adrenoceptor stimulation on positive inotropic effect in control and diabetic rat ventricles.

Figure 23 shows the effect of increasing concentrations of norepinephrine (in the presence of propranolol) on the change in tension (% of maximum) in right ventricles obtained from control and diabetic rats. It can be seen that norepinephrine induced a concentration-dependent

increase in percentage of maximum change in tension in both groups of right ventricles. However, concentration-response curves to norepinephrine obtained from diabetic rats were shifted to the left of the corresponding age-matched control groups, indicative of supersensitivity to the inotropic effect of norepinephrine. The increase in sensitivity to norepinephrine was reflected by higher estimated PD_2 values (3.26 ± 0.19) obtained in the diabetic group relative to the age-matched control group (2.43 ± 0.21) (Figure 24).

When data were expressed as Δ change in tension in response to increasing concentrations of norepinephrine, similar results were obtained (Figure 25). Norepinephrine produced a positive inotropic effect in both groups of right ventricles. The increase in tension was concentration-dependent and significantly greater in the diabetic group compared with controls. At the highest concentration employed (10^{-2} M), Δ change in tension in right ventricles from diabetic animals was shown to be most prominently increased compared with controls (Figure 26).

(3) Effect of alpha$_1$-adrenoceptor stimulation on Ins(1,4,5)P$_3$ formation in control and diabetic rat ventricles.

When the concentration-response curves were completed, tissues were immediately freeze-clamped. Ins(1,4,5)P$_3$ levels were determined using a protein binding assay. It is shown that norepinephrine induced a significantly greater

increase in $Ins(1,4,5)P_3$ levels in diabetic right ventricles (2.98 ± 0.19) compared with controls (0.56 ± 0.17) (Figure 27), which paralleled the $\triangle$ change in tension at 10^{-2} M of norepinephrine.

(4)   Effect of prazosin on $alpha_1$-adrenoceptor-mediated $Ins(1,4,5)P_3$ formation and inotropic responses in control and diabetic right ventricles.

To assess the role of alpha-adrenoceptors in the contractile effect of norepinephrine, ventricles were exposed to prazosin (in the presence of propranolol) before addition of norepinephrine. The results shown in Table 7 indicate that in the presence of prazosin, the positive inotropic action of norepinephrine was antagonized in both control and diabetic right ventricles. As well, norepinephrine-stimulated $Ins(1,4,5)P_3$ formation was blocked by the addition of prazosin.

3.8. TIME COURSE OF $ALPHA_1$-ADRENOCEPTOR-MEDIATED POSITIVE INOTROPISM AND INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P_3] FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

Figure 28 depicts the time course of norepinephrine-stimulated inotropism in control and diabetic rat hearts. Stimulation of $alpha_1$-adrenoceptors in the right ventricles of rats resulted in a biphasic inotropic response. The initial decrease in contractile force appeared about 10 sec

after the stimulation and reached a maximum level at 30 sec. The contractile force then started to increase at about 45 to 60 sec, reaching a maximum level at 3 min. The contractile force developed in the diabetic heart [Figure 28 (b)] was significantly higher compared with controls [Figure 28 (a)].

The level of $Ins(1,4,5)P_3$ in both control and diabetic rat hearts increased significantly within 15 sec after the stimulation, reached maximum at 60 sec and gradually declined thereafter (Figure 29). Starting from 30 sec, $Ins(1,4,5)P_3$ levels in the diabetic hearts were significantly higher compared with controls.

When $Ins(1,4,5)P_3$ formation was expressed as percentage change of $Ins(1,4,5)P_3$ over basal levels (basal level=100%) at different time points of norepinephrine stimulation, it was shown that % change in $Ins(1,4,5)P_3$ levels in response to norepinephrine stimulation for the time periods longer than 30 sec was also significantly greater in diabetic ventricles compared with controls (Table 8).

Correlation coefficients for the time course of $alpha_1$-adrenoceptor-mediated positive inotropic effect and $Ins(1,4,5)P_3$ formation were 0.33 for the controls and 0.39 for the diabetics, which were not significantly different from each other. The low correlation coefficients might be due to transient negative inotropic effect induced by norepinephrine. The correlation between tissue $Ins(1,4,5)P_3$ levels and the extent of the transient negative inotropic

effect as documented by the present study may suggest that the transient negative inotropic effect of norepinephrine may also be triggered by $Ins(1,4,5)P_3$ formation. One possible mechanism by which $Ins(1,4,5)P_3$ formation causes a negative inotropic effect is Ca^{2+} overload induced by intracellular Ca^{2+} mobilization. In fact, caffeine and Ca^{2+} ionophore, which could cause release of intracellular Ca^{2+}, produced negative inotropic effects following a transient positive inotropic effect (Otani et al., 1988a). It is also possible that $alpha_1$-adrenoceptor-mediated reduction of cardiac cAMP levels may be associated with the transient negative inotropic effect.

3.9. CONCENTRATION DEPENDENCE OF $ALPHA_1$-ADRENOCEPTOR-MEDIATED POSITIVE INOTROPIC EFFECT AND INOSITOL 1,4,5-TRISPHOSPHATE [$INS(1,4,5)P_3$] FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

(1) Concentration responsiveness of $alpha_1$-adrenoceptor-mediated positive inotropic effect in control and diabetic rats.

The effect of increasing concentrations of norepinephrine (in the presence of propranolol) on the change in tension (% of maximum) in right ventricles obtained from control and diabetic rats is shown in Figure 30. Norepinephrine induced a concentration-dependent increase in percentage of maximum change in tension in both groups of right ventricles. However, concentration-response

curves to norepinephrine obtained from diabetic right ventricles were shifted to the left of the corresponding age-matched controls, indicative of supersensitivity to the inotropic effect of norepinephrine in diabetic animals.

(2) Concentration responsiveness of $alpha_1$-adrenoceptor-mediated inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] formation in control and diabetic rats.

Tissues were freeze-clamped following stimulation by different concentrations of norepinephrine. Ins(1,4,5)P_3 was determined using a protein binding assay. Norepinephrine induced a concentration-dependent increase in Ins(1,4,5)P_3 levels in right ventricles from control rats (basal 0.22 ± 0.09; peak 0.66 ± 0.09) (Figure 31). In right ventricles of diabetic animals, Ins(1,4,5)P_3 levels also increased in norepinephrine concentration-dependent manner and were significantly greater compared with control hearts. The peak level of Ins(1,4,5)P_3 formation in diabetic hearts was 1.13 ± 0.13. The basal levels of Ins(1,4,5)P_3 formation were similar in right ventricles of both control and diabetic groups (control, 0.22 ± 0.09; diabetic, 0.30 ± 0.08).

When Ins(1,4,5)P_3 formation was expressed as percentage change of Ins(1,4,5)P_3 over basal levels (basal level=100%) at different concentrations of norepinephrine stimulation, it was shown that % change in Ins(1,4,5)P_3 levels in response to 10^{-6} to 10^{-3} M of norepinephrine stimulation was

significantly greater in diabetic ventricles compared with controls (Table 9).

Correlation curves for the concentration-responses of alpha$_1$-adrenoceptor-mediated positive inotropic effect and Ins(1,4,5)P$_3$ formation (Figure 32) indicated that the positive inotropic effect and Ins(1,4,5)P$_3$ formation were indeed correlated with each other in control and diabetic right ventricles.

3.10. INFLUENCE OF INDOMETHACIN ON ALPHA$_1$-ADRENOCEPTOR-MEDIATED POSITIVE INOTROPIC EFFECT AND INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P$_3$] FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

(1) Influence of indomethacin on concentration responsiveness of alpha$_1$-adrenoceptor-mediated positive inotropic effect.

Figure 33 depicts concentration-response curves for the change in tension (% of maximum) evoked by norepinephrine in right ventricles of control and diabetic rats. Norepinephrine produced a positive inotropic effect in both control and diabetic groups. The increase in tension was concentration-dependent and was significantly greater in the diabetic group than in controls.

In the presence of the cyclooxygenase inhibitor indomethacin, the positive inotropic effect induced by norepinephrine was significantly increased in control right ventricles. In fact, the change in tension (% of maximum)

in control right ventricles pretreated with indomethacin was similar to that in diabetic right ventricles. On the other hand, indomethacin failed to modify the contractile stimulatory action of norepinephrine in the diabetic right ventricles.

The increase in sensitivity to norepinephrine in diabetic right ventricles was reflected by higher estimated PD_2 values obtained in the diabetic group (3.25 ± 0.23) relative to the age-matched control group (2.43 ± 0.25) (Figure 34). Estimated PD_2 values in the control group treated with indomethacin were significantly increased (3.51 ± 0.25) compared with controls, whereas estimated PD_2 values remained elevated in the diabetic group pretreated with indomethacin (3.36 ± 0.23).

When data were expressed as Δ change in tension in response to increasing concentrations of norepinephrine stimulation, norepinephrine also produced a positive inotropic effect in all groups of right ventricles (Figure 35). The increase in tension was concentration-dependent, and was significantly greater in the diabetic group compared with controls. In the presence of indomethacin, Δ change in tension induced by norepinephrine was significantly increased in control right ventricles. However, indomethacin was unable to modify Δ change in tension to norepinephrine in the right ventricles from diabetic animals.

At the highest norepinephrine concentration employed $(10^{-2}$ M), $\triangle$ change in tension in diabetic right ventricles was increased compared with controls (Figure 36). The tension change remained the same in the diabetic right ventricles pretreated with indomethacin. On the other hand, the change in tension increased in the control group treated with indomethacin.

(2) Influence of indomethacin on $alpha_1$-adrenoceptor-stimulated $Ins(1,4,5)P_3$ formation in control and diabetic rats.

At the end of the concentration-response curves, right ventricles were freeze-clamped and $Ins(1,4,5)P_3$ levels were measured in these tissues. The changes in $Ins(1,4,5)P_3$ corresponded with the above changes in the tension development. $Ins(1,4,5)P_3$ levels were elevated in the diabetic group (2.98 ± 0.59) as well as in the diabetic group pretreated with indomethacin (3.36 ± 0.42) compared with the control group (0.56 ± 0.17) (Figure 37). $Ins(1,4,5)P_3$ levels in the control group treated with indomethacin (2.79 ± 0.68) were, however, similar to the $Ins(1,4,5)P_3$ values in diabetic hearts.

3.11. INFLUENCE OF IMIDAZOLE ON $ALPHA_1$-ADRENOCEPTOR-MEDIATED POSITIVE INOTROPIC EFFECT AND INOSITOL 1,4,5-TRISPHOSPHATE [$INS(1,4,5)P_3$] FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

(1) Influence of imidazole on concentration responsiveness of alpha$_1$-adrenoceptor-mediated positive inotropic effect.

The exposure of control right ventricles to the thromboxane synthetase inhibitor imidazole increased the positive inotropic effect of norepinephrine to values similar to those in the diabetic group (Figure 38). However, it should be noted that imidazole did not modify significantly the tension development in the diabetic group.

The increase in sensitivity to norepinephrine in diabetic right ventricles was reflected by higher estimated PD$_2$ values obtained in the diabetic group (3.25 ± 0.18) relative to the age-matched control group (2.43 ± 0.20) (Figure 39). Estimated PD$_2$ values in the control group treated with imidazole were significantly increased (3.70 ± 0.25) compared with the control group, whereas estimated PD$_2$ values remained elevated in the imidazole-treated diabetic group (4.11 ± 0.22).

Similar results were shown when data were expressed as Δ change in tension (Figure 40). At 10^{-2} M of norepinephrine stimulation, the diabetic group as well as the diabetic group pretreated with imidazole had enhanced positive inotropic effect compared with the control group. Interestingly, imidazole enhanced the positive inotropic effect significantly in the control animals.

(2) Influence of imidazole on alpha$_1$-adrenoceptor-stimulated Ins(1,4,5)P$_3$ formation in control and diabetic rats.

At the end of the concentration-response curves, right ventricles were freeze-clamped immediately and Ins(1,4,5)P$_3$ levels were measured in these tissues using a protein binding assay. Ins(1,4,5)P$_3$ levels were significantly elevated in the control group treated with imidazole (3.67 ± 0.27) compared with the control group (0.56 ± 0.17) (Figure 41). Also, Ins(1,4,5)P$_3$ levels were increased in the diabetic group (2.98 ± 0.27). Ins(1,4,5)P$_3$ levels in the diabetic group treated with imidazole (1.51 ± 0.21) were higher than the ones in the control group but were lower than the ones in the imidazole-treated control group as well as the diabetic group.

3.12. INFLUENCE OF TRANYLCYPROMINE ON ALPHA$_1$-ADRENOCEPTOR-MEDIATED POSITIVE INOTROPIC EFFECT AND INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P$_3$] FORMATION IN CONTROL AND DIABETIC RAT VENTRICLES.

(1) General features of control and diabetic rats 6 weeks after administration of streptozotocin.

Injecting rats with STZ resulted in a diabetic state, with an elevated urine glucose (>2%) throughout the study period. No detectable glucose was present in the urine of control animals. Parameters used to assess the diabetic state of animals are summarized in Table 10. At the time of

sacrifice, the diabetic rats exhibited reduced body weights compared with controls. Plasma glucose levels were markedly elevated in STZ-injected rats accompanied by a depression in plasma insulin levels.

Table 11 shows the plasma lipid profile of the experimental rats. Plasma triglyceride and cholesterol were significantly higher in the Wistar diabetic group compared with controls.

(2) Influence of tranylcypromine on concentration responsiveness of $alpha_1$-adrenoceptor-mediated positive inotropic effect in control and diabetic rats.

$\wedge$ Change in tension in response to 10^{-2} M norepinephrine obtained from the diabetic group was significantly elevated compared with the control group (Figure 42). Incubation with tranylcypromine, a prostacyclin synthetase inhibitor, reduced the inotropic effect of norepinephrine in diabetic right ventricles, but failed to modify it in the control right ventricles.

(3) Influence of tranylcypromine on $alpha_1$-adrenoceptor-stimulated $Ins(1,4,5)P_3$ formation in control and diabetic rats.

Changes in $Ins(1,4,5)P_3$ levels (Figure 43) corresponded with the changes in tension development discussed above. The diabetic group showed a significant increase in $Ins(1,4,5)P_3$ levels (2.98 ± 0.15) compared with controls

(0.41 ± 0.13). The prostacyclin synthetase inhibitor tranylcypromine diminished norepinephrine-induced Ins(1,4,5)P_3 formation in diabetic preparations (0.50 ± 0.13) without altering norepinephrine-induced Ins(1,4,5)P_3 formation in the controls (0.48 ± 0.15).

3.13. PROTEIN KINASE C ACTIVITY IN CONTROL AND DIABETIC RAT VENTRICLES.

Table 12 shows the distribution of protein kinase C in cytosolic and membrane fractions in the right ventricles of control and diabetic rats. In the control hearts, the basal kinase activity was similar in the cytosolic and membrane fractions. The activated kinase activity (kinase activity in the presence of Ca^{2+}, PS and DG) was higher in the cytosol fraction compared with the membrane fraction. The specific protein kinase C activity was also higher in the cytosol fraction compared with the membrane fraction. In the diabetic hearts, the basal kinase activity was also similar in the cytosolic and membrane fractions. However, the activated kinase activity was higher in the membrane fraction compared with the cytosolic fraction. As well, the specific protein kinase C activity was higher in the membrane fraction compared with the cytosolic one. Therefore, the activated kinase activity as well as the specific protein kinase C activity in the cytosolic fraction in the diabetic hearts was significantly lower than that in the control hearts, whereas the activated kinase activity

and the specific protein kinase C activity in the membrane fraction in the diabetic hearts was significantly higher than that in the control hearts. It was also noted that both basal kinase activity and the kinase activity in the presence of Ca^{2+} alone in the membrane fraction were higher in the diabetic hearts compared with controls. Total protein kinase C activity, calculated as the sum of the cytosolic and membrane fractions, was not significantly different between diabetic and control animals.

It is also demonstrated in Table 12 that Ca^{2+} and phosphatidylserine are required for protein kinase C activity in both the cardiac cytosolic and membrane fractions. It can be seen that when Ca^{2+} alone was added, there was no significant change in the kinase activity, either in the cytosolic or membrane fractions. Both the cytosolic and membrane fractions showed a significant increase in kinase activity when both Ca^{2+} and phosphatidylserine were added to the incubation mixture.

A significant amount of protein kinase C activity in the membrane fraction was detected by the addition of EGTA and Triton X-100, since both EGTA and Triton X-100 were found to be essential in the exposure of the Ca^{2+}- and phosphatidylserine-binding sites of protein kinase C in cardiac membrane preparations (Yuan and Sen, 1986). Triton X-100, having little or no effect when used alone, has been found to enhance the ability of EGTA to solubilize a large amount of the enzyme from the membrane fraction (Katoh and

Kuo, 1982). Triton X-100 at the concentration of 0.075% was chosen since it has been reported to be the optimum one required to expose the kinase C activity (Yuan and Sen, 1986). EGTA was used at a concentration of 2 mM since higher concentrations (up to 10 mM) did not release additional enzyme (Katoh and Kuo, 1982).

Table 13 demonstrats the protein kinase C activity of control and diabetic rat ventricles with or without norepinephrine stimulation. Results showed that protein kinase C activity was not altered significantly by norepinephrine stimulation.

Figure 4

Basal inositol monophosphate (IP_1) formation in right ventricles of control and diabetic rats. Tissues were incubated with propranolol (10 μM) for 30 min. IP_1 levels were then measured using the radioisotope method. Results are mean ± S.E.M. n, number of ventricles.

BASAL IP$_1$ FORMATION
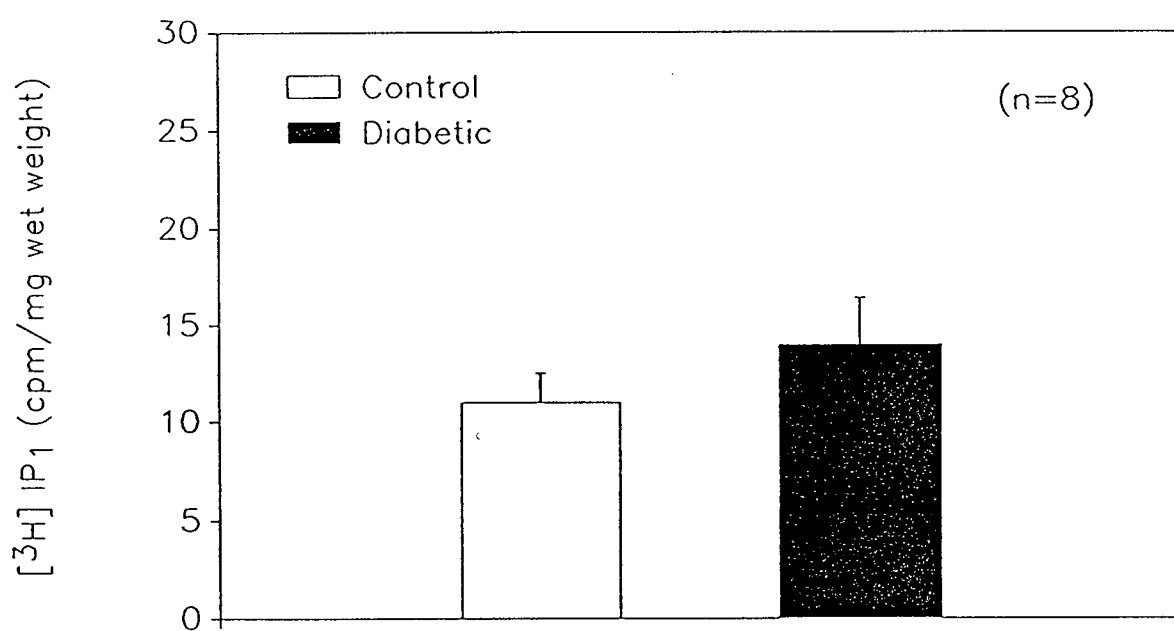
Table 2

GENERAL FEATURES OF EXPERIMENTAL RATS
(Time Course Study)

	Control (n=15)	Diabetic (n=15)
Initial Body Weight (g)	228±3	221±3
Final Body Weight (g)	458±8	362±8*
Δ Body Weight (g)	230±8	141±8*
Plasma Glucose (mmol/l)	5.49±0.18	20.45±0.23*
Plasma Insulin (μU/ml)	49±6	25±4*

* Significantly different from controls; P<0.05.

Figure 5

Time course of body weight changes in control and six-week diabetic rats. Points represent mean ± S.E.M. n, number of hearts. (Time course study)

* Significantly different from controls, p<0.05.
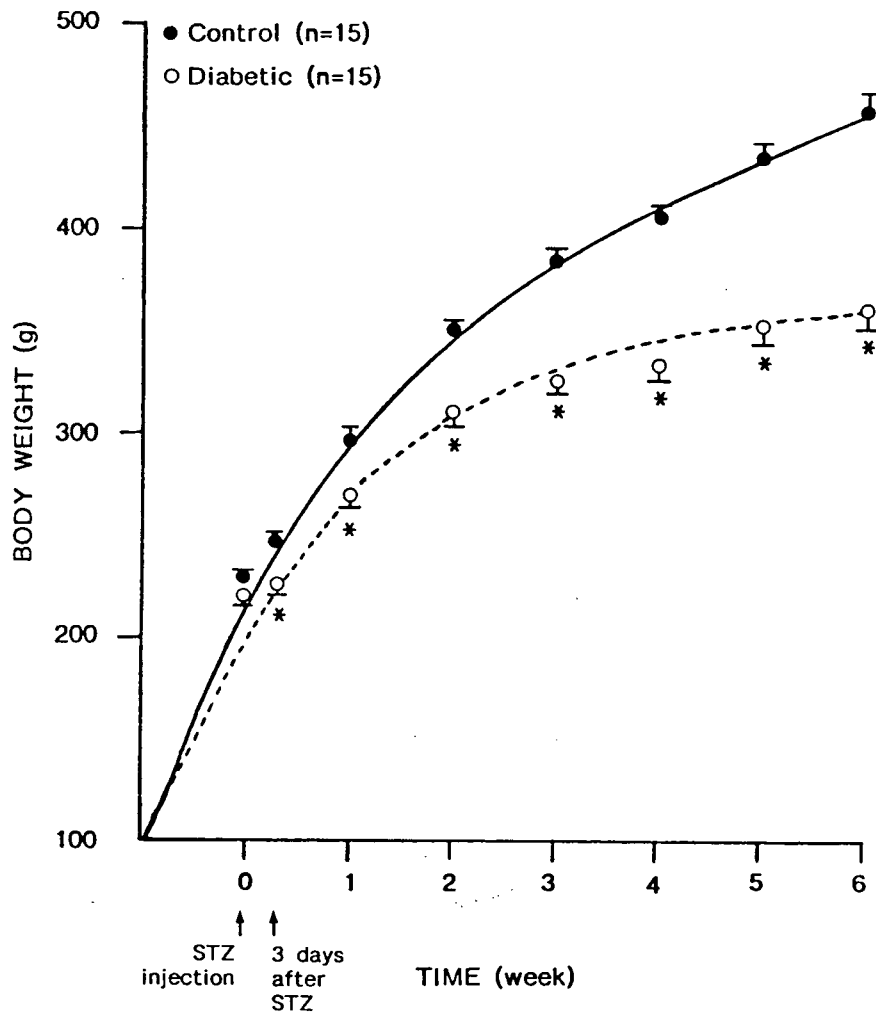
Table 3

PLASMA LIPID PROFILE OF THE EXPERIMENTAL RATS
(Time Course Study)

	Control (n=15)	Diabetic (n=15)
Plasma triglyceride (mmol/l)	1.38±0.13	3.92±0.15*
Plasma cholesterol (mmol/l)	1.77±0.22	3.31±0.23*

* Significantly different from controls; P<0.05.

Figure 6

Right ventricular wet weights of control and STZ-diabetic rats.   Results   are   mean   ±   S.E.M.   for   the   number   of ventricles shown in parentheses. (Time course study)

* Significantly different from controls, p<0.05.

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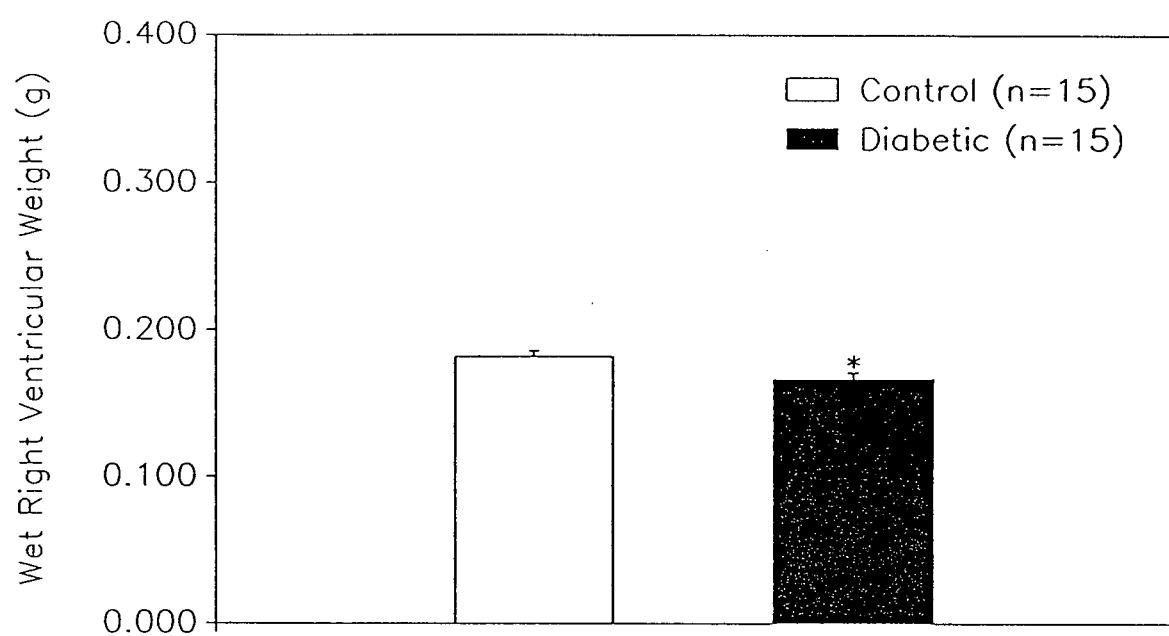
Figure 7

Time course of the effect of norepinephrine (10 μM) on inositol monophosphate (IP_1) formation in right ventricles from control and diabetic animals.   Norepinephrine was applied 15 min after addition of propranolol (10 μM). Tissues were incubated for the fixed time period and IP_1 levels were measured using the radioisotope method.   Points represent mean ± S.E.M.   n, number of ventricles.

* Significantly different from controls, p<0.05.
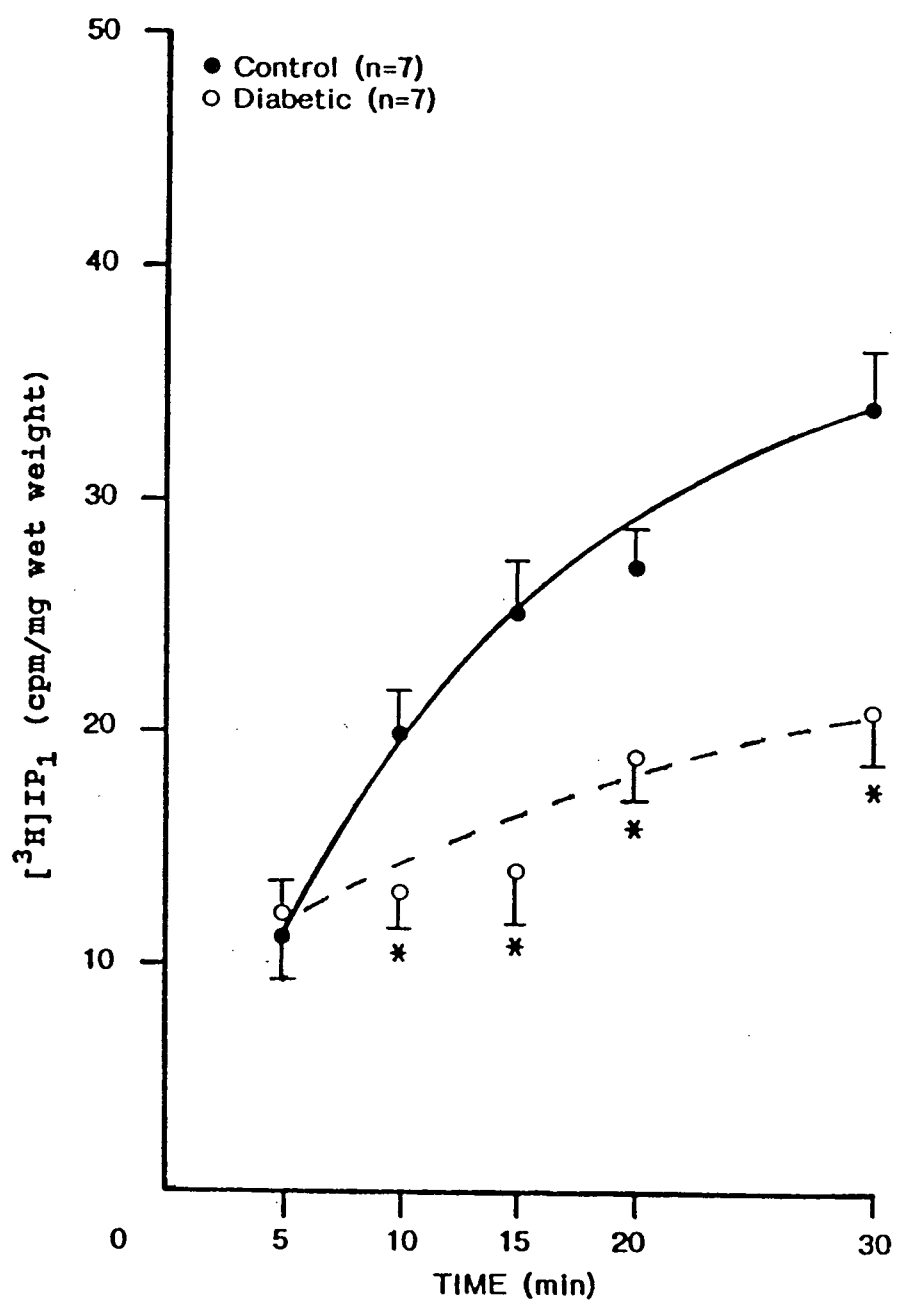
Table 4

GENERAL FEATURES OF EXPERIMENTAL RATS
(Concentration-Response Study)

	Control (n=15)	Diabetic (n=15)
Initial Body Weight (g)	202±2	200±2
Final Body Weight (g)	423±10	327±10*
Δ Body Weight (g)	221±10	127±10*
Plasma Glucose (mmol/l)	5.99±0.19	20.21±0.24*
Plasma Insulin (μU/ml)	44±5	25±3*

* Significantly different from controls; $P < 0.05$.

Figure 8

Time course of body weight changes in control and six-week diabetic rats. Points represent mean ± S.E.M. n, number of rats. (Concentration-response study)

* Significantly different from controls, $p < 0.05$.
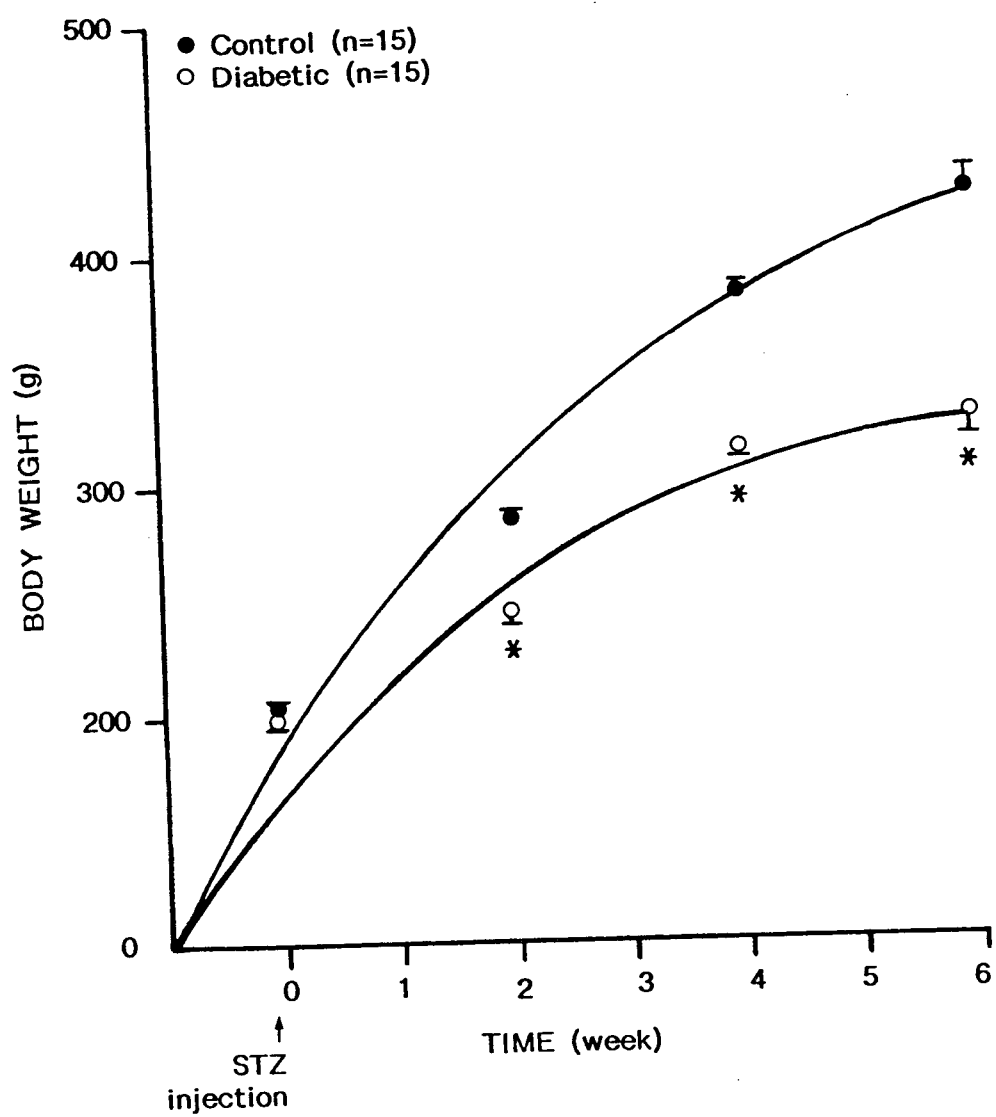
Table 5

PLASMA LIPID PROFILE OF THE EXPERIMENTAL RATS
(Concentration-Response Study)

	Control (n=15)	Diabetic (n=15)
Plasma triglyceride (mmol/l)	1.28±0.13	3.74±0.14*
Plasma cholesterol (mmol/l)	1.69±0.13	2.58±0.16*

* Significantly different from controls; $P<0.05$.

Figure 9

Right ventricular wet weights of control and STZ-diabetic rats. Results are mean ± S.E.M. for the number of ventricles shown in parentheses. (Concentration-response study)

* Significantly different from controls, p<0.05.
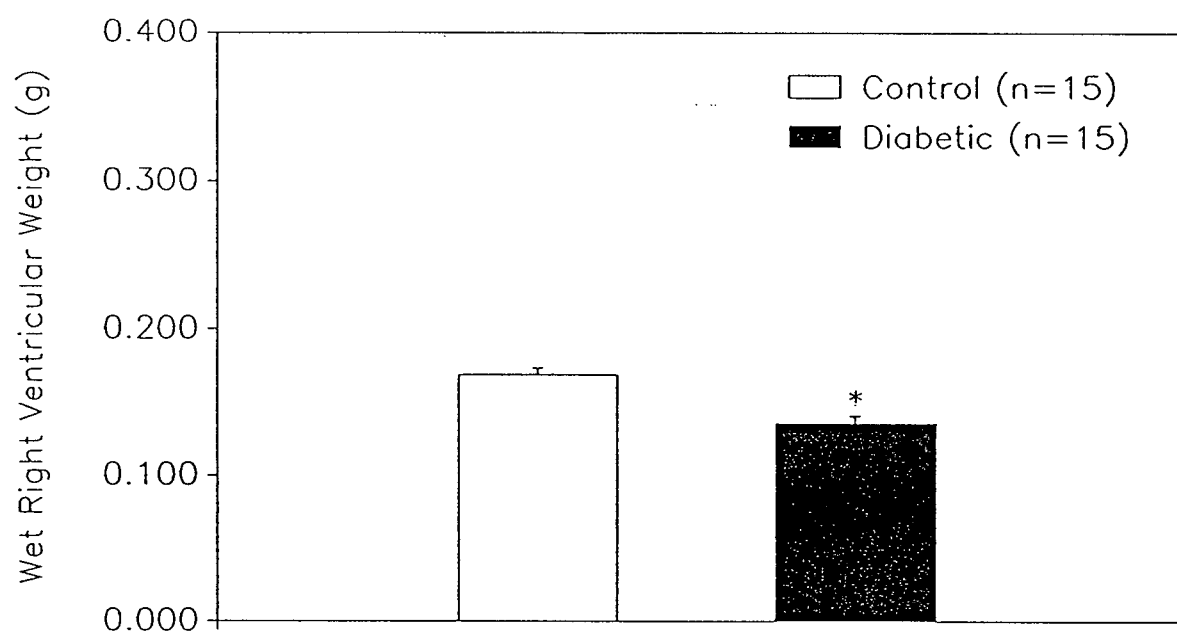
Figure 10

Effect of different concentrations of norepinephrine (in the presence of propranolol) on inositol monophosphate (IP_1) production in control and diabetic right ventricles. Norepinephrine was applied 15 min after addition of propranolol (10 μM). The time of exposure to norepinephrine was 15 min. IP_1 levels were measured using the radioisotope method. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, $p < 0.05$.
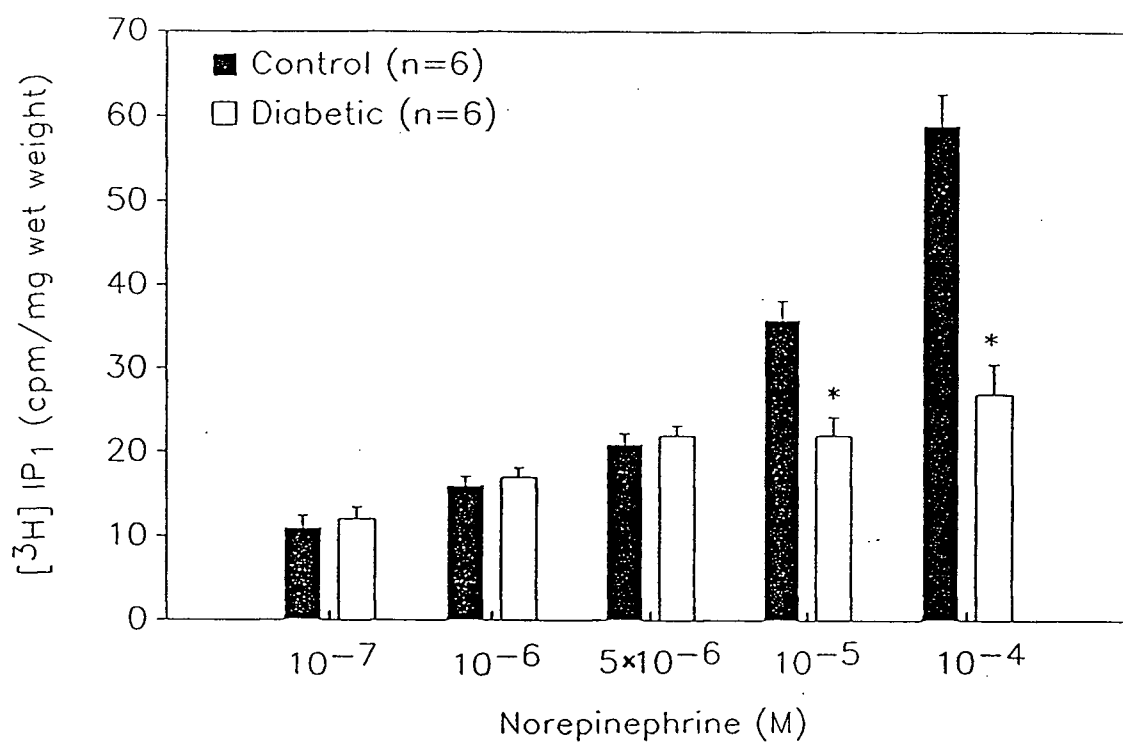
Figure 11

Effect of prazosin (10 μM) and propranolol (10 μM) on norepinephrine-stimulated inositol monophosphate (IP_1) formation in right ventricles of control and diabetic rats. Norepinephrine (10 μM) was applied 15 min after addition of prazosin or propranolol. The time of exposure to norepinephrine was 15 min. IP_1 levels were measured using the radioisotope method. Results are mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from basal values from control rats, $p < 0.05$.

+ Significantly different from basal values from diabetic rats, $p < 0.05$.
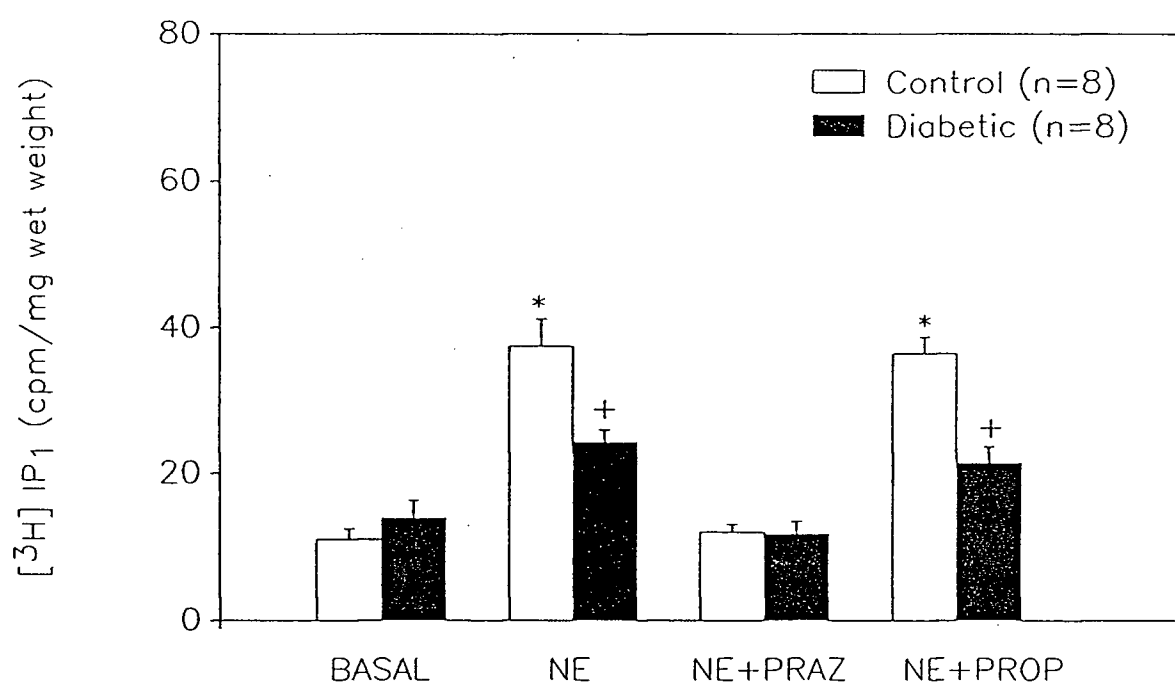
Figure 12

Effect of prazosin, propranolol or DMSO alone on basal inositol monophosphate (IP_1) formation in right ventricles of control and diabetic rats. Tissues were incubated with prazosin (10 μM) for 15 min and IP_1 levels were measured using the radioisotope method. Results are mean ± S.E.M. for the number of ventricles shown in parentheses.
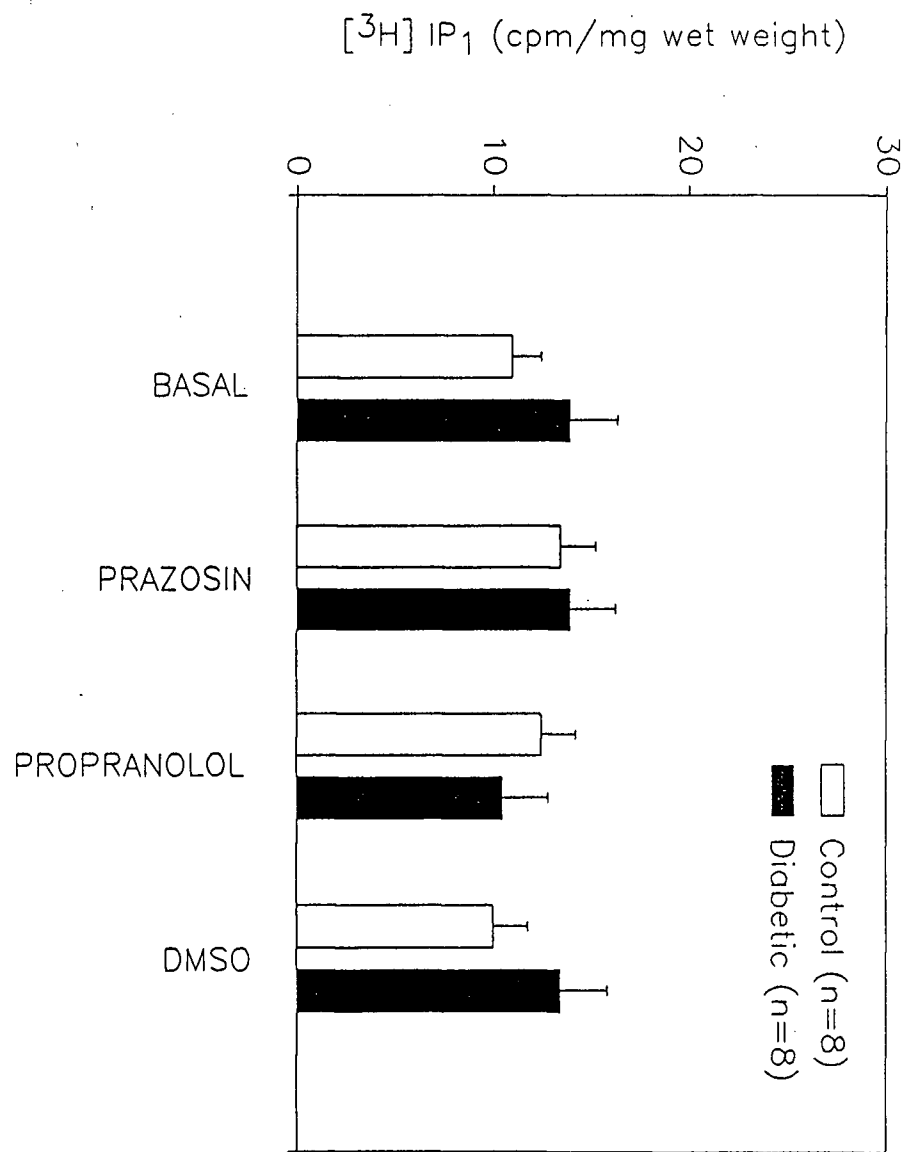
Figure 13

Body weights of control and STZ-diabetic rats treated with omega-3 fatty acid (Promega, 0.5 ml/kg/day). Results are mean ± S.E.M. for the number of animals shown in parentheses.

* Significantly different from controls, $p < 0.05$.
+ Significantly different from controls treated with omega-3 fatty acid, $p < 0.05$.
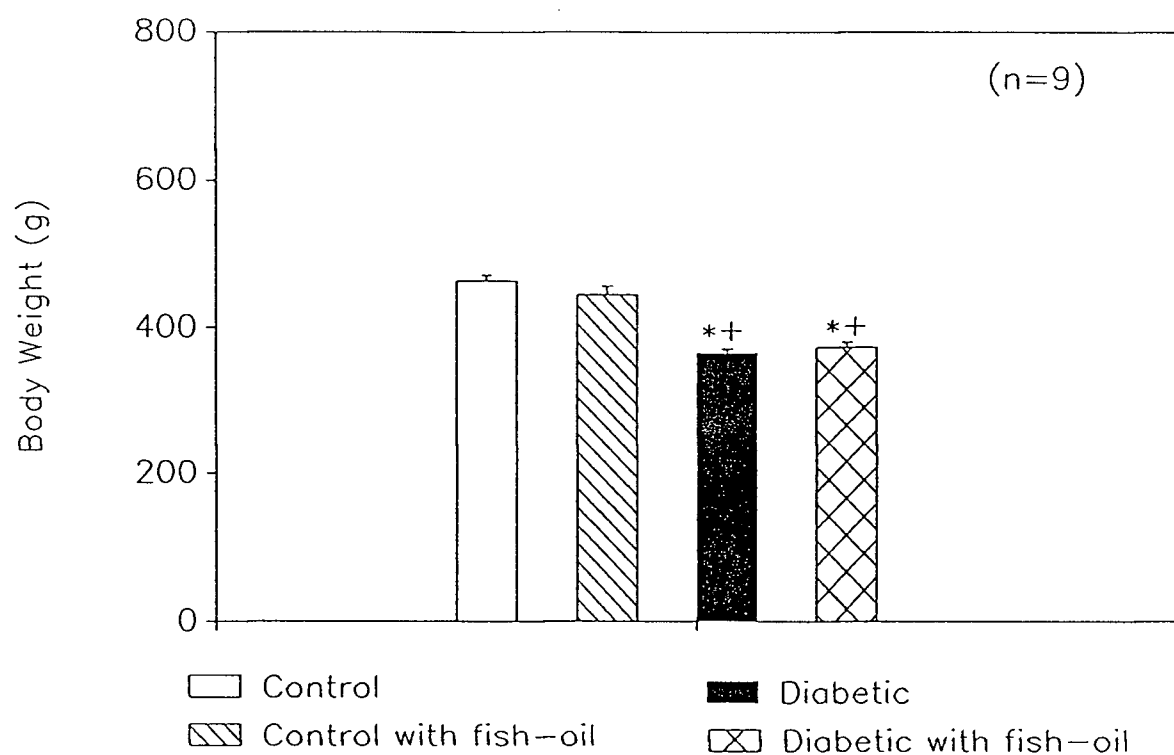
Figure 14

Plasma glucose levels of control and STZ-diabetic rats treated with omega-3 fatty acid (Promega, 0.5 ml/kg/day). Results are mean ± S.E.M. for the number of animals shown in parentheses.

* Significantly different from controls, p<0.05.
+ Significantly different from controls treated with omega-3 fatty acid, p<0.05.
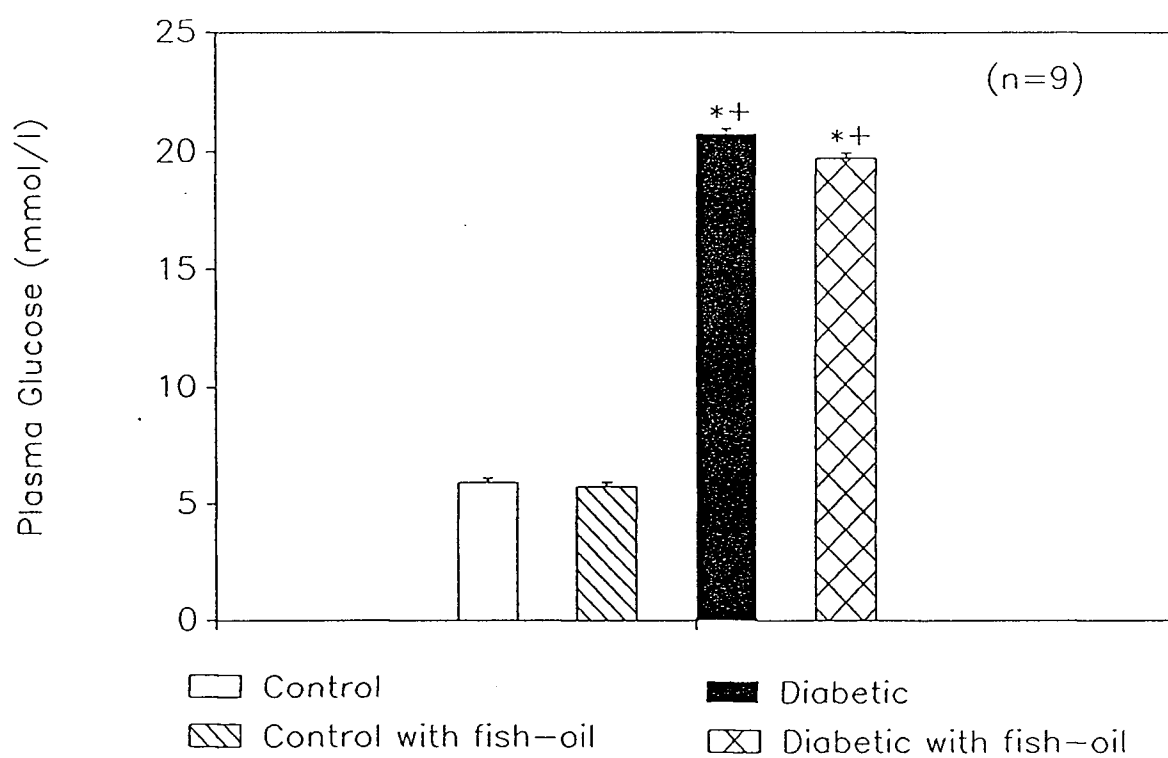
Figure 15

Plasma insulin levels of control and STZ-diabetic rats treated with omega-3 fatty acid (Promega, 0.5 ml/kg/day). Results are mean ± S.E.M. for the number of animals shown in parentheses.

* Significantly different from controls, $p < 0.05$.
\+ Significantly different from controls treated with omega-3 fatty acid, $p < 0.05$.
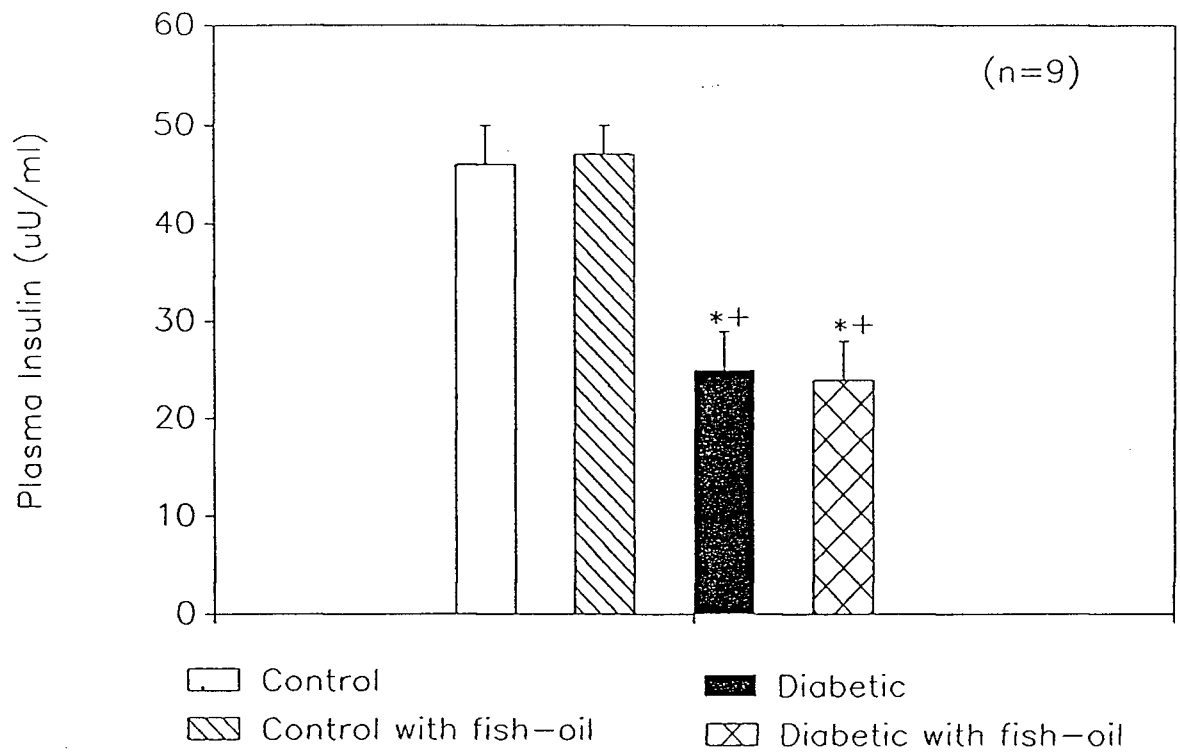
Figure 16

Plasma triglyceride levels of control and STZ-diabetic rats treated with omega-3 fatty acid (Promega, 0.5 ml/kg/day). Results are mean ± S.E.M. for the number of animals shown in parentheses.

* Significantly different from controls, $p<0.05$.
+ Significantly different from controls treated with omega-3 fatty acid, $p<0.05$.
+ Significantly different from diabetics, $p<0.05$.
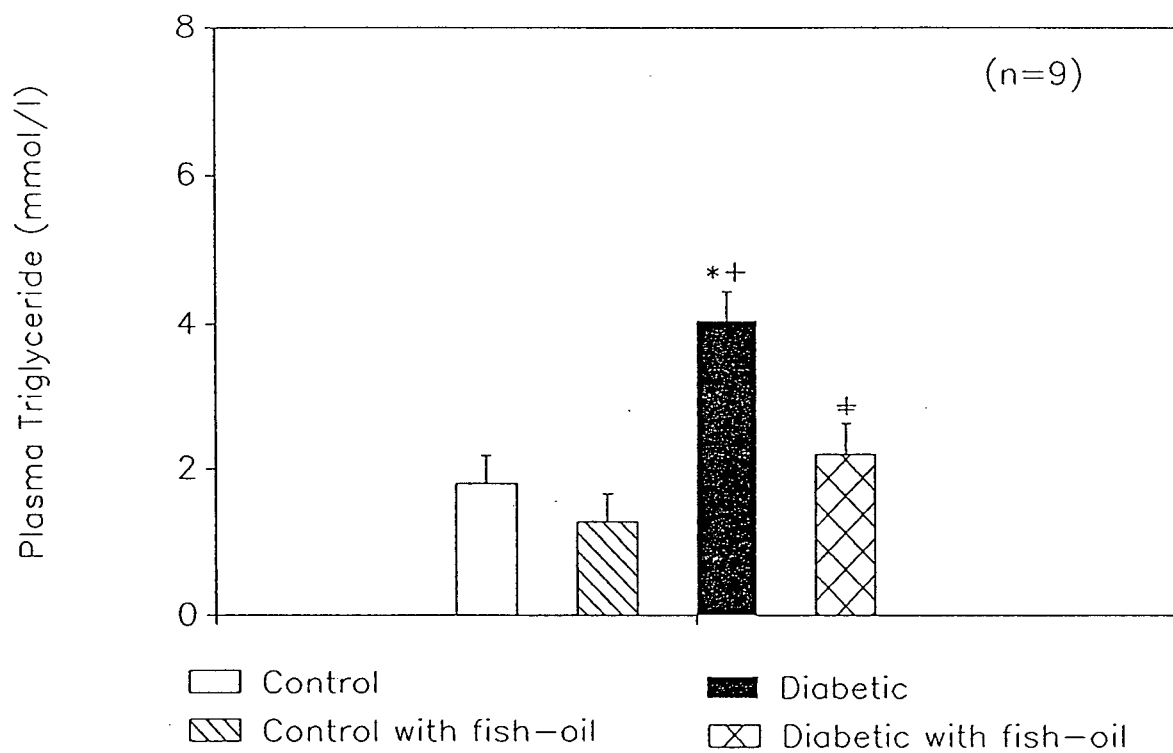
Figure 17

Plasma cholesterol levels of control and STZ-diabetic rats treated with omega-3 fatty acid (Promega, 0.5 ml/kg/day). Results are mean ± S.E.M. for the number of animals shown in parentheses.

* Significantly different from controls, $p<0.05$.
+ Significantly different from controls treated with omega-3 fatty acid, $p<0.05$.
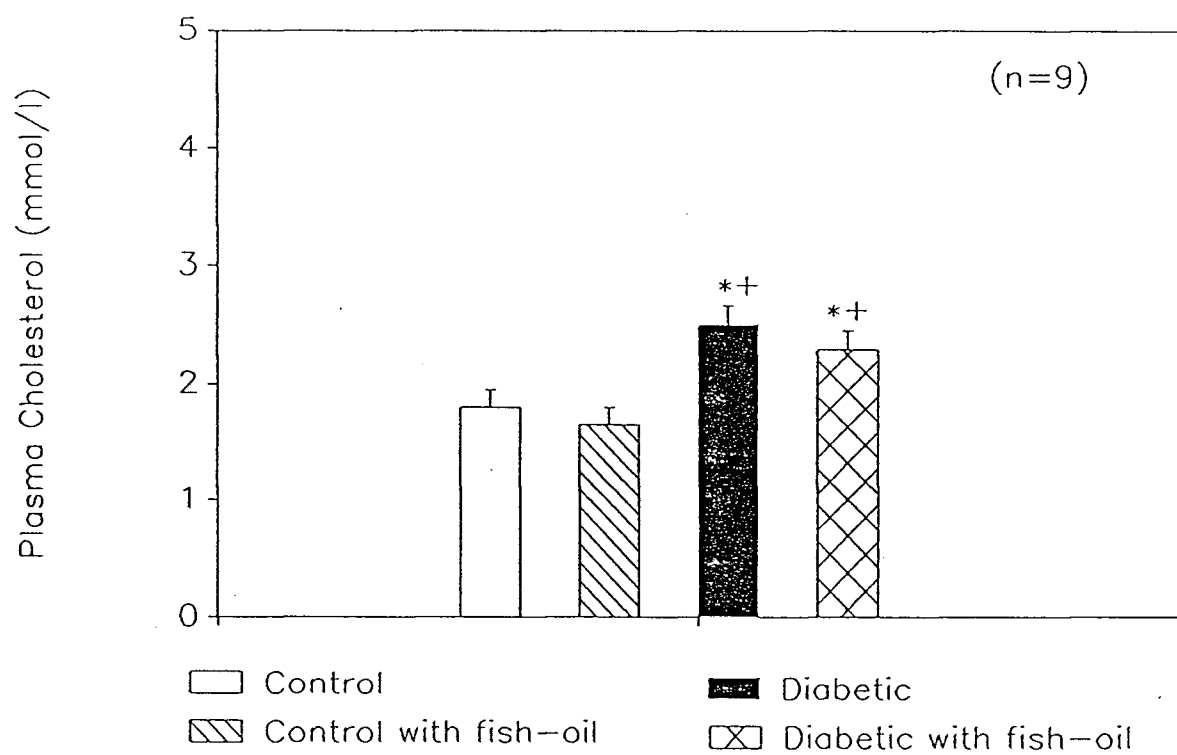
Figure 18

Right ventricular wet weights of control and STZ-diabetic rats treated with omega-3 fatty acid (Promega, 0.5 ml/kg/day). Results are mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, p<0.05.
+ Significantly different from controls treated with omega-3 fatty acid, p<0.05.
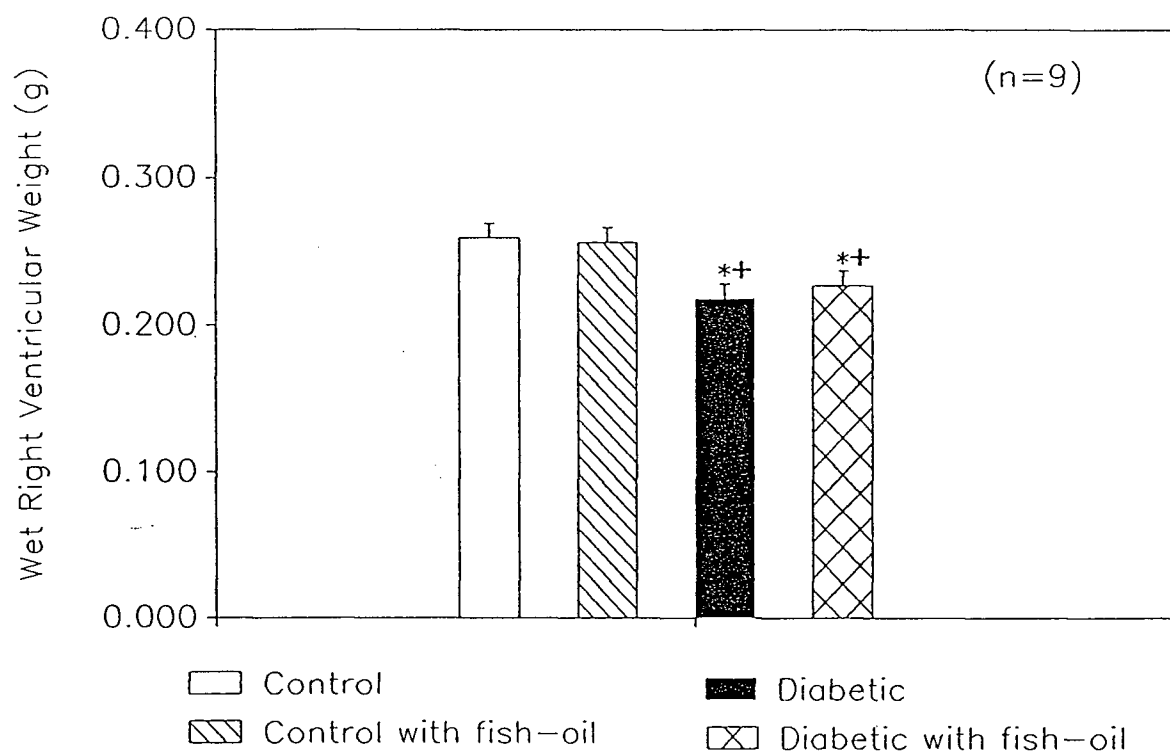
Figure 19

Effect of omega-3 fatty acid (Promega, 0.5 ml/kg/day) on cardiac norepinephrine-stimulated inositol monophosphate (IP_1) formation in control and diabetic rats. Norepinephrine was applied 15 min after addition of propranolol (10 μM). The time of exposure to norepinephrine was 15 min. IP_1 levels were measured using the radioisotope method. CON, controls; DIA, diabetics; F, omega-3 fatty acid-treated. Points represent mean ± S.E.M. n, number of ventricles.

* Significantly different from untreated controls, $p < 0.05$.

+ Significantly different from treated controls, $p < 0.05$.

Y-axis: [³H] IP₁ (cpm/mg wet wt)

X-axis categories: CON, CON-F, DIA, DIA-F

n=6

Legend: □ NE 10⁻⁶ M, ☑ NE 10⁻⁵ M, ⊠ NE 10⁻⁴ M
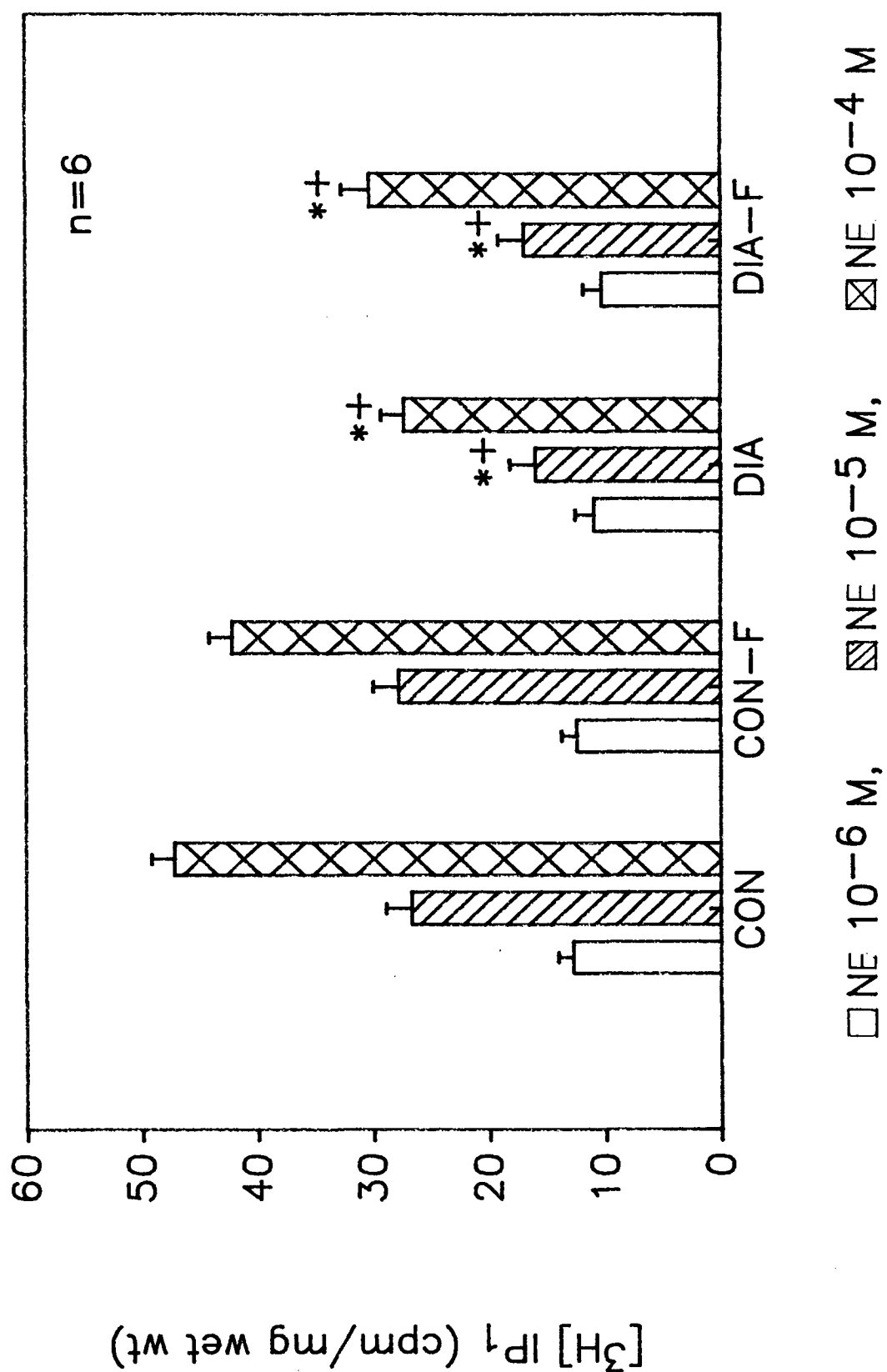
Figure 20

Effect of omega-3 fatty acid (Promega, 0.5 ml/kg/day) on cardiac norepinephrine-stimulated inositol bisphosphate (IP_2) formation in control and diabetic rats. Norepinephrine was applied 15 min after addition of propranolol (10 μM). The time of exposure to norepinephrine was 15 min. IP_2 levels were measured using the radioisotope method. CON, controls; DIA, diabetics; F, omega-3 fatty acid-treated. Points represent mean ± S.E.M. n, number of ventricles.

* Significantly different from untreated controls, $p<0.05$.

+ Significantly different from treated controls, $p<0.05$.
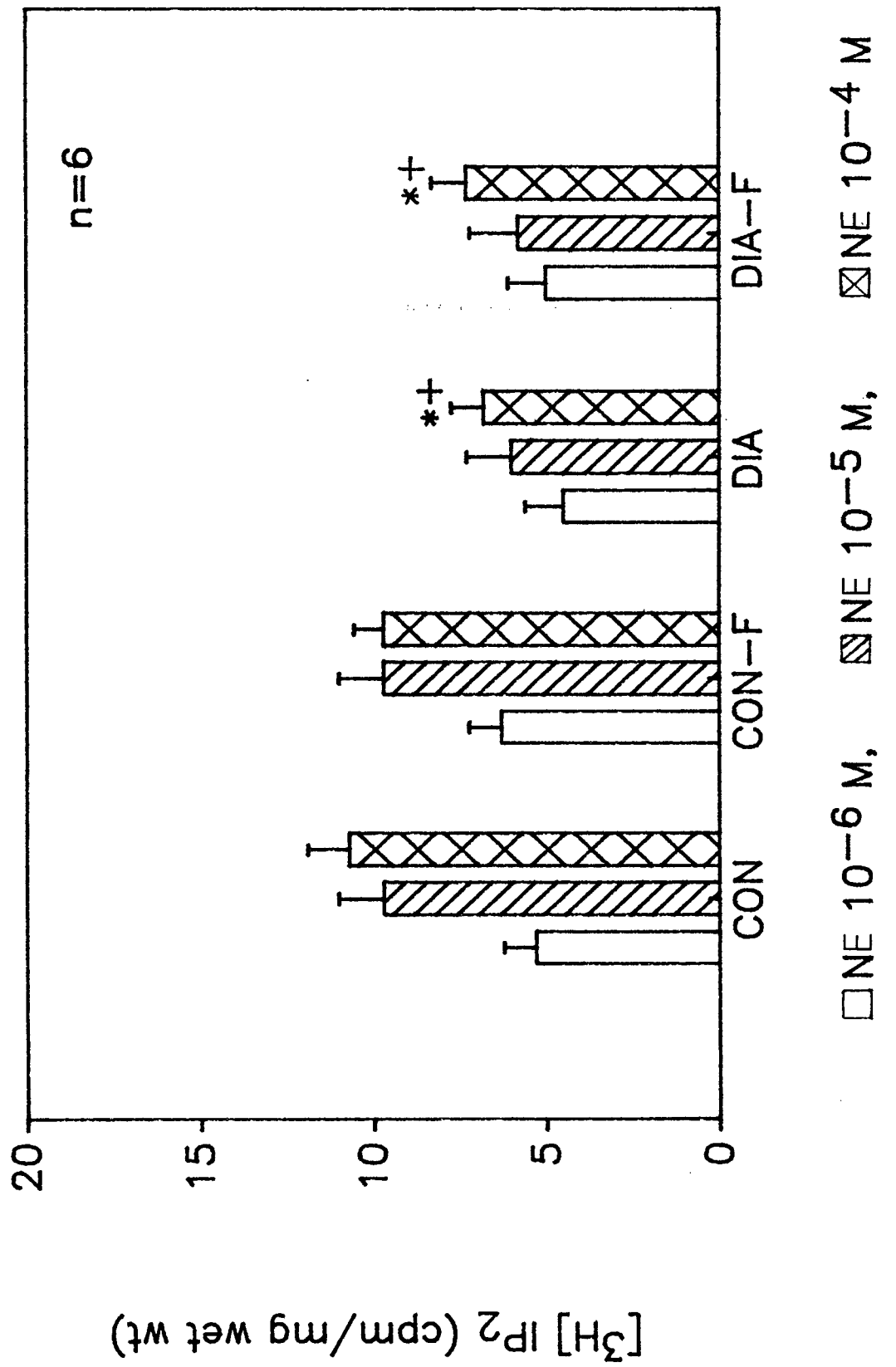
Figure 21

Effect of omega-3 fatty acid (Promega, 0.5 ml/kg/day) on cardiac norepinephrine-stimulated inositol trisphosphate (IP_3) formation in control and diabetic rats. Norepinephrine was applied 15 min after addition of propranolol (10 μM). The time of exposure to norepinephrine was 15 min. IP_3 levels were measured using the radioisotope method. CON, controls; DIA, diabetics; F, omega-3 fatty acid-treated. Points represent mean ± S.E.M. n, number of ventricles.

$[^3H]$ IP$_3$ (cpm/mg wet wt)
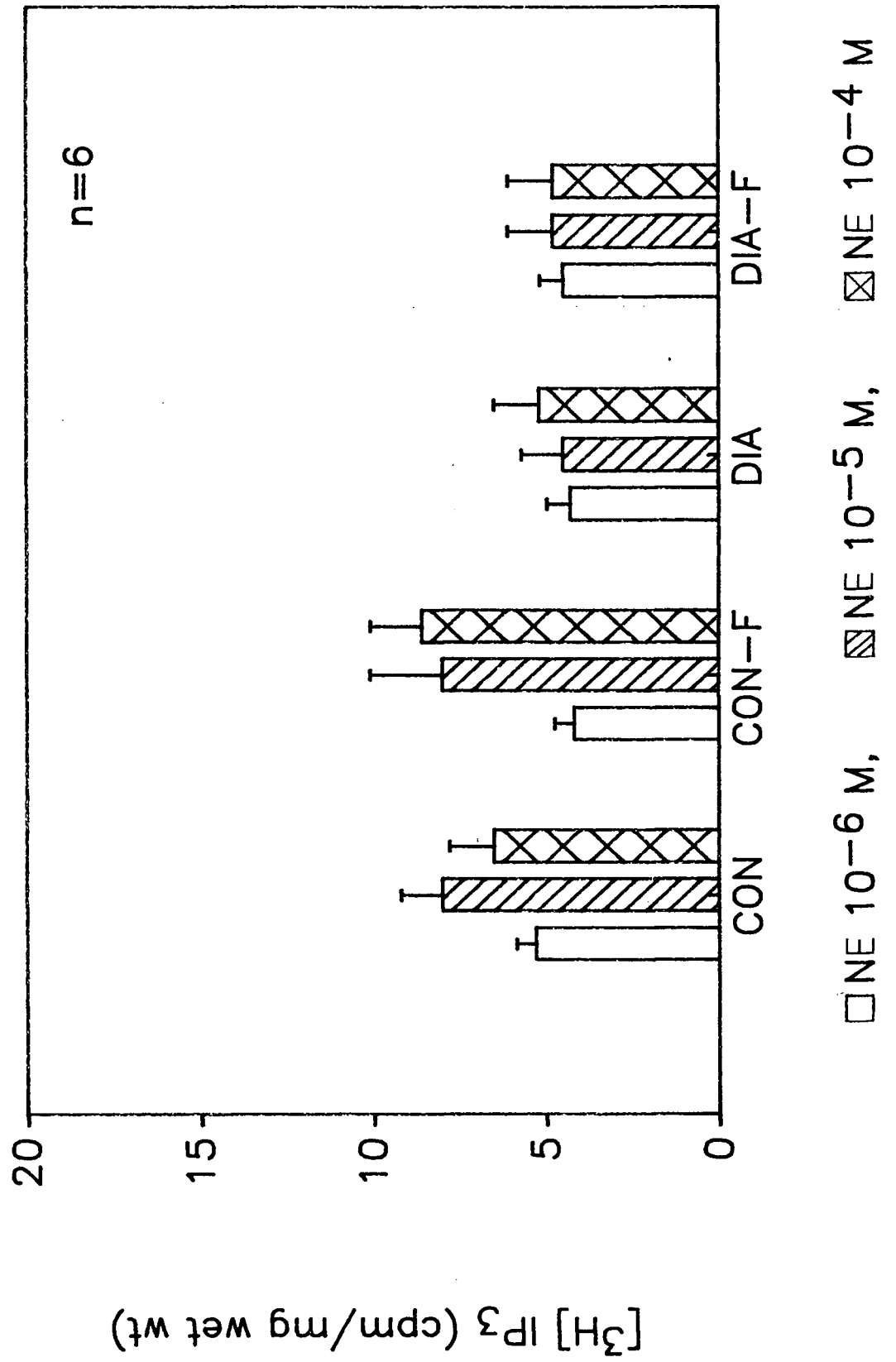
Figure 22

Effect of omega-3 fatty acid (Promega, 0.5 ml/kg/day) on cardiac norepinephrine-stimulated inositol tetrakisphosphate (IP_4) formation in control and diabetic rats. Norepinephrine was applied 15 min after addition of propranolol (10 μM). The time of exposure to norepinephrine was 15 min. IP_4 levels were measured using the radioisotope method. CON, controls; DIA, diabetics; F, omega-3 fatty acid-treated. Points represent mean ± S.E.M. n, number of ventricles.
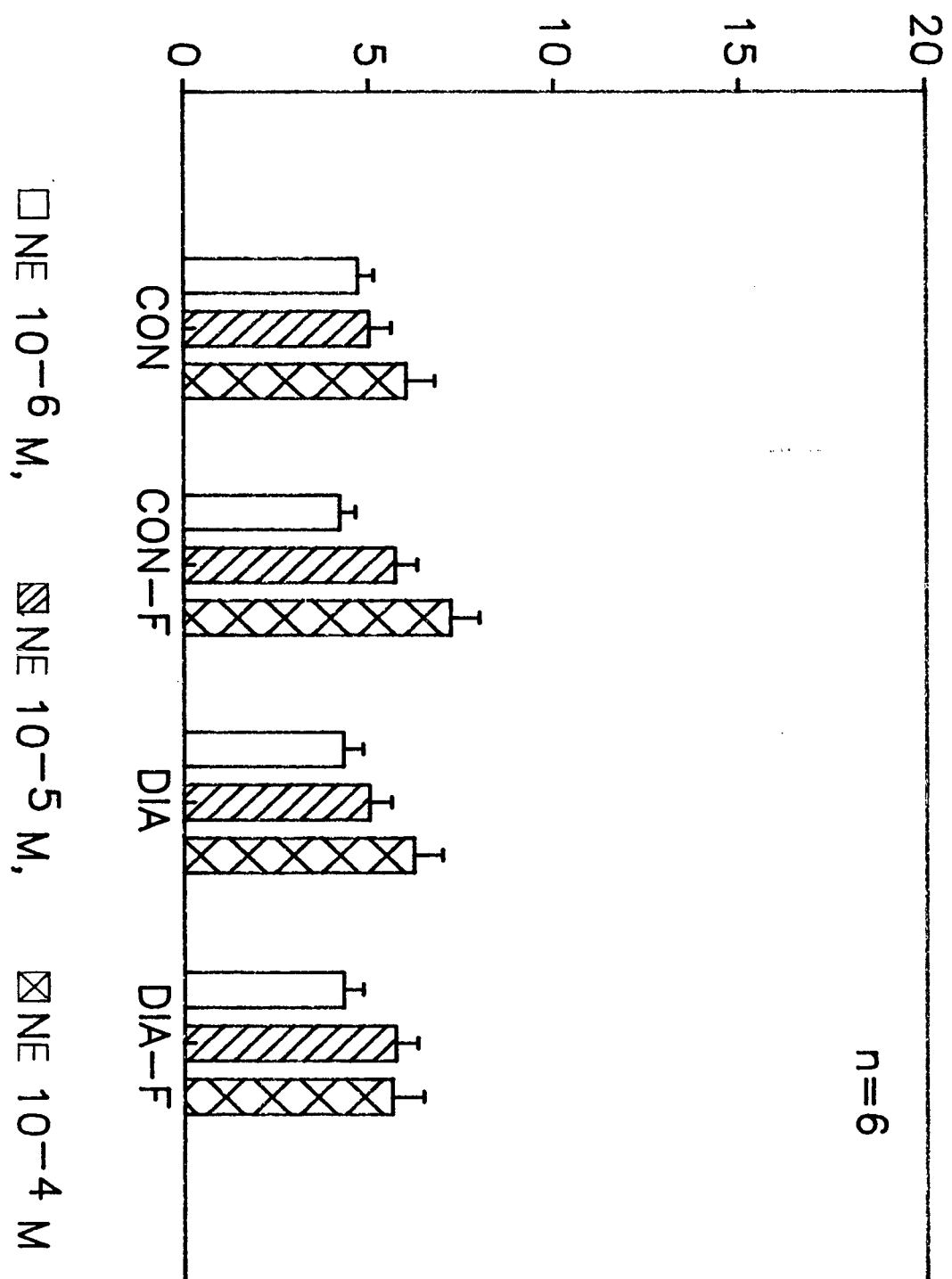
Table 6

**GENERAL FEATURES OF CONTROL AND DIABETIC RATS
6 WEEKS AFTER ADMINISTRATION OF STREPTOZOTOCIN**

	Control (n=12)	Diabetic (n=12)
Body Weight (g)	456±8	365±8*
Plasma glucose (mmol/l)	6.04±0.13	18.13±0.15*
Plasma insulin (μU/ml)	45±5	27±3*

* Significantly different from controls; P<0.05.

Figure 23

Concentration-response curves for the effect of norepinephrine (in the presence of propranolol) on change in tension (% of maximum) in right ventricles of control and diabetic rats. Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM). At each concentration, the inotropic response to norepinephrine was allowed to stabilize before it was recorded. The stabilization time was approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, $p < 0.05$.
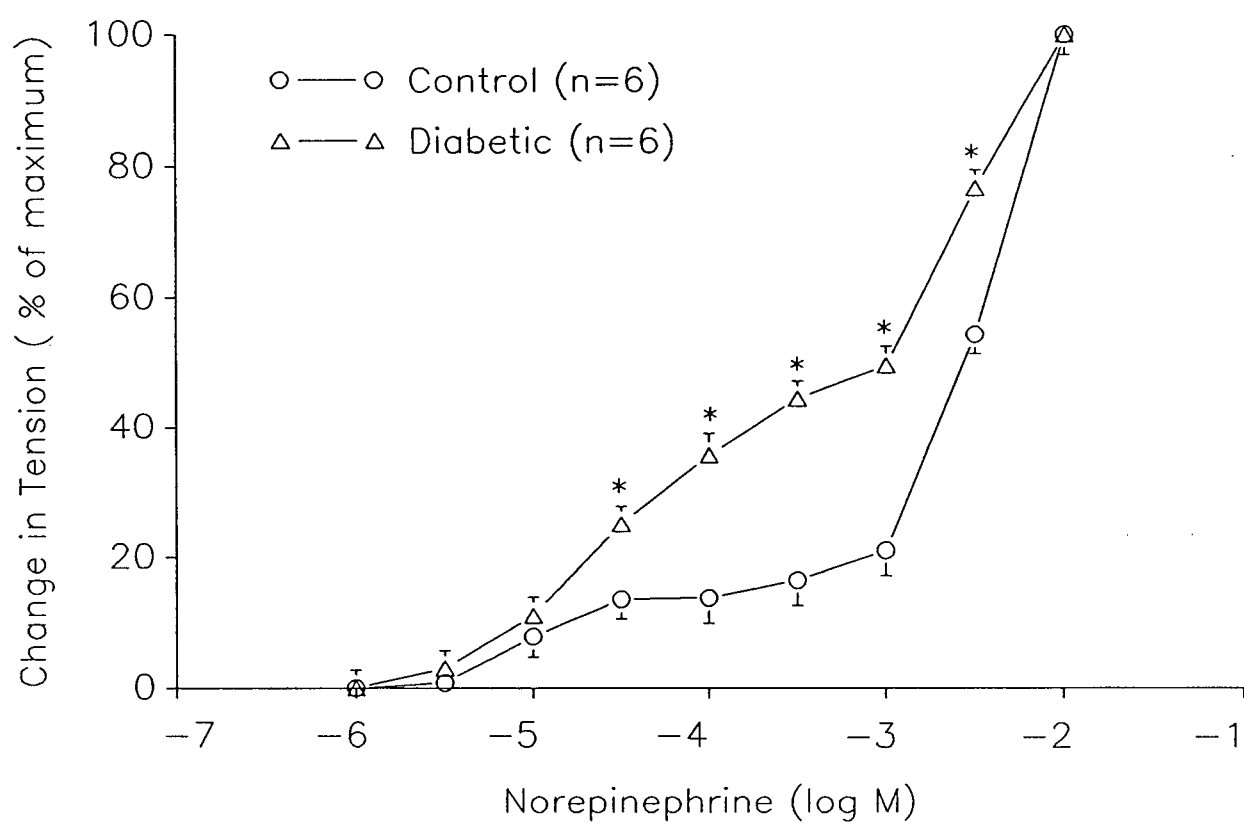
Figure 24

Estimated PD_2 values for norepinephrine (in the presence of propranolol) in control and diabetic right ventricles. Results are mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from PD_2 values for norepinephrine in control rat hearts, p<0.05.

Figure 25

Concentration-response curves for the effect of norepinephrine (in the presence of propranolol) on Δ change in tension in control and diabetic right ventricles. Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM). At each concentration, the inotropic response to norepinephrine was allowed to stabilize before it was recorded. The stabilization time was approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, $p < 0.05$.

Figure 26

Δ Change in tension in response to 10^{-2} M norepinephrine (in the presence of propranolol) in control and diabetic right ventricles. Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM). The inotropic response to norepinephrine was allowed to stabilize before it was recorded. The stabilization time was approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, $p < 0.05$.
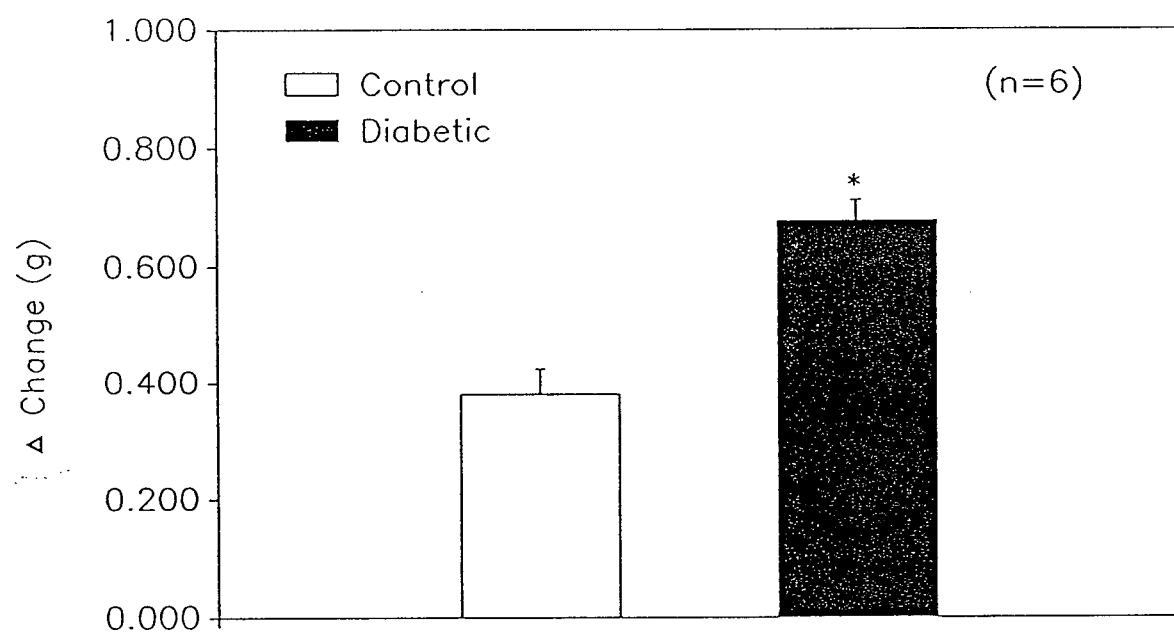
Figure 27

Norepinephrine (10 mM)-stimulated changes in inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] levels in right ventricles from control and STZ-diabetic rats. Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM). At the end of the determination of concentration-response curves, tissues were freeze-clamped and Ins(1,4,5)P_3 levels were determined using a protein binding assay kit. The basal Ins(1,4,5)P_3 levels [Ins(1,4,5)P_3 levels in the absence of norepinephrine] were 0.19 ± 0.02 in control right ventricles and 0.18 ± 0.02 in diabetics. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, $p<0.05$.
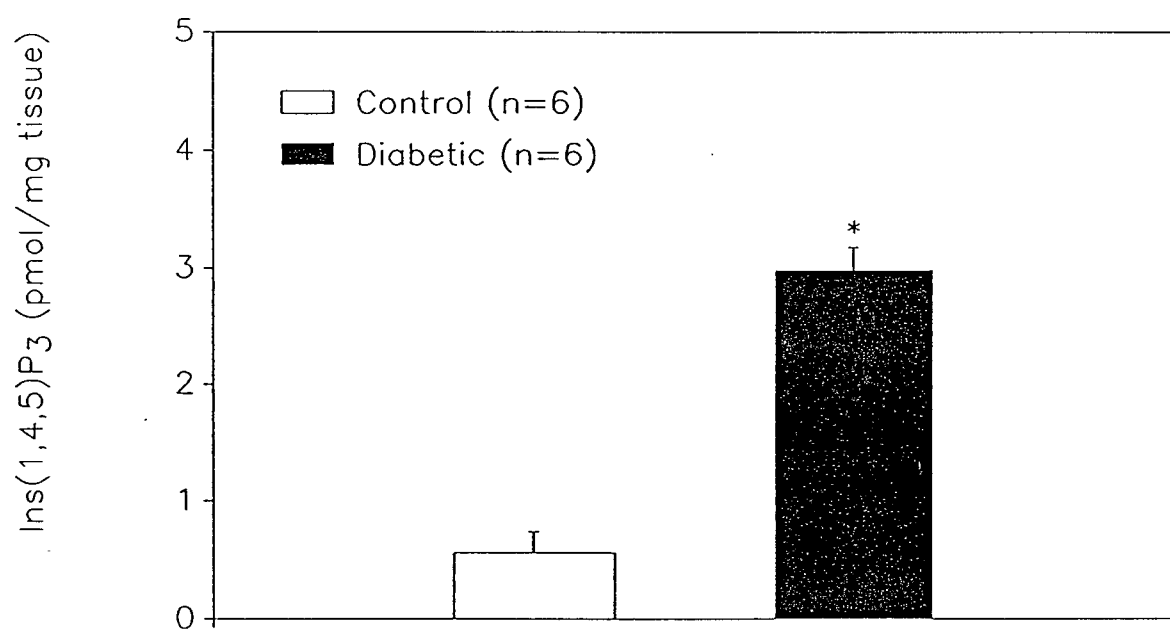
Table 7

EFFECT OF PRAZOSIN ON ALPHA$_1$-ADRENOCEPTOR-MEDIATED INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P$_3$, IP$_3$] (PMOL/MG TISSUE) FORMATION AND INOTROPIC RESPONSES (G) IN CONTROL AND DIABETIC RIGHT VENTRICLES.

	Control (n=8)		Diabetic (n=8)	
	IP$_3$	Δ Change in tension	IP$_3$	Δ Change in tension
Basal	0.20±0.08	-	0.30±0.08	-
NE	0.76±0.10	0.38±0.10	2.98±0.19*	0.72±0.08*
Prazosin + NE	0.22±0.09	0.02±0.00	0.31±0.07	0.01±0.00

Rat right ventricles were treated with prazosin (10 μM) for 15 min in the presence of propranolol (10 μM) and exposed to 10 mM norepinephrine (NE) for 3 min.

* Significantly different from controls; $p < 0.05$.

Figure 28

Time course of positive inotropic effects mediated by alpha$_1$-adrenoceptor stimulation in right ventricles from control (a) and diabetic (b) rats. Norepinephrine (NE) (10^{-3} M) was applied to the tissue bath 15 min after addition of propranolol (10 μM).

a. Control

b. Diabetic

10 30 60   180 (sec)

NE
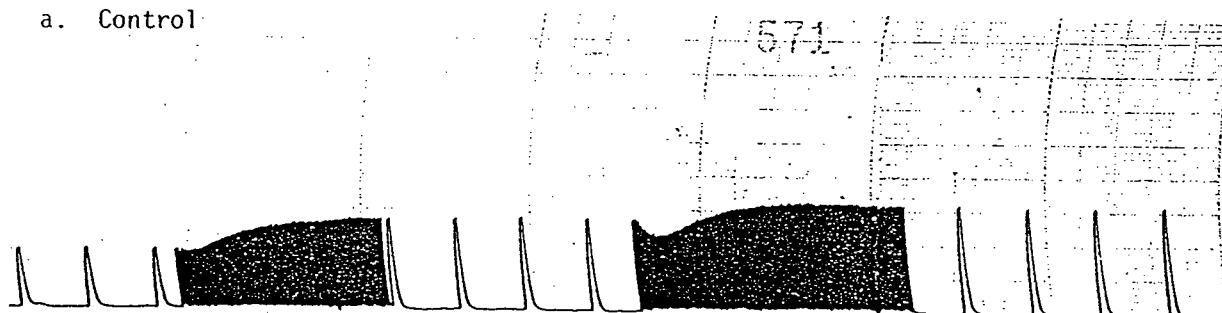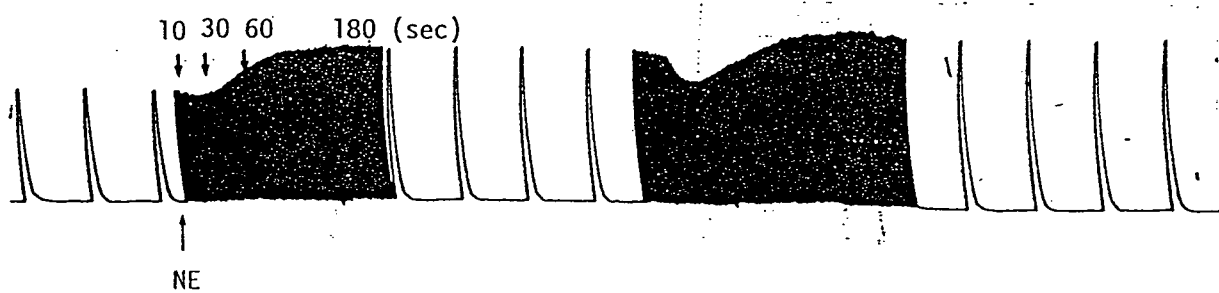
Figure 29

Time course of the effect of norepinephrine (10 μM) on inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] formation in right ventricles of control and diabetic rats. Norepinephrine (10 μM) was applied to the tissue bath 15 min after addition of propranolol (10 μM). Tissues were freeze-clamped at indicated times after addition of norepinephrine. Ins(1,4,5)P$_3$ levels were determined using a protein binding assay kit. The basal values of Ins(1,4,5)P$_3$ [Ins(1,4,5)P$_3$ levels in the absence of norepinephrine] at zero time were 0.17 $\pm$ 0.02 in the control group and 0.18 $\pm$ 0.02 in the diabetic group. The basal levels over the time periods studied did not change significantly compared with the basal values at zero time. Points represent mean $\pm$ S.E.M. n, the number of ventricles.

* Significantly different from controls at the same time point, $p < 0.05$.
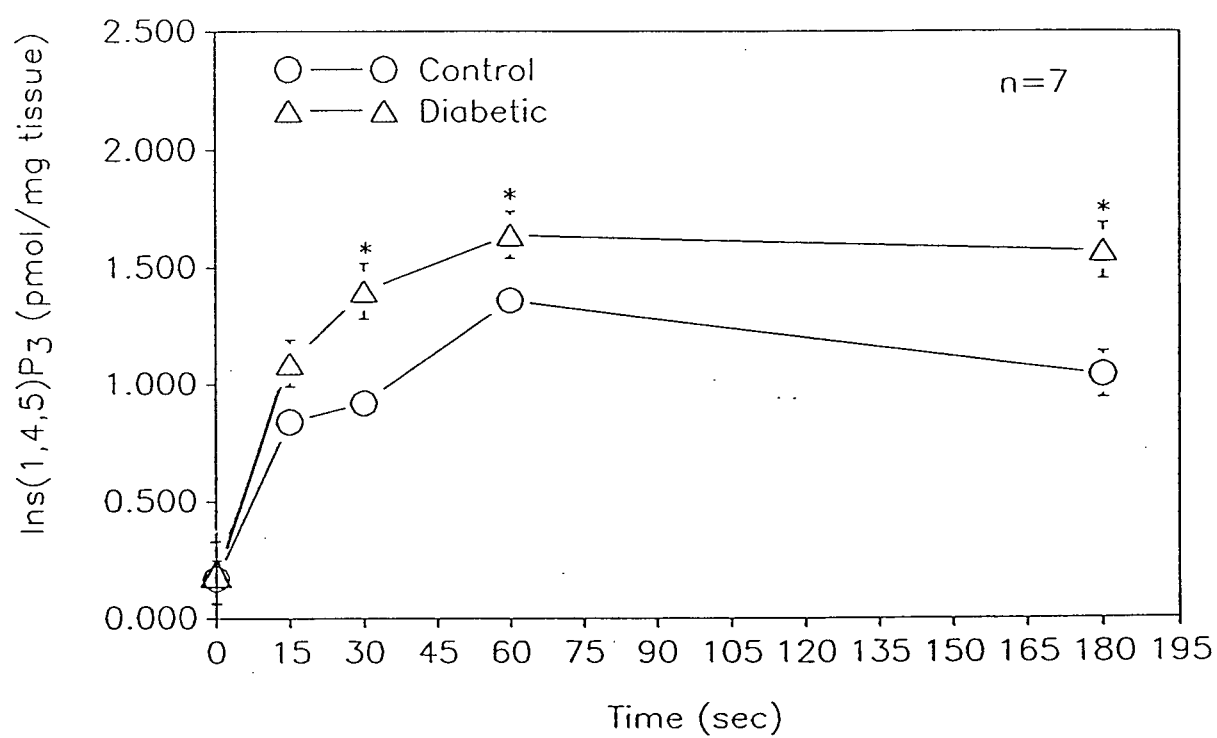
Table 8

% CHANGE IN INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P$_3$] LEVELS AT DIFFERENT TIME POINTS OF NOREPINEPHRINE STIMULATION IN CONTROL AND DIABETIC RIGHT VENTRICLES.

	% Change in Ins(1,4,5)P$_3$	
	Control (n=7)	Diabetic (n=7)
0 sec	100	100
15 sec	492±25	607±70
30 sec	537±95	774±31*
60 sec	858±11	911±35*
180 sec	609±26	872±88*

Norepinephrine (10 μM) was applied to the tissue bath 15 min after addition of propranolol (10 μM). Tissues were freeze-clamped at indicated time periods of norepinephrine stimulation. Ins(1,4,5)P$_3$ was determined using a protein binding assay kit. Values are expressed as mean ± S.E.M. n, number of right ventricles. Basal level=100 %.

* Significantly different from controls at the same time point; $P<0.05$.

Figure 30

Concentration-response curves for the effect of norepinephrine (in the presence of propranolol) on change in tension (% of maximum) in right ventricles of control and diabetic rats. Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM). At each concentration, the inotropic response to norepinephrine was allowed to stabilize before it was recorded. The stabilization time was approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.
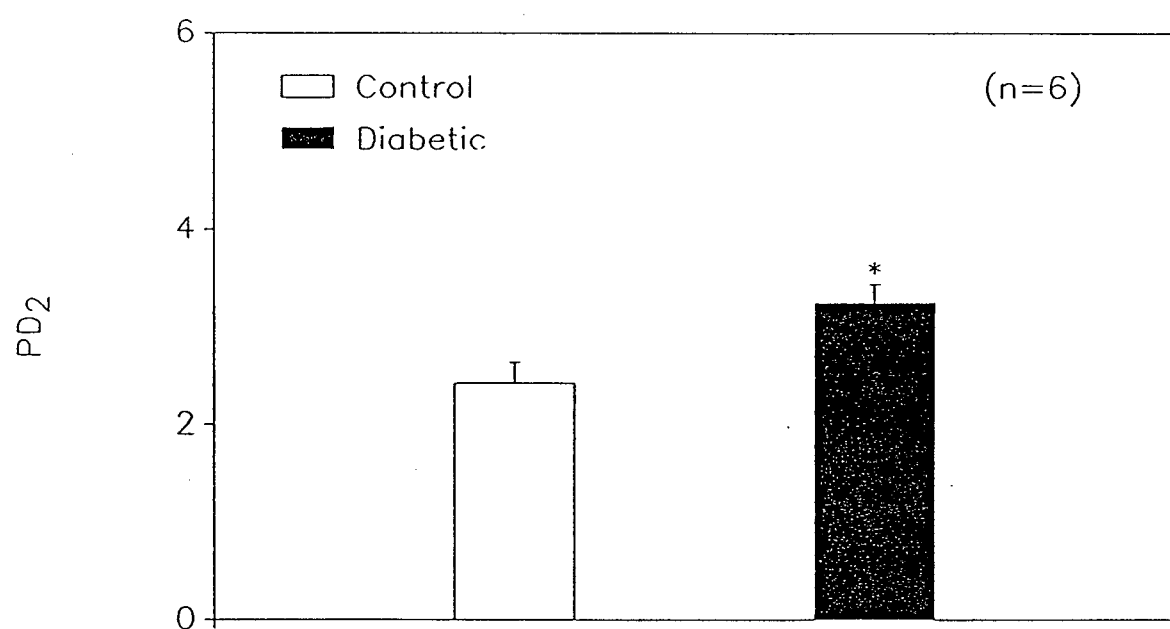
* Significantly different from controls, p<0.05.
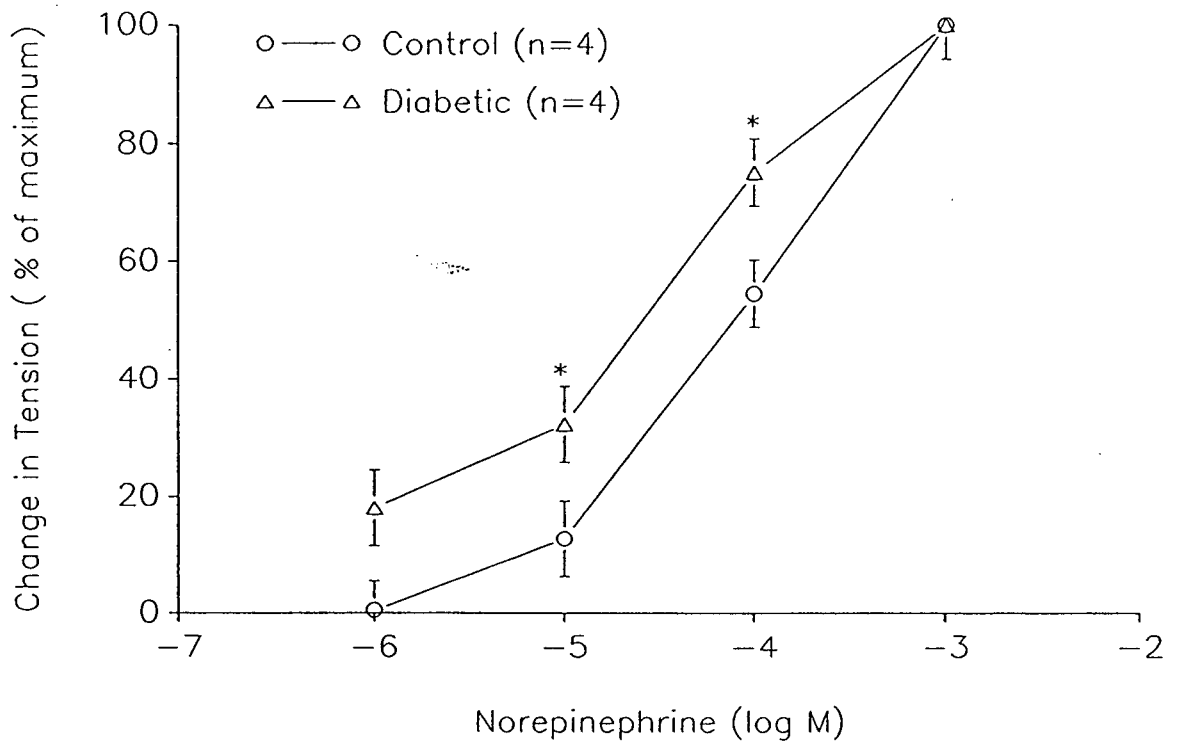
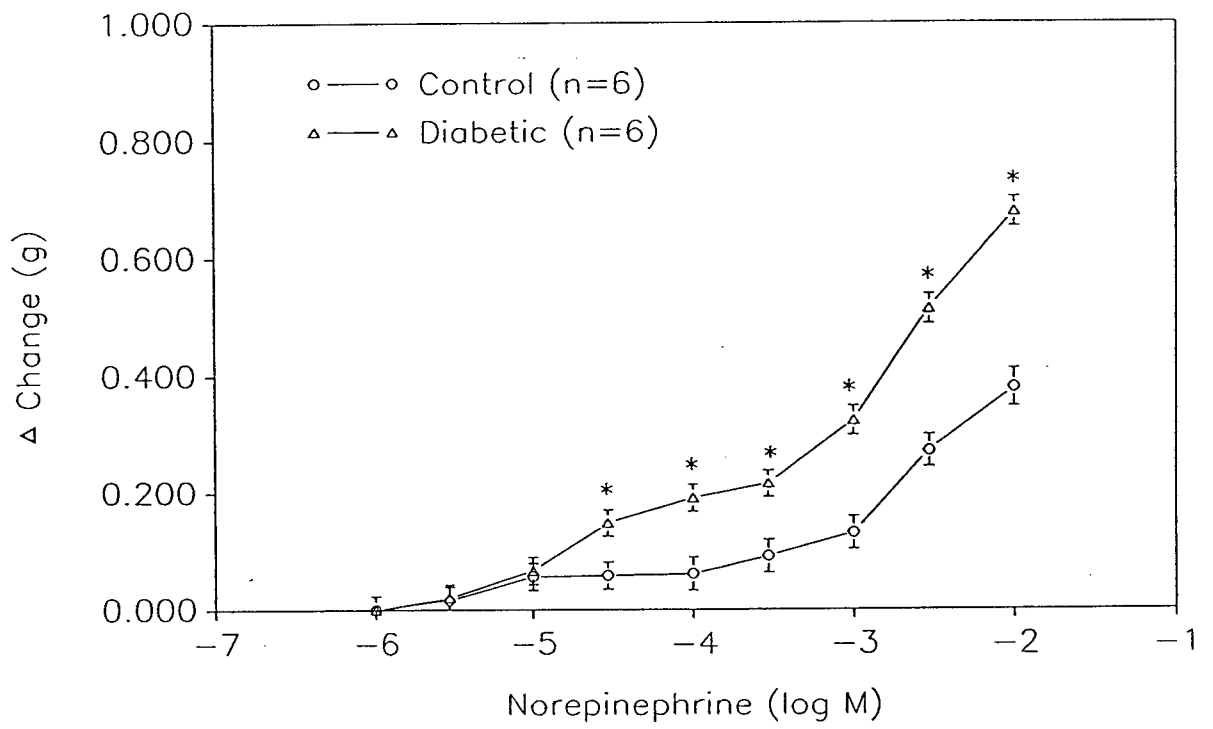## Figure 31

Concentration-response curves for the effect of norepinephrine (in the presence of propranolol) on inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] formation in right ventricles from control and diabetic rats. Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM). Tissues were freeze-clamped when the steady inotropic responses to each concentration of norepinephrine were obtained. Ins(1,4,5)P_3 levels were determined using a protein binding assay kit. The basal values of Ins(1,4,5)P_3 [Ins(1,4,5)P_3 levels in the absence of norepinephrine] over the concentration range studied did not change significantly compared with basal levels shown on the graph. Points represent mean $\pm$ S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, $p < 0.05$.
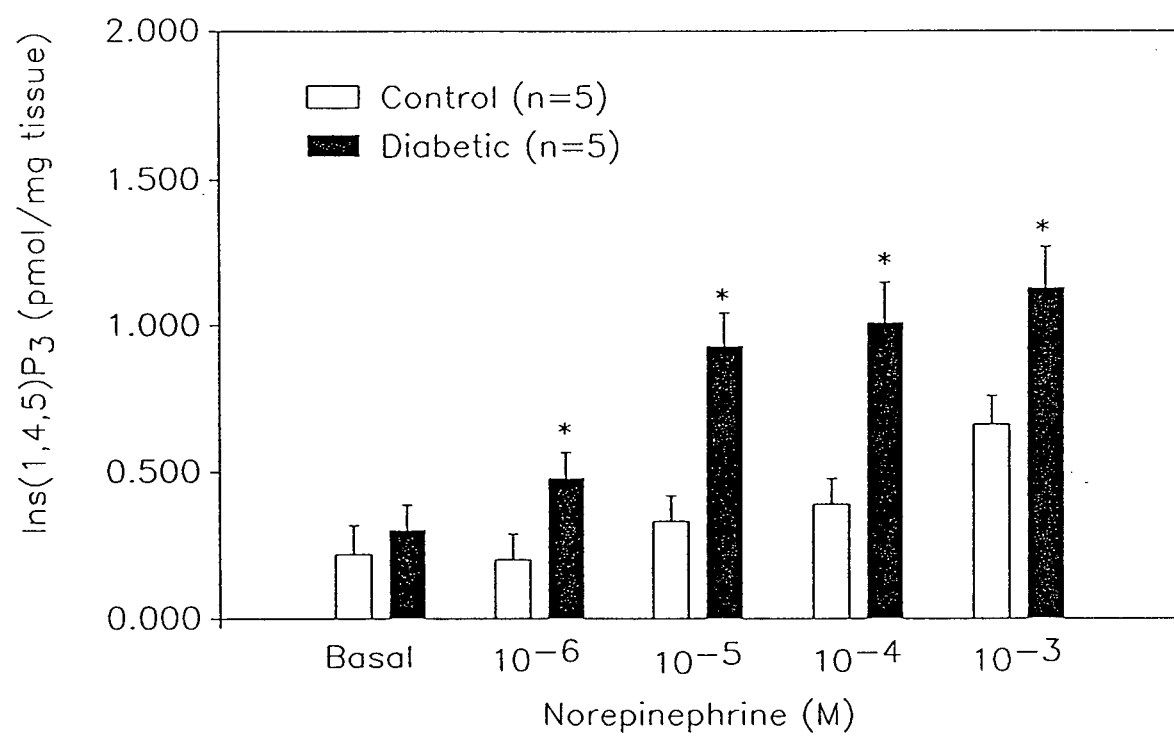
Table 9

% CHANGE IN INOSITOL 1,4,5-TRISPHOSPHATE [INS(1,4,5)P$_3$] FORMATION STIMULATED BY DIFFERENT CONCENTRATIONS OF NOREPINEPHRINE IN CONTROL AND DIABETIC RIGHT VENTRICLES.

	% Change in Ins(1,4,5)P$_3$	
	Control (n=5)	Diabetic (n=5)
10^{-6} M	110±11	165±10*
10^{-5} M	176±46	310±49*
10^{-4} M	195±33	336±15*
10^{-3} M	331±18	376±17*

Norepinephrine (10 μM) was applied to the tissue bath 15 min after addition of propranolol (10 μM). Tissues were freeze-clamped when the steady inotropic responses to each concentration of norepinephrine stimulation were obtained. Ins(1,4,5)P$_3$ was determined using a protein binding assay kit. Values are expressed as mean ± S.E.M. n, number of right ventricles. Basal level=100 %.

* Significantly different from controls; $P<0.05$.

Figure 32

Correlation curves for the concentration-responses of alpha$_1$-adrenoceptor-mediated positive inotropic effect and inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] formation in control and diabetic right ventricles.   r, correlation coefficient.
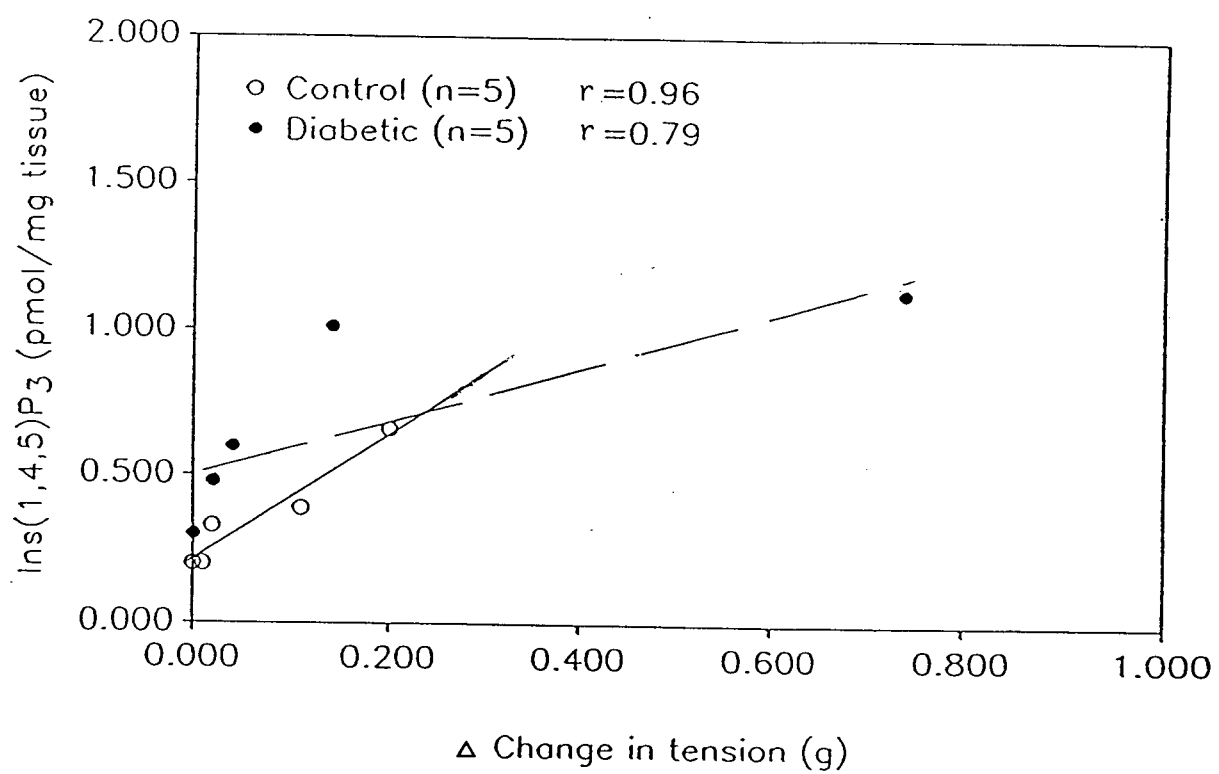
Figure 33

Concentration-response curves for the effect of norepinephrine (in the presence of propranolol) on change in tension (% of maximum) in right ventricles of control and diabetic rats. Influence of the cyclooxygenase inhibitor indomethacin (10 μM). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and indomethacin. At each concentration, the inotropic response to norepinephrine was recorded after it was stabilized which took approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from the other three groups, $p < 0.05$.
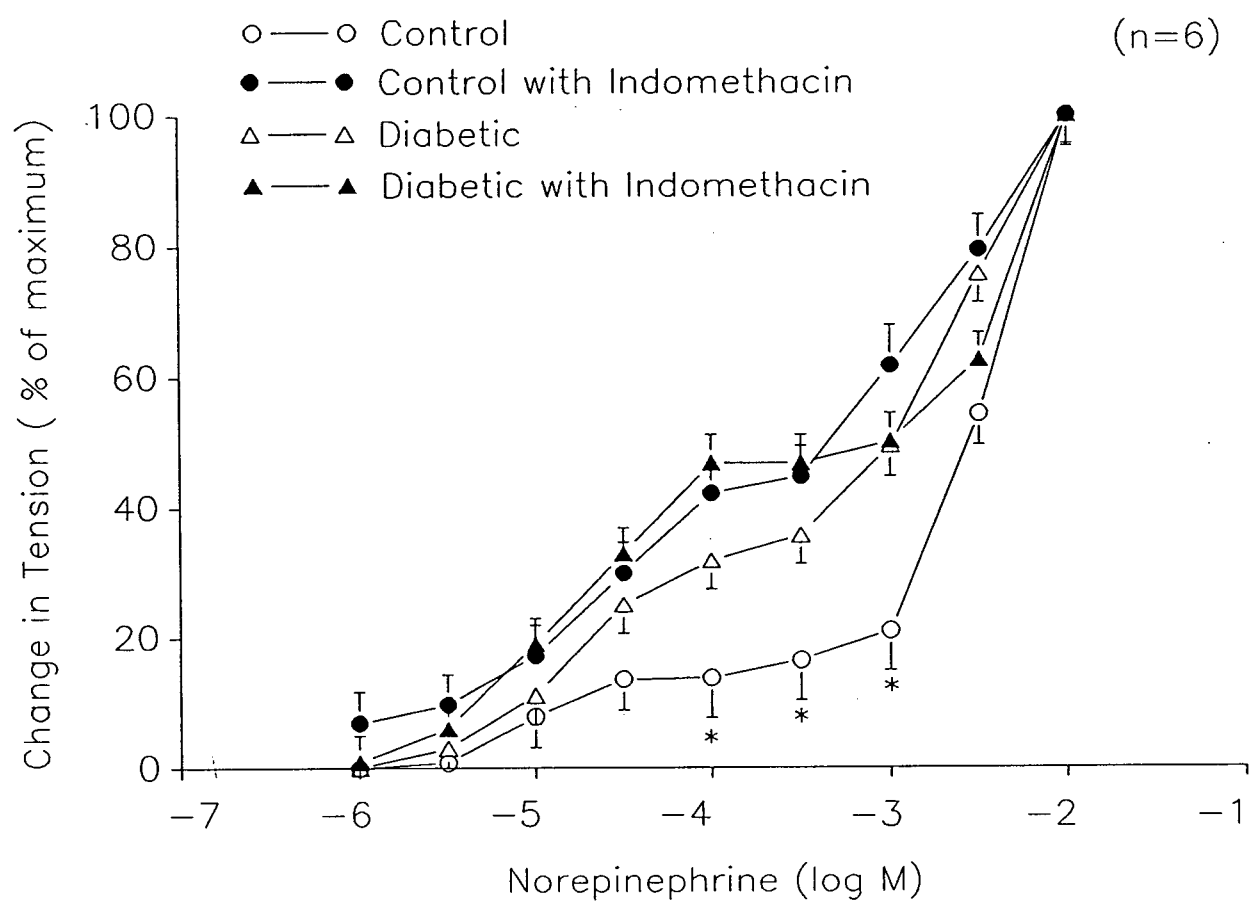
Figure 34

Influence of indomethacin on the estimated PD_2 values for norepinephrine (in the presence of propranolol) in control and diabetic right ventricles. Results are mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from PD_2 values for norepinephrine in control rat ventricles, $p < 0.05$.

PD$_2$

(n=6)

☐ Control
▨ Control with indomethacin
■ Diabetic
⊠ Diabetic with indomethacin
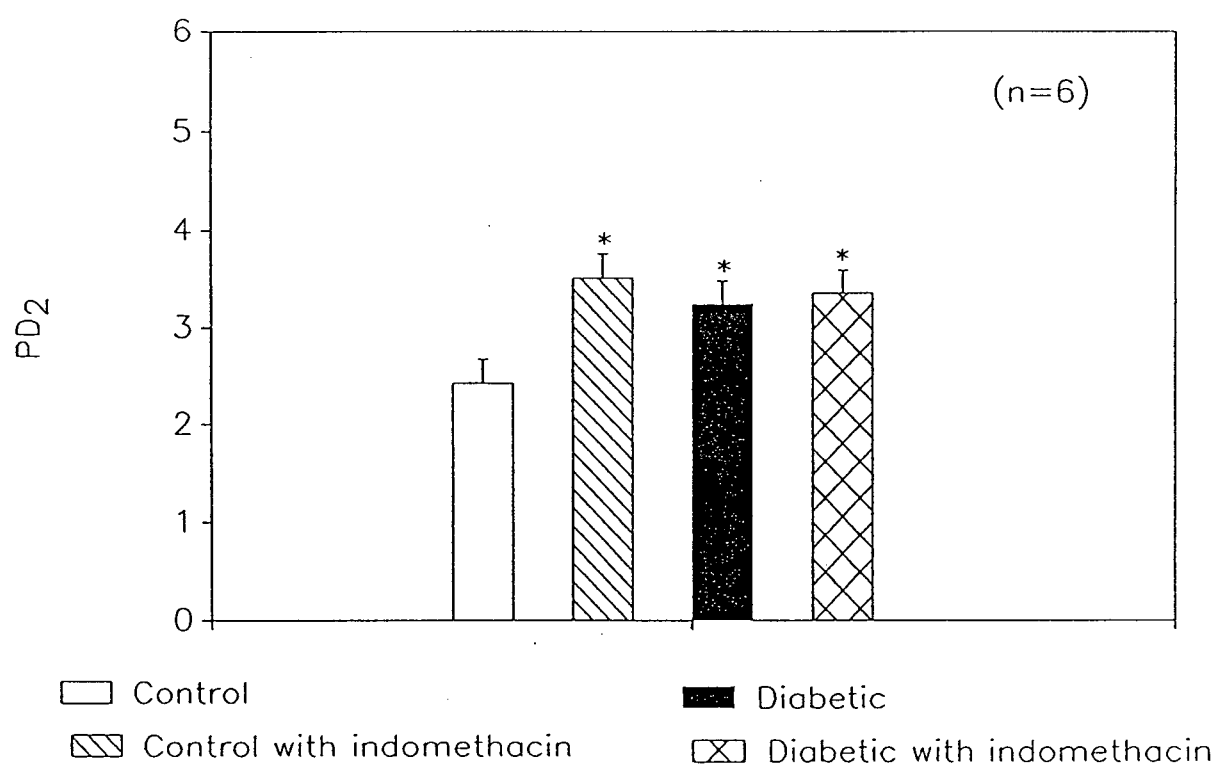
Figure 35

Concentration-response curves for the effect of norepinephrine (in the presence of propranolol) on $\triangle$ change in tension in right ventricles of control and diabetic rats. Influence of indomethacin (10 μM). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and indomethacin. At each concentration, the inotropic response to norepinephrine was recorded after it was stabilized which took approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from the other three groups, $p < 0.05$.

Figure 36

$\triangle$ Change in tension in response to 10^{-2} M of norepinephrine (in the presence of propranolol) in control and diabetic right ventricles.   Influence of indomethacin (10 μM). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and indomethacin.   The inotropic response to norepinephrine was recorded after it was stabilized which took approximately 3 min.   Points represent mean $\pm$ S.E.M. for the number of ventricles shown in parentheses.

*   Significantly   different   from   controls   without indomethacin, $p<0.05$.

Legend:

□ Control　　　　　■ Diabetic

▨ Control with indomethacin　　▨ Diabetic with indomethacin
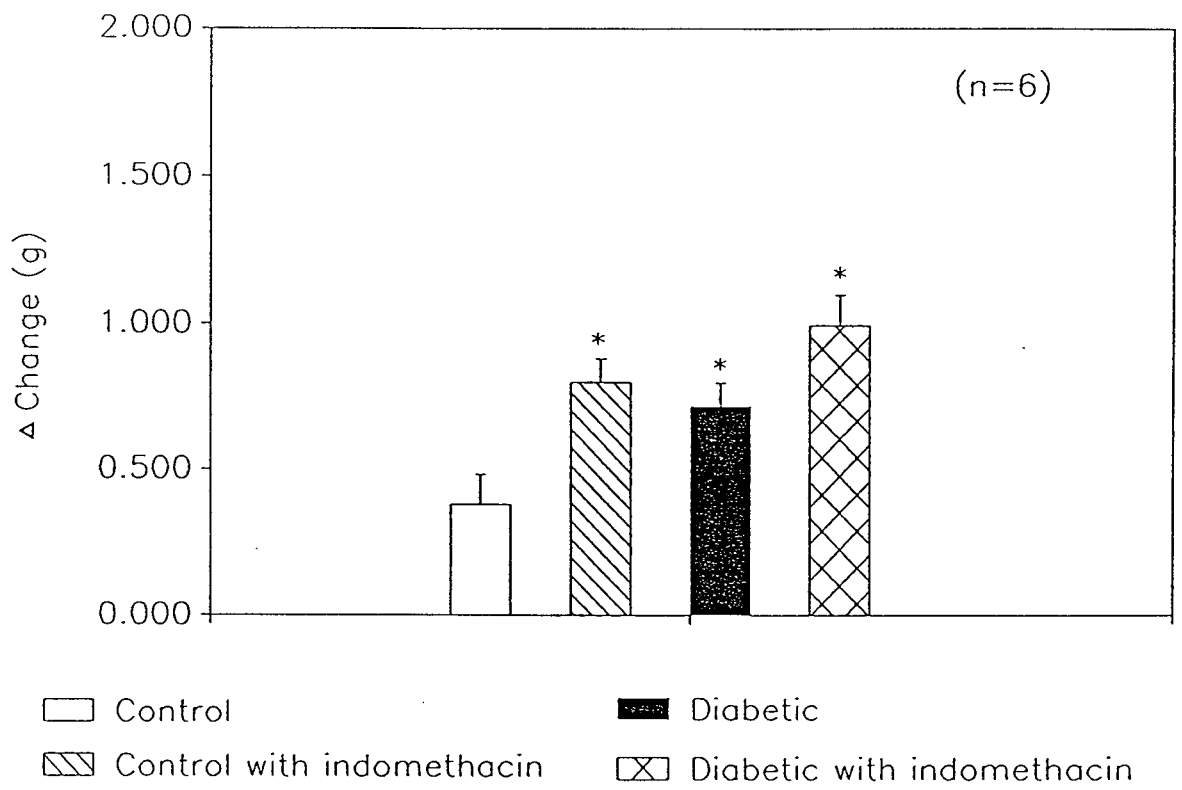
Figure 37

Norepinephrine (10 mM)-stimulated changes in inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] levels in right ventricles of control and diabetic rats. Influence of indomethacin (10 μM). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and indomethacin. At the end of the determination of concentration-response curves, tissues were freeze-clamped and Ins(1,4,5)P$_3$ levels were determined using a protein binding assay kit. The basal Ins(1,4,5)P$_3$ levels [Ins(1,4,5)P$_3$ levels in the absence of norepinephrine] were 0.14 ± 0.02 in the control group, 0.12 ± 0.01 in the control with indomethacin group, 0.13 ± 0.02 in the diabetic group, and 0.12 ± 0.02 in the diabetic with indomethacin group. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls without the inhibitor, $p < 0.05$.
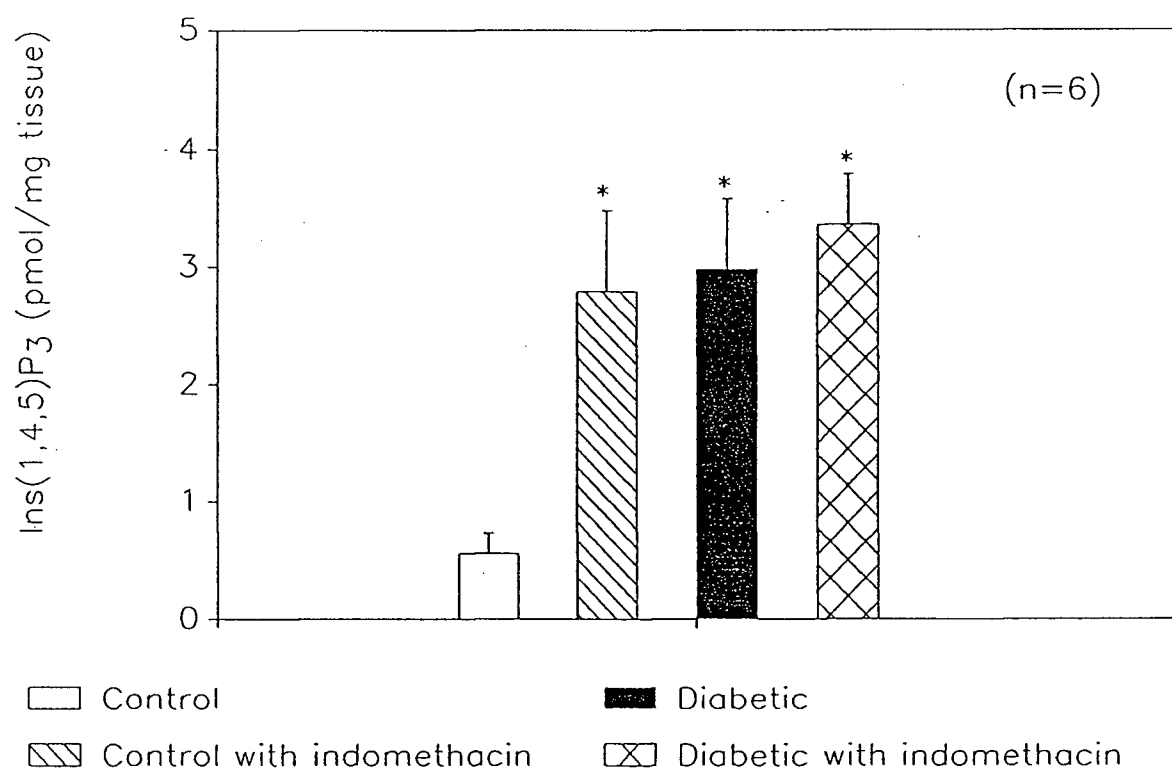
Figure 38

Concentration-response curves for the effect of norepinephrine (in the presence of propranolol) on change in tension (% of maximum) in right ventricles of control and diabetic rats. Influence of the thromboxane synthetase inhibitor imidazole (10 μM). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and imidazole. At each concentration, the inotropic response to norepinephrine was recorded after it was stabilized which took approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from the other three groups, $p < 0.05$.
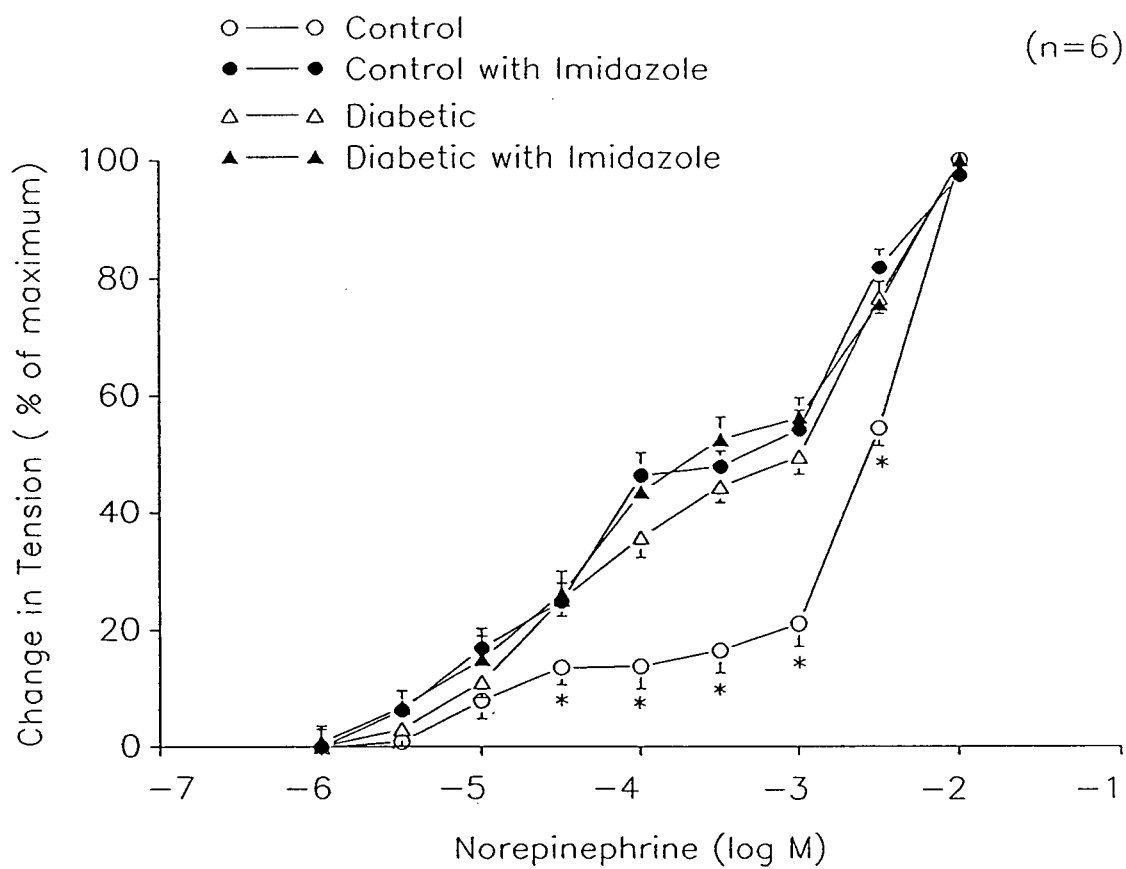
Figure 39

Influence of imidazole on the estimated PD_2 values for norepinephrine (in the presence of propranolol) in control and diabetic right ventricles. Results are mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from PD_2 values for norepinephrine in control rat ventricles, $p<0.05$.

PD_2

$(n=6)$

Control
Control with imidazole
Diabetic
Diabetic with imidazole
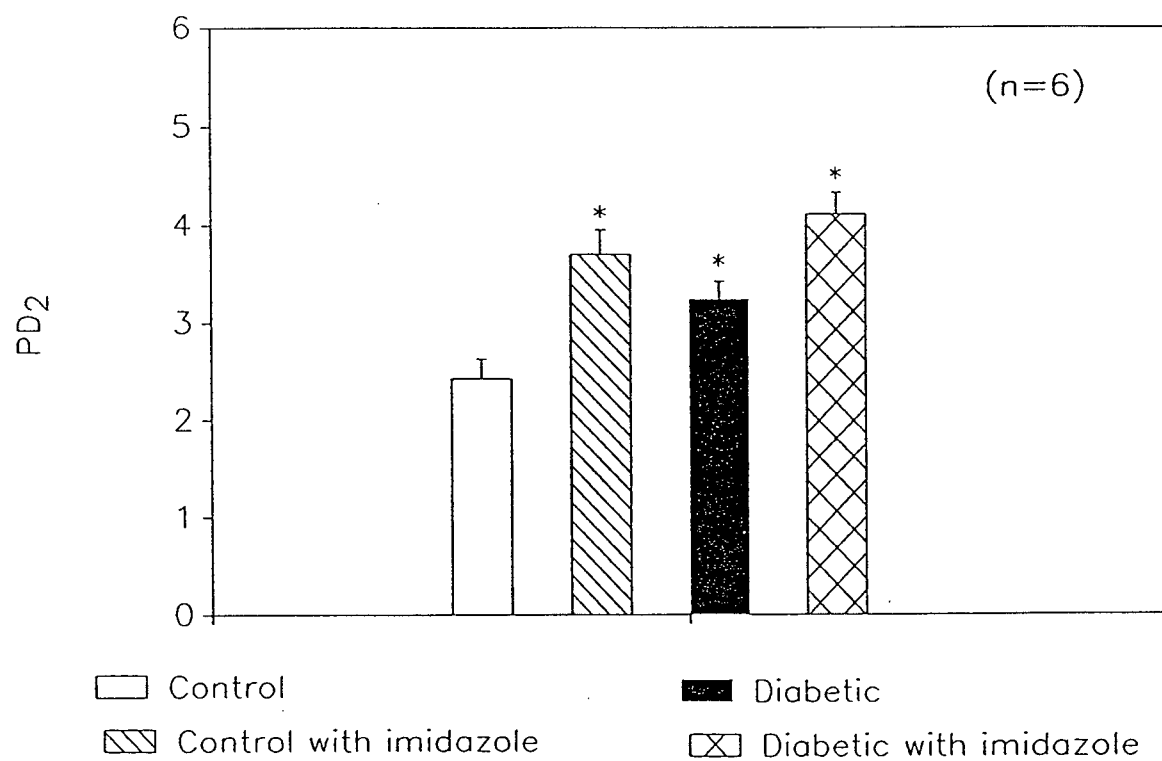
Figure 40

$\triangle$ Change in tension in response to 10^{-2} M of norepinephrine (in the presence of propranolol) in control and diabetic right ventricles. Influence of imidazole. Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and imidazole. The inotropic response to norepinephrine was recorded after it was stabilized which took approximately 3 min. Points represent mean $\pm$ S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls without imidazole, $p < 0.05$.
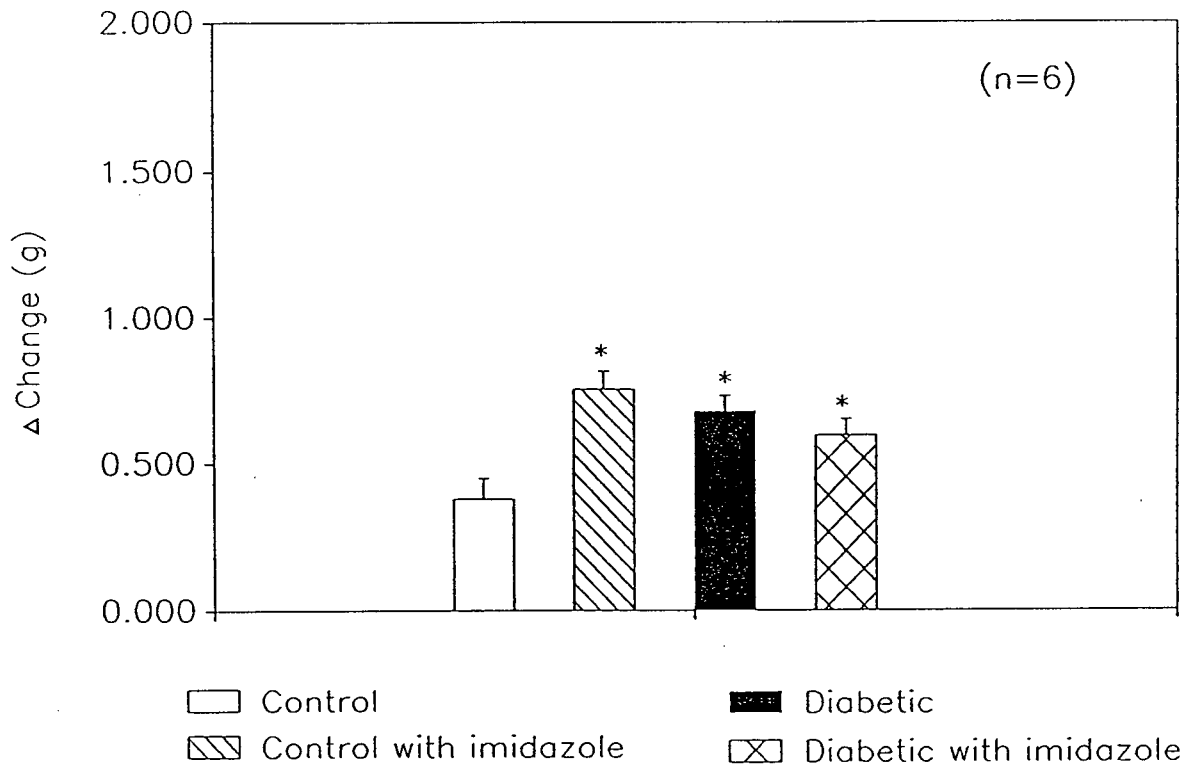
Figure 41

Norepinephrine (10 mM)-stimulated changes in inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] levels in right ventricles from control and diabetic rats. Influence of imidazole (10 μM). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and imidazole. At the end of the determination of concentration-response curves, tissues were freeze-clamped and Ins(1,4,5)P$_3$ levels were determined using a protein binding assay kit. The basal values of Ins(1,4,5)P$_3$ [Ins(1,4,5)P$_3$ levels in the absence of norepinephrine] were 0.14 $\pm$ 0.01 in the control group, 0.13 $\pm$ 0.02 in the control with imidazole group; 0.13 $\pm$ 0.01 in the diabetic group and 0.12 $\pm$ 0.02 in the diabetic with imidazole group. Points represent mean $\pm$ S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from controls, $p < 0.05$.
+ Significantly different from controls with imidazole and diabetic group, $p < 0.05$.
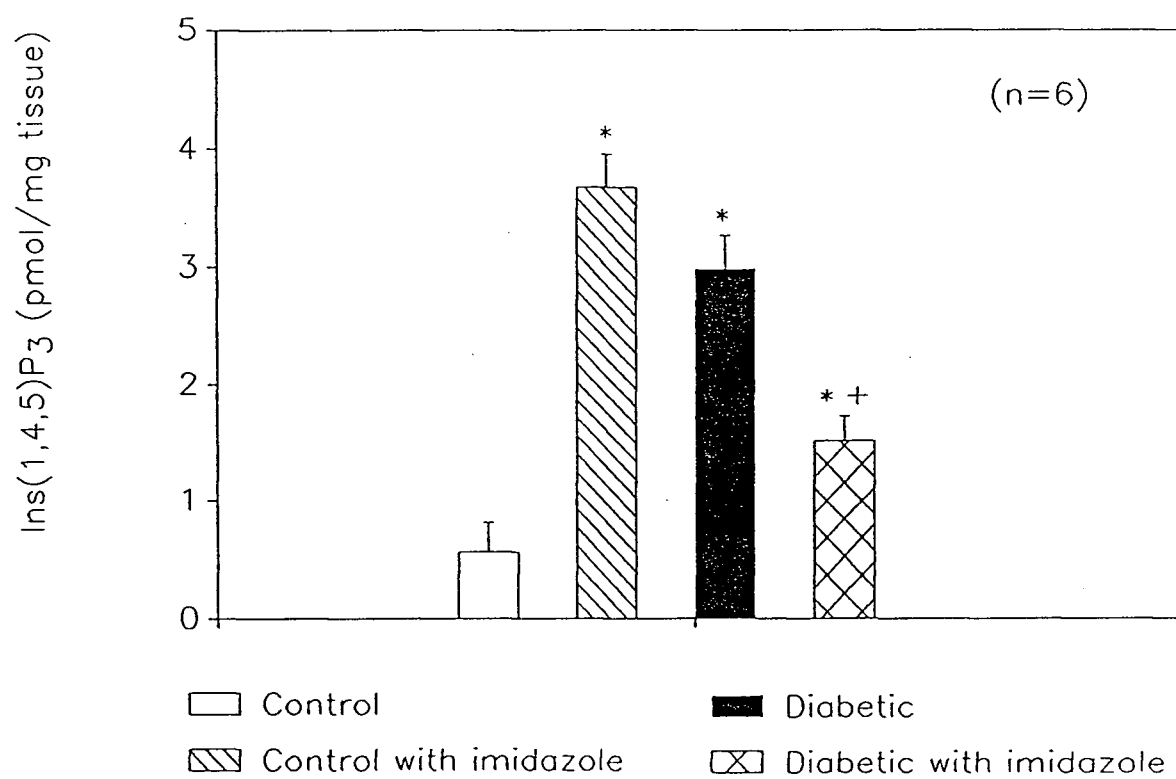
Table 10

**GENERAL FEATURES OF CONTROL AND DIABETIC RATS
6 WEEKS AFTER ADMINISTRATION OF STREPTOZOTOCIN
(Tranylcypromine Study)**

	Control (n=6)	Diabetic (n=6)
Body Weight (g)	457±8	347±8*
Plasma glucose (mmol/l)	7.25±0.61	25.27±0.61*
Plasma insulin (μU/ml)	48±6	26±5*

* Significantly different from controls; $P<0.05$.

Table 11

PLASMA LIPID PROFILE OF THE EXPERIMENTAL RATS
(Tranylcypromine Study)

	Control (n=6)	Diabetic (n=6)
Plasma triglyceride (mmol/l)	1.54±0.39	3.73±0.39*
Plasma cholesterol (mmol/l)	1.70±0.15	2.51±0.16*

* Significantly different from controls; P<0.05.

Figure 42

$\triangle$ Change in tension in response to 10^{-2} M of norepinephrine (in the presence of propranolol) in control and diabetic right ventricles. Influence of the prostacyclin synthetase inhibitor tranylcypromine (1.25 x 10^{-4} M). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and tranylcypromine. The inotropic response to norepinephrine was recorded after it was stabilized which took approximately 3 min. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from the other three groups, $p < 0.05$.

Legend:
- □ Control
- ▨ Control with tranylcypromine
- ■ Diabetic
- ▧ Diabetic with tranylcypromine
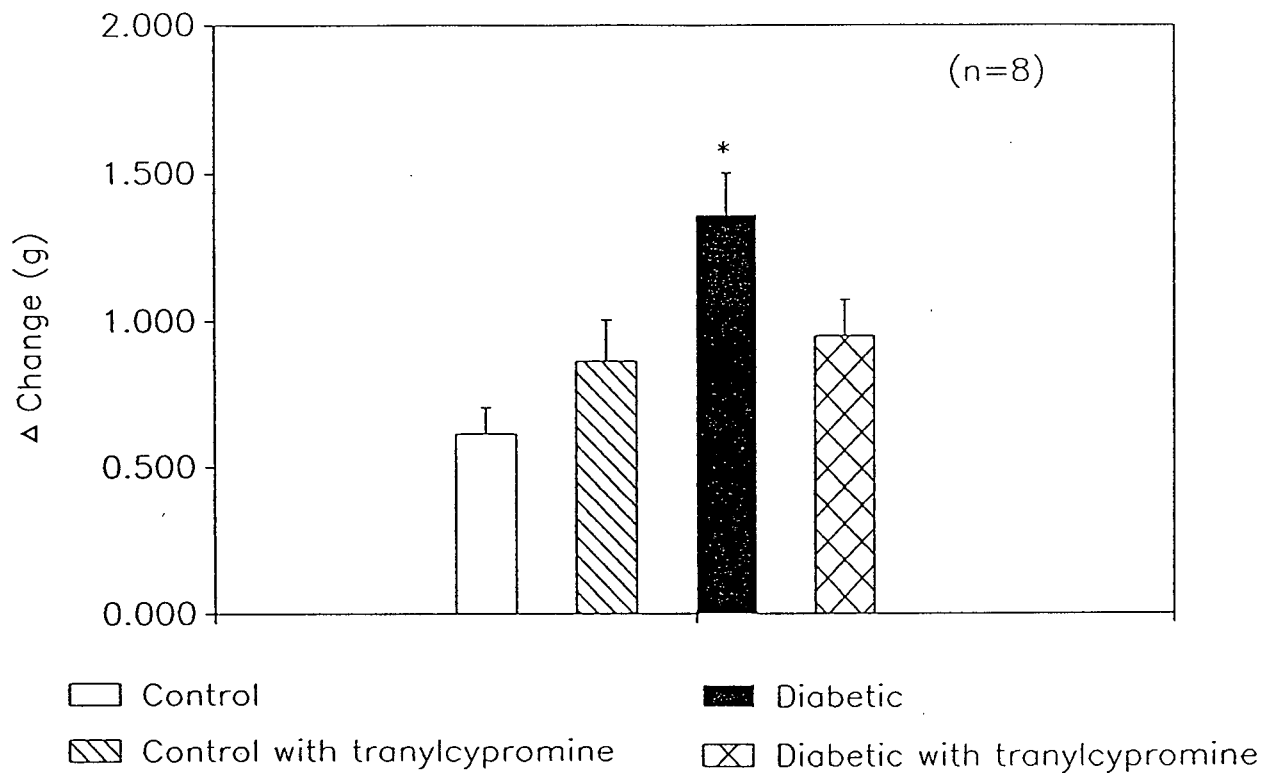
Figure 43

Norepinephrine (10 mM)-stimulated changes in inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] levels in right ventricles of control and diabetic rats. Influence of tranylcypromine (1,25 x 10^{-4} M). Norepinephrine was applied to the tissue bath 15 min after addition of propranolol (10 μM) and tranylcypromine. At the end of the determination of concentration-response curves, tissues were freeze-clamped and Ins(1,4,5)P$_3$ levels were determined using a protein binding assay kit. The basal values of Ins(1,4,5)P$_3$ [Ins(1,4,5)P$_3$ levels in the absence of norepinephrine] were 0.13 ± 0.01 in the control group, 0.12 ± 0.01 in the control with tranylcypromine group, 0.12 ± 0.02 in the diabetic group, and 0.13 ± 0.01 in the diabetic with tranylcypromine group. Points represent mean ± S.E.M. for the number of ventricles shown in parentheses.

* Significantly different from the other three groups, $p < 0.05$.
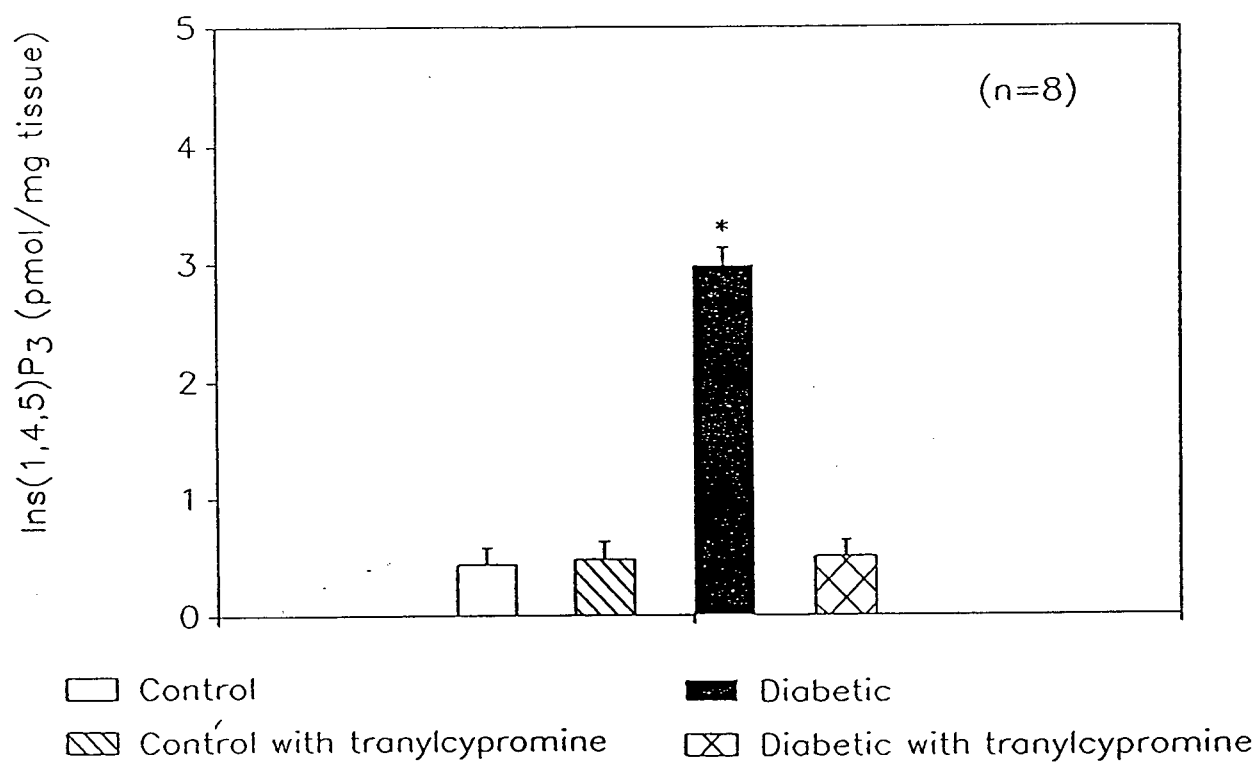
Table 12

**PROTEIN KINASE C ACTIVITY
OF CONTROL AND DIABETIC RAT VENTRICLES
(pmol Pi/mg tissue/min)**

	Control (n=8)	Diabetic (n=8)
Protein Kinase Activity		
Cytosol		
-Ca,-PS,-DG	12.2±2.3	16.1±1.4
+Ca,-PS,-DG	16.2±2.3	16.2±1.4
+Ca,+PS,+DG	38.2±2.3*	20.5±1.4+
Membrane		
-Ca,-PS,-DG	13.7±1.6	20.7±2.6+
+Ca,-PS,-DG	15.5±1.6	20.3±2.6+
+Ca,+PS,+DG	21.5±1.6*	37.8±2.6*+
Protein Kinase C Activity		
Cytosol	22.0±1.9	4.4±1.3+
Membrane	5.9±2.4	17.6±1.8+
Total Activity	27.4±2.9	22.3±2.8

PS, phosphatidylserine.
DG, D-1-stearoyl-2-arachidonyl glycerol.

* Significantly different from "$-Ca^{2+}$,-PS,-DG" values in the same preparations in control or diabetic animals, $p<0.05$.

+ Significantly different from the relative control values, $p<0.05$.

Table 13

PROTEIN KINASE C (PKC) ACTIVITY OF CONTROL AND DIABETIC RAT VENTRICLES WITH OR WITHOUT NOREPINEPHRINE (NE) STIMULATION.

(pmol Pi/mg tissue/min)

	Control		Diabetic	
	With NE (n=4)	Without NE (n=8)	With NE (n=4)	Without NE (n=8)
PK Activity				
Cytosol				
$-Ca^{2+}$,-PS,-DG	11.2±2.1	12.2±2.3	15.2±2.1	16.1±1.4
$+Ca^{2+}$,-PS,-DG	14.3±1.9	16.2±2.3	16.3±2.1	16.2±1.4
$+Ca^{2+}$,+PS,+DG	36.0±2.0*	38.2±2.3*	21.1±2.1+	20.5±1.4+
Membrane				
$-Ca^{2+}$,-PS,-DG	13.0±1.7	13.7±1.6	19.4±2.3+	20.7±2.6+
$+Ca^{2+}$,-PS,-DG	14.5±1.7	15.5±1.6	20.5±2.3+	20.3±2.6+
$+Ca^{2+}$,+PS,+DG	20.0±1.7*	21.5±1.6*	38.6±2.3*+	37.8±2.6*+
PKC Activity				
Cytosol	21.7±1.8	22.0±1.9	4.8±1.5+	4.4±1.3+
Membrane	5.6±1.2	5.9±2.4	17.9±2.0+	17.6±1.8+
Total Activity	27.5±2.1	27.4±2.9	22.8±2.6	22.3±2.8

Norepinephrine (10 mM), where used, was applied to the tissue bath 15 min after addition of propranolol (10 μM). Tissues were frozen in liquid nitrogen for the subsequent measurement of PKC activity on the same day.

PS, phosphatidylserine.
DG, D-1-stearoyl-2-arachidonyl glycerol.

* Significantly different from "$-Ca^{2+}$,-PS,-DG" values in the same preparations in control or diabetic animals, p<0.05.

+ Significantly different from the relative control values, p<0.05.

DISCUSSION

1. Alpha$_1$-adrenoceptor-mediated Inositol Phosphates Formation and Positive Inotropic Effect in the Control Rat Heart

Extensive studies have been made of hormone-stimulated phosphatidylinositol turnover in many different tissues, but detailed studies on alpha$_1$-adrenoceptor-mediated phosphatidylinositol hydrolysis in heart tissue have been limited. The observations presented here demonstrate that stimulation of alpha$_1$-adrenoceptors with norepinephrine increased the formation of IP$_1$ and IP$_2$ (measured by a radioisotope method) and Ins(1,4,5)P$_3$ [measured by a Ins(1,4,5)P$_3$ protein binding assay kit] in rat right ventricles, which is in general agreement with the data from embryonic chick heart cells (Brown and Jones, 1986); neonatal rat cardiomyocytes (Karliner and Simpson, 1986); rat cardiomyocytes (Brown et al., 1985; Kaku et al., 1986); mouse atria (Scherer and Sastre, 1985); rat papillary muscles (Otani et al., 1986); rat ventricles (Poggioli et al., 1986); and intact perfused rat hearts (Woodcock et al., 1987; Woodcock et al., 1988).

The molecular mechanism by which the activated receptor couples to phospholipase C-mediated hydrolysis of phosphoinositide is unknown. Recent evidence (Cockcroft and Gomperts, 1985; Gonzales and Crews, 1985; Straub and Gershengorn, 1986) suggests that a guanine nucleotide-

binding protein (G-protein) regulates phospholipase C activity, a molecular mechanism possibly analogous to that by which the activity of adenylate cyclase is regulated (Gilman, 1984). The hypothesis that a G-protein regulates phospholipase C activity in the heart is supported by the observation that guanosine-5'-O-(3-thiotriphosphate) (GTPγS) and other guanine nucleotides stimulate the accumulation of [^{3}H]IP$_3$ in permeabilized chick heart cells (Jones, 1988). Masters et al. (1985) have previously shown in the intact chick heart cell that pertussis toxin blocks muscarinic receptor-mediated inhibition of adenylate cyclase, but does not block muscarinic stimulation of PI hydrolysis. Pertussis toxin pretreatment also fails to block GTPrS-stimulated IP$_1$ formation in the permeabilized heart. Recently, it was shown that pertussis toxin could not inhibit the alpha$_1$-adrenoceptor-mediated effect on IP$_1$ production in the heart (Schmitz et al., 1987b), nor could it influence the alpha$_1$-adrenoceptor-mediated positive inotropic response (Bohm et al., 1987). The putative G-protein to regulate phosphlipase C in the heart would therefore appear to be a protein other than G$_i$ or G$_o$, both of which can be ribosylated by pertussis toxin (Martin et al., 1985).

Our results showed that norepinephrine (in the presence of propranolol) resulted in the formation of IP$_1$ and Ins(1,4,5)P$_3$ in a time and concentration dependent manner. The increase in Ins(1,4,5)P$_3$ preceded the increase in the

alpha$_1$-adrenoceptor-mediated positive inotropic effect. Moreover, stimulation of alpha$_1$-adrenoceptors increases force of contraction and Ins(1,4,5)P$_3$ approximately to the same extent and all effects were blocked by the alpha$_1$-adrenoceptor antagonist prazosin. This provided evidence that the alpha$_1$-adrenoceptor-mediated increase in Ins(1,4,5)P$_3$ and force of contraction may be related causally. The molecular mechanism of the alpha$_1$-adrenoceptor-mediated positive inotropic effect in the heart is poorly understood. It has been suggested to be partly due to a cAMP-independent increase in transmembrane slow calcium inward current and an increase in calcium sensitivity of the myofibrils. Recently, the involvement of the phosphoinositide pathway with the resulting increase in IP$_3$ in the positive inotropic effect of alpha$_1$-adrenoceptor agonists has aroused great interest. Micromolar concentrations of IP$_3$ have been shown to release calcium from a nonmitochondrial intracellular calcium store in pancreatic cells (Streb et al., 1983). This initial observation has been confirmed for many other permeabilized cells (e.g. hepatocytes, GH$_3$ cells, Swiss 3T3, neutrophils, smooth muscles and macrophages) and microsomes (Berridge and Irvine, 1984; Burgess et al., 1984; Joseph et al., 1984; Dawson and Irvine, 1984). In isolated cardiac (Movsesian et al., 1985) and skeletal muscle (Scherer and Ferguson, 1985) some authors failed to observe an effect of exogenously added IP$_3$ on calcium release from the sarcoplasmic

reticulum. But there is also evidence that IP_3 is indeed able to release calcium from skeletal (Vergara et al., 1985; Volpe et al., 1985) as well as from cardiac sarcoplasmic reticulum (Hirata et al., 1984; Fabiato, 1986; Nosek et al., 1986). Further, there is evidence that IP_3 thereby increases force of contraction in skeletal (Vergara et al., 1985; Volpe et al., 1985) and cardiac muscle (Nosek et al., 1986).

As mentioned above, $alpha_1$-adrenoceptor-stimulated phosphoinositide breakdown has been observed in several different preparations of the heart. However, it was only recently that the relationship between $alpha_1$-receptor-linked phosphoinositide breakdown and the regulation of contractile activity in cardiac muscle has been studied.

The first evidence was provided by Scholz et al. (1987) who showed that stimulation of $alpha_1$-adrenoceptors in rat left auricles increased force of contraction and IP_3 formation to approximately the same extent. Subsequently, it was observed that the increase in IP_3 preceded the $alpha_1$-adrenoceptor-induced increase in force of contraction in cardiac muscle (Schmitz et al., 1987a).

Furthermore, Otani et al. (1988a) showed that $alpha_1$-adrenoceptor stimulation of rat left ventricular papillary muscles by phenylephrine in the presence of propranolol resulted in rapid inositol phosphates formation and a triphasic inotropic response in a concentration-dependent manner. The $alpha_1$-adrenoceptor antagonist prazosin or the

phospholipase C inhibitor neomyocin inhibited PIP_2 hydrolysis and blocked all components of the inotropic responses. Furthermore, they suggest that IP_3 may produce transient inotropic effects by mobilizing intracellular calcium which is supported by the fact that combined addition of 2,3-diphosphoglyceric acid (a competitive inhibitor of IP_3 phosphatase) with phenylephrine doubled the IP_3 formation and potentiated the initial phases of inotropic responses, as well as the fact that the transient inotropic responses were insensitive to calcium entry blockers. On the other hand, diacylglycerol, along with some cofactors, may provoke a sustained positive inotropic effect by potentiating slow calcium channels through activation of protein kinase C (Figure 46). This is evidenced by the fact that 4-phorbol-12,13-dibutyrate (PDBU) at concentrations similar to those in stimulating protein kinase C activity *in vitro* (Nishizuka, 1984a), in combination with caffeine or A23187 resulted in dose-dependent potentiation of an $alpha_1$-adrenoceptor-mediated sustained positive inotropic effect, which was blocked by the calcium channel blockers, nifedipine or Mn^{2+}. The relationship between PI hydrolysis and $alpha_1$-adrenoceptor-mediated positive inotropic effect was also studied by Scholz et al. (1988) using electrically driven rat left auricles. It was shown that the decrease in phosphatidylinositol bisphosphate and increase in IP_3 preceded the increase in force of contraction.

Figure 44

The inositol phospholipid cycle replenishes the supply of second messengers, Ins(1,4,5)P_3 and DG. PLC, phospholipase C; PtdIns, phosphatidylinositol; CDP-DG, cytidine-5′-diphospho-1,2-diacyl-sn-glycerol; DG, diacylglycerol; Ins(1)P, D-inositol 1-phosphate; Ins(4)P, D-inositol 4-phosphate; PtdIns(4)P, phosphatidylinositol 4-phosphate; Ins(1,4)P_2, D-inositol 1,4-bisphosphate; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; Ins(1,4,5)P_3, D-inositol 1,4,5-trisphosphate; Ins(1,3,4)P_3, D-inositol 1,3,4-trisphosphate; Ins(1,3,4,5)P_3, D-inositol 1,3,4,5-tetrakisphosphate.
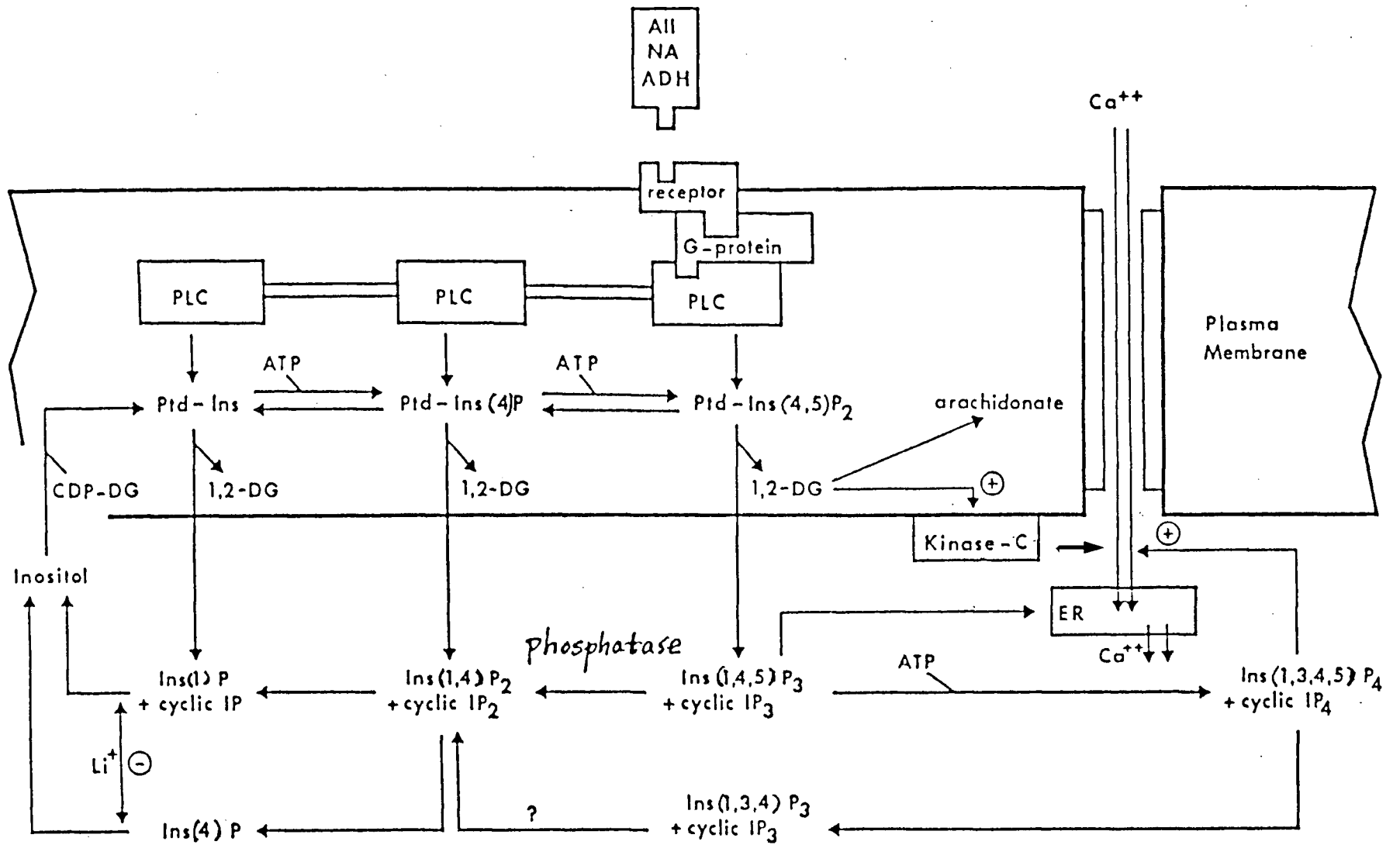
Very recently, Kohl et al.(1989) provided evidence for alpha$_1$-adrenoceptor-mediated increase of IP$_3$ in the nonfailing human heart, which is associated with a similar extent of increase in the force of contraction, further suggesting a physiological role of IP$_3$ hydrolysis in the regulation of myocardial contractility.

A few problems were encountered in the measurement of inositol phosphates with the radioisotope method. First, with the specific activity of the myo[2-^{3}H]inositol (4 μCi/mmol) employed in our study, large standard errors and very low activities of IP$_3$ and IP$_4$ were found. Secondly, unless high performance liquid chromatography (HPLC) separation of inositol phosphates is used, any quantitation of the IP$_3$ fraction may consist of two isomers, Ins(1,4,5)P$_3$ and Ins(1,3,4)P$_3$ (Irvine et al., 1984), and also may contain their cyclic forms (Wilson et al., 1985) as well as the Ins(1,3,4,5)P$_3$ (Batty et al., 1985). In fact, Ins(1,3,4)P$_3$ accumulation predominates over Ins(1,4,5)P$_3$. Therefore, [^{3}H]IP$_3$ accumulation is a poor index of Ins(1,4,5)P$_3$ formation. Thirdly, there are problems with agonist-stimulated incorporation of [^{3}H]inositol into the phosphoinositides (Chilvers et al., 1988). Fourthly, the radioisotope method has proven to be arduous and time-consuming.

Since only Ins(1,4,5)P$_3$ has been demonstrated to release Ca^{2+} in several tissues whereas other inositol phosphates were ineffective in this respect, it is necessary

to specifically measure Ins(1,4,5)P_3. Therefore, we employed an Ins(1,4,5)P_3 protein binding assay kit in our study. The method provides a simple, rapid, sensitive and specific assay for the measurement of intracellular Ins(1,4,5)P_3. The Ins(1,4,5)P_3 protein binding assay kit was developed using a microsomal preparation of bovine adrenal cortex which demonstrates Ins(1,4,5)P_3-specific binding (Palmer et al., 1989). The microsomal preparation has been used to quantitate Ins(1,4,5)P_3 in acid extracts of rat hepatocytes. Direct evidence for the presence of high affinity, intracellular Ins(1,4,5)P_3 binding sites has been obtained in a number of systems - bovine adrenal cortex homogenate (Baukal et al., 1985), permeabilised rabbit neutrophils (Spat et al., 1986), permeabilised guinea pig hepatocytes (Spat et al., 1986), liver microsomal fractions and plasma membrane fractions (Guillemette et al., 1987), bovine adrenal cortex microsomes (Guillemette et al., 1987), bovine anterior pituitary microsomes (Guillemette et al., 1987), rat cerebellar membranes (Willcocks et al., 1987), and rat cerebral cortex membranes (Lo and Hughes, 1987). In addition, Willcocks et al. (1987) have shown that the sites in rat cerebellum are stereospecific for Ins(1,4,5)P_3. More recently, the purification of an Ins(1,4,5)P_3 binding protein, which may be the receptor from rat cerebella, has been reported (Supattapone et al., 1988).

A prerequisite for a second messenger role of Ins(1,4,5)P_3 in the alpha$_1$-adrenoceptor-mediated increase in

force of contraction is that the norepinephrine-induced increase in $Ins(1,4,5)P_3$ should precede the increase in force of contraction. As shown in Figure 30 and 31, whereas the increase in $Ins(1,4,5)P_3$ could already be detected at 15 sec the increase in force of contraction did not start before 45 sec. Thus, it is evident that the increase in $Ins(1,4,5)P_3$ preceded the increase in force of contraction.

Norepinephrine seemed to be more potent in increasing force of contraction (effect significant at 10^{-5} M; Figure 33) than in enhancing $Ins(1,4,5)P_3$ in which a pronounced and significant effect was observed only at 10^{-3} M norepinephrine (Figure 34). This, however, does not necessarily suggest a dissociation between both parameters but may indicate that, if both events are related causally, a relatively small increase in $Ins(1,4,5)P_3$ is capable of producing a relatively great increase in contractile force.

We carried out experiments to study PI hydrolysis by using isolated rat hearts, in which $alpha_1$-adrenoceptors are relatively rich (Mukherjee et al., 1983; Bristow et al., 1988). A potent alpha-adrenoceptor antagonist, [3H]-dihydroergocryptine ([3H]DHE), has been shown to bind to the particulate membrane fraction derived from the rat myocardium (Williams and Lefkowitz, 1978; Wei and Sulakhe, 1979) at sites which have the characteristics expected of alpha-adrenoceptors. In addition, [3H]WB4101 has been reported to selectively label postsynaptic $alpha_1$-adrenoceptors in the rat heart (Yamada et al., 1980). In

fact, although there is a substantial amount of physiological evidence to support the presence of alpha-receptors in the hearts of various species - including rat, guinea pig, rabbit, and dog - to the present, most of the binding studies have been done in rat (Mugge et al., 1985) and guinea pig hearts (Karliner et al., 1979). Furthermore, it was shown that rat cardiac ventricular tissue contains a relative larger number of $alpha_1$-adrenoceptors than guinea-pig ventricular muscle (Rugevics and Schumann, 1987).

It is important to note that there may be a wide range of species variation in the extent of PI elevation after myocardial alpha-adrenoceptor activation, since in the rabbit papillary muscle IP turnover is increased much less than that in the rat heart by activation of alpha adrenoceptors (Hiramoto and Endoh, 1988).

Ventricular tissue of the rat was employed in the present study and may offer several advantages. First, rat ventricular alpha-adrenergic receptor density is greater than in the atrial region (Wei and Sulakhe, 1979). Therefore, alpha-adrenergic receptor-mediated effects of catecholamines may be more readily seen with the rat ventricle compared to atrial preparations. Secondly, compared with atrial muscle, ventricular preparations are less affected by the variations in experimental conditions (Endoh, 1982).

In the measurement of inositol phosphates formation using the radioisotope method, lithium was employed, which

is based on the report by Berridge et al. (1982) that IP_3 turnover is very rapid and that lithium, by inhibiting inositol 1-phosphatase, could be used as an amplification technique to facilitate the measurement of phosphoinositide products. In fact, without lithium, no or only small stimulatory effects of agonist on IP_3 content are detectable (Batty and Nahorski, 1985). The rapid generation of IP_3 in tissues and its degradation by sequential dephosphorylation to inositol is, in particular, characteristic of a cellular second messenger (Nohorski and Batty, 1986). On the other hand, a dissociation between an increase in IP_3 and an increase in force of contraction with the addition of lithium has been suggested because it appears that the presence of lithium is required to produce any measurable increase in inositol phosphate formation induced by phenylephrine. But lithium did not enhance the positive inotropic effect of phenylephrine (Scholz et al., 1988).

Based on the published data on a variety of tissues (e.g. brain and salivary glands)(Berridge et al., 1982) including the heart (Brown and Brown, 1983; Scholz et al., 1988), a concentration of 10 mM lithium and an incubation time of 15 minutes were employed in the radioisotope method because at this concentration lithium itself had only a small effect on force of contraction, but made the $alpha_1$-adrenoceptor-mediated stimulation of PI turnover easily detected. Higher concentrations of lithium and longer times of incubation with lithium suppressed the phenylephrine-

induced increase in inositol phosphates (Batty and Nahorski, 1985), suggesting a complex effect of lithium on phosphoinositide metabolism.

2. Changes in $Alpha_1$-adrenoceptor-induced Phosphoinositide Breakdown and Positive Inotropic Effect in the Diabetic Rat Heart

It is now well known that there are abnormalities in the myocardium in experimental as well as in clinical human diabetes. The mechanism(s) involved in this diabetic cardiomyopathy is still not clear, but one defect appears to occur in the $alpha_1$-adrenoceptor system. In diabetes, it has been reported that both the sensitivity and responsiveness of the diabetic myocardium to alpha adrenoceptor agonists are altered. Generally, increased sensitivity and responsiveness of atrial (Jackson et al., 1986; Goyal et al., 1987) and ventricular preparations (unpublished results from our laboratory) in response to $alpha_1$-adrenoceptor agonists have been reported in diabetes, although a decrease in the contractile force generation by methoxamine in isolated papillary muscles has also been shown (Heyliger et al., 1982). We found that the diabetic right ventricles were supersensitive (increase in PD_2 values) and showed a higher maximum response to norepinephrine stimulation when compared with controls, which is in accord with previous observations from most authors.

It is now well known that stimulation of $alpha_1$-adrenoceptors causes the hydrolysis of phosphoinositides in a variety of tissues including the heart. It is therefore possible that changes in $alpha_1$-adrenoceptor stimulated phosphoinositides turnover in the diabetic heart may be involved in the changes in the responsiveness to $alpha_1$-adrenoceptor stimulation. Results from our study show that norepinephrine induced a time- and concentration-dependent increase in $Ins(1,4,5)P_3$ content in both control and diabetic myocardium. $Ins(1,4,5)P_3$ levels in the diabetic heart were significantly higher than in the control heart. Since $Ins(1,4,5)P_3$ has been suggested to be one of the important intracellular second messengers, the data may indicate the involvement of $Ins(1,4,5)P_3$ and, possibly, the resulting increase in Ca^{2+} release in the supersensitivity of the diabetic myocardium to alpha-adrenoceptor agonists. The increase in $Ins(1,4,5)P_3$ may exacerbate Ca^{2+} overload in the diabetic state which could contribute to the development of diabetic cardiomyopathy. These postulated effects of $Ins(1,4,5)P_3$ and Ca^{2+} on the diabetic heart require further investigation.

As suggested by Otani et al. (1988), $Ins(1,4,5)P_3$ may only be able to produce transient inotropic effects by mobilizing intracellular Ca^{2+}, while DG may provoke a sustained positive inotropic effect by activating slow Ca^{2+} channels through stimulation of protein kinase C. Protein kinase C is present in the heart (Kuo et al., 1980; Wise et

al., 1982a), and a variety of myofibrillar, sarcoplasmic reticular and plasma membrane proteins are putative substrates for this enzyme (Lim et al., 1985; Lindemann, 1986). We therefore measured diacylglycerol-stimulated protein kinase C activity in the diabetic heart. Results showed that the diabetic heart had a higher activated protein kinase C acitvity in the membrane fraction compared with controls, accompanied by a decrease in cytosolic protein kinase C activity. Moreover, total enzyme activity of the soluble plus membrane fraction did not differ between the hearts from diabetics and controls when expressed on the basis of tissue weight. Thus, the increase in membranous protein kinase C appeared to be due to translocation of the enzyme from the cytosolic to the membrane fraction as suggested by May et al. (1985). Nevertheless, alternative interpretations of these findings must be considered. Several isoenzymic forms of protein kinase C have been described that differ in their tissue distribution and subcellular localization (Yoshida et al., 1988). Each isozyme is known to be encoded by a different gene and thus may be differentially expressed. We cannot exclude the possibility that the shift in subcellular distribution of protein kinase C that we observed in the hearts from diabetic rats reflects an altered pattern of protein kinase C isozyme expression. The observed increase in the membranous protein kinase C activity has also been shown in diabetic nerve. Also, Lee et al. (1989) have reported that

the membranous pool of protein kinase C activity was increased 100% by elevation of the glucose level in cultured bovine retinal capillary endothelial cells.

Recent models envision the activated protein kinase C as a component of a quaternary complex consisting of the enzyme, Ca^{2+}, acid phospholipid (such as phosphatidylserine) and DG, presumably associated with a cellular membrane structure (Garong et al., 1986). Thus, the observed increase in membranous protein kinase C in the diabetic heart may be due to increased membranous DG, Ca^{2+} and/or acidic phospholipid levels. It might be postulated that the increased responsiveness of the diabetic heart to $alpha_1$-adrenoceptor stimulation may enhance the activity of phospholipase C, resulting in the increase in phosphoinositides turnover and the increase in the level of DG which is known to be an important second messenger to activate protein kinase C. In fact, an increase in DG level in the hearts of diabetic Wistar rats has recently been reported (Okumura et al. 1988). Nevertheless, the relationship between the increase in responsiveness to $alpha_1$-adrenoceptor stimulation and the increase in DG formation in the diabetic heart requires further investigation. DG, in addition to being derived from the breakdown of phosphoinositides, can also be synthesized *de novo* from the glycolytic intermediates dihydroxyacetone phosphate and glycerol 3-phosphate (Peter-Riesch et al., 1988) by stepwise acylation. Hyperglycemia in diabetes may

therefore enhance the glycolytic pathway with a resulting increase in DG levels which further stimulates protein kinase C. There is also the possibility of an effect of glucose to enhance phospholipase C-mediated hydrolysis of phosphatidylcholine (Besterman et al., 1986), or phospholipase C-induced hydrolysis of inositol phospholipids (Dunlop et al., 1984). This is supported by the fact that elevation of glucose concentration can cause an elevation of membranous protein kinase C activity in cultured bovine retinal capillary endothelial cells (Lee et al., 1989). However, insulin treatment failed to normalize DG content in the diabetic myocardium (Okumura et al., 1988). Therefore, it is not likely that DG is activated by an increase in glucose levels in the diabetic heart.

Diabetes mellitus is associated with an altered Ca^{2+} disposition. It has been shown that the ability of the sarcoplasmic reticulum to take up Ca^{2+} is impaired in the diabetic heart (Penpargkul et al., 1981; Lopaschuk et al., 1983). Glucose has been shown to increase cytosolic Ca^{2+} in pancreatic islets (Nilsson et al., 1988) and recent evidence suggests that an increase in cytosolic Ca^{2+} alone may be sufficient to trigger protein kinase C activation (Ho et al., 1988). There remains the possibility that increases in cytosolic Ca^{2+} in diabetes may contribute to the observed activation of protein kinase C.

Whatever the mechanisms by which protein kinase C is activated in the heart of diabetic rats, this finding is of

considerable interest and potential importance. The increase in protein kinase C activity associated with the membrane fraction, an index of enzyme activation (Kim et al., 1988), may be involved in the sustained increase in the positive inotropic effect of the diabetic heart. However, since $alpha_1$-adrenoceptor stimulation did not further increase protein kinase C activity in these hearts, the involvement of protein kinase C in the supersensitivity of the diabetic hearts to $alpha_1$-adrenoceptor stimulation requires further investigation.

The mechanisms involved in the supersensitivity of the diabetic heart to $alpha_1$-adrenoceptor stimulation and in the increased inositol phosphates formation are still unclear. A number of possibilities could be forwarded. Firstly, since treatment of diabetic rats with T_3 were able to abolish the increased sensitivity to alpha-agonists (Goyal et al., 1987), changes in alpha-adrenoceptors may be the result of hypothyroidism. Secondly, the supersensitivity to alpha-agonist could be due to high activity of phospholipase C with an increase in DG production and protein kinase C activity. This is supported by the fact that, in the acute diabetic state, inhibition of phospholipase C blocked the ventricular response to methoxamine in control as well as in diabetic hearts. Synthetic DG potentiated the inotropic action of the alpha-agonist in control ventricles. On the other hand, inhibition of protein kinase C partially reduced the supersensitivity to alpha-agonists in diabetic

ventricles and prevented the stimulatory action of DG on the positive inotropic effect of methoxamine in control ventricles (Wald et al., 1988). Thirdly, recent evidence indicates that a rise in cytosolic Ca^{2+} can also activate phospholipase C (Eberhard and Holz, 1988). It is known that there is a decreased uptake of Ca^{2+} into diabetic SR (Penpargkul et al., 1981). The resulting increase in intracellular Ca^{2+} could then activate phospholipase C. Fourthly, in diabetes the response to alpha-adrenoceptor stimulation could be mediated by oxidative products generated from arachidonic acid metabolism.

With the radioisotope method, however, we showed a decrease in IP_1 and IP_2 formation in the diabetic heart compared with controls. This appears to contradict the results obtained with the use of $Ins(1,4,5)P_3$ protein binding assay kits. There are several possible explanations for this discrepancy. Firstly, the radioisotope method is not as sensitive a method as the $Ins(1,4,5)P_3$ protein binding assay kit and the measurement may thus not be as accurate. Secondly, there could be a reduced metabolism from $Ins(1,4,5)P_3$ to IP_1 in the diabetic state. In insulin-secreting ß-cells, it has been shown that endogeneous rate of $Ins(1,4,5)P_3$ production is not fast enough to account for the rapid increased rate of $Ins(1,4,5)P_3$ formation that has been measured in response to glucose (Turk et al., 1986). Therefore it has been suggested that some of the glucose metabolites, particularly 2,3-diphosphoglyceric acid and

fructose 1,6-bisphosphate, which increase markedly in the presence of glucose, may act to inhibit $Ins(1,4,5)P_3$ 5-phosphatase that degrades $Ins(1,4,5)P_3$ (Rana et al., 1986). It is possible that the increased glucose level as well as its metabolites in the diabetic heart may inhibit $Ins(1,4,5)P_3$ 5-phosphatase, which may then result in an increase in $Ins(1,4,5)P_3$ and a decrease in IP_2 and IP_1 levels.   Thirdly, IP_1 can also be produced from the breakdown of PtdIns and $PtdIns(4)P$ (via IP_2).   Similarly, IP_2 can also be produced directly from the breakdown of $PtdIns(4)P$.   It is possible that, in diabetic hearts, the breakdown of $PtdIns(4,5)P_2$ is much greater than the breakdown of $PtdIns(4)P$ and PtdIns, which could result in an increase in $Ins(1,4,5)P_3$ formation with a decrease in IP_2 and IP_1 formation.

3.   Effects of Omega-3 Fatty Acid Treatment on Cardiac $Alpha_1$-adrenoceptor-mediated Inositol Phosphates Formation

We found that omega-3 fatty acid supplementation had no effect on the plasma glucose level of STZ-diabetic rats compared with the untreated diabetics.   Plasma glucose levels in the treated nondiabetics were also not affected which is in accord with previous observations by Black et al. (1988).   There are however also reports describing a decrease in plasma glucose level (Popp-Snijders et al., 1987) or worsening of hyperglycemia (Friday et al., 1987;

Kasim et al., 1988) by omega-3 fatty acid treatment in type II diabetic patients.

We also found that omega-3 fatty acid treatment decreased plasma triglyceride levels in diabetic rats compared with untreated diabetics. This response is similar to that observed in nondiabetic normolipidemic (Sanders and Roshanai, 1983) and nondiabetic hypertriglyceridemic individuals (Sanders et al., 1985). In the present study, omega-3 fatty acid supplementation did not affect plasma cholesterol level of the diabetic rats. Kasim et al. (1988) have shown similar results. However, the data are in contrast with previous studies (Harris et al., 1983; Phillipson et al., 1985) which reported decreases in serum cholesterol and triglyceride levels during omega-3 fatty acid treatment. However, these previous studies were carried out in patients with either familial hypertriglyceridemia or familial combined hyperlipidemia. There are also reports that LDL cholesterol is increased by omega-3 fatty acid supplementation in subjects with hyperlipidemia (Simons et al., 1985) or insulin-dependent diabetes (Haines et al., 1986). Although a suitable explanation for the variable effects of omega-3 fatty acid supplementation on plasma cholesterol concentrations cannot at this time be provided, the variable response to omega-3 fatty acids may relate to the population studied, the dose and/or duration of supplementation.

The decrease in serum triglyceride levels observed in the present study could be attributed to decreased hepatic triglyceride synthesis (Nestel et al., 1984), and/or accelerated triglyceride clearance due to increased LPL activity (Kasim et al., 1988).

An important finding was that some of the changes during omega-3 fatty acid supplementation were persistent, e.g. those on blood pressure and serum apo B levels, whereas others were transient, e.g. plasma LPL activity (Kasim et al., 1988). A previous study (Simons et al., 1985) reported that the most prominent changes in the lipid profile during omega-3 fatty acid treatment were in the early phase of the study. Currently, most of the studies in the literature are limited to 4-6 weeks duration. An obvious question is the degree of compliance. Therefore, it is possible that the long term effects of omega-3 fatty acid may differ from their short term effects.

Our results show that dietary omega-3 fatty acid supplementation had no effect on cardiac $alpha_1$-adrenoceptor-mediated inositol phosphates production in diabetic or non-diabetic Wistar rats. This could be related to several factors. It is known that, in relative terms, the arachidonic acid content is decreased and omega-3 fatty acid content is often increased in the tissue phospholipids of diabetic rats (Holman et al., 1983). If this is the case, additional supplementation with omega-3 fatty acids may be of little benefit. Secondly, potentially deleterious

effects of omega-3 fatty acids have been reported, including increased plasma glucose (Friday et al., 1987; Kasim et al., 1988), glycosylated hemoglobin (Glauber et al., 1988; Schectman et al., 1988), plasma total cholesterol and low density lipoprotein cholesterol (Haines et al, 1986; Stacpoole et al., 1988). These adverse effects of omega-3 fatty acids have almost invariably been achieved with large doses (4-10 g/day). In the present study, a relatively small dose of omega-3 fatty acid was employed. However, the dose of omega-3 fatty acids that will yield the optimal ratio of beneficial to deleterious effects is unknown. Whether small quantities taken over a prolonged period will produce the same effects as large quantities consumed over a brief period is also unknown. It is possible that the potential beneficial effects of the omega-3 fatty acid treatment employed in the present study was negated by putative adverse effects of the omega-3 fatty acid treatment, such that a favourable effect of omega-3 fatty acid on $alpha_1$-adrenoceptor-stimulated PI turnover in the diabetic heart was prevented. Thirdly, it may be difficult to predict the effect of omega-3 fatty acids on the diabetic heart and its metabolism, because the pathogenesis of these complications involves multiple variables.

In summary, we have shown that dietary omega-3 fatty acid supplementation did not alter the reduced $alpha_1$-adrenoceptor-stimulated inositol phosphates formation in STZ-diabetic rats. These observations indicate that the

changes in cardiac alpha$_1$-adrenoceptor-mediated inositol phosphates formation cannot contribute to the previously described improved cardiac function of omega-3 fatty acid treated STZ-diabetic rats.

4. Effect of Indomethacin, Imidazole and Tranylcypromine on Alpha$_1$-adrenoceptor-mediated Ins(1,4,5)P$_3$ Formation and Positive Inotropic Effect in Control and Diabetic Rat Heart.

Besides activating protein kinase C, DG can be converted to AA. AA, a fatty acid bound to membrane phospholipids, is metabolized by cyclooxygenase, thromboxane synthetase and prostacyclin synthetase to form thromboxanes and prostaglandins, and is metabolized by lipoxygenase to form leukotrienes. There is evidence that one or more metabolites of AA can produce positive inotropic effects (Otani et al., 1988b) and these could be implicated in the pathophysiological alterations observed in diabetes mellitus (Chen and Robertson, 1979; Metz, 1981). Indeed, studies with rats rendered diabetic with STZ showed an altered metabolism of platelet AA (Johnson and Harrison, 1979). We therefore proposed a study to investigate the possibility that some products derived from AA metabolism could be involved in the altered cardiac alpha$_1$-effect of norepinephrine. Our results have shown that the cyclooxygenase inhibitor indomethacin had no effect on either alpha$_1$-adrenoceptor-mediated positive inotropic

effect or Ins(1,4,5)P_3 formation in the diabetic heart, but did have a stimulatory effect on both the function and Ins(1,4,5)P_3 production in control hearts. The thromboxane inhibitor imidazole produced effects similar to indomethacin. In contrast, the prostacyclin synthetase inhibitor tranylcypromine reduced the alpha$_1$-adrenoceptor-mediated positive inotropic effect and Ins(1,4,5)P_3 formation only in the diabetic heart and had no effect in the controls.

The enhancement by indomethacin and imidazole of the alpha$_1$-adrenoceptor-mediated positive inotropic effect in the control heart could be attributed to the increase in the level of cytosolic arachidonic acid and in this way an enlarged quantity of calcium could be mobilized from the sarcoplasmic reticulum. In favour of this assumption is the observation that arachidonic acid, which might result from the diacylglycerol metabolism, is able to mobilize Ca^{2+} from isolated organelles such as mitochondria and sarcoplasmic reticulum (Hokin, 1985; Zeitler et al., 1986). It could be that, in control hearts, the arachidonic acid level is relatively low (Figure 45a). With the addition of indomethacin and imidazole, arachidonic acid levels increase and this could be involved in the increased inotropic responses to alpha$_1$-adrenoceptor stimulation. It is also possible that thromboxane under normal condition may have an inhibitory effect on alpha$_1$-inotropic-effect of the heart.

Figure 45

The possible involvement of arachidonic acid metabolites in the changes in contractility and $Ins(1,4,5)P_3$ formation in control and diabetic hearts.

CONTROL HEARTS

DIABETIC HEARTS

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            ┌─────────────────────────┐
            │  Alpha₁-Adrenoceptor    │
            │      Stimulation        │
            └─────────────────────────┘
                        │
                        ▼
            ┌─────────────────────────┐
            │  PtdIns(4,5)P₂ Turnover │
            └─────────────────────────┘
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$$\text{Alpha}_1\text{-Adrenoceptor Stimulation}$$

$$\text{PtdIns(4,5)P}_2 \text{ Turnover}$$

DG ↑↑ → PKC Activation ↑↑ → Conversion of PI to PIP₂ ↑↑ → Ins(1,4,5)P₃ ↑↑

Arachidonic Acid ↑↑ → Ca²⁺ Release from SR ↑↑

Indomethacin

PGH₂

Tranylcypromine | Imidazole

PGI₂ ↑↑   TXA₂

cAMP ↓↓

Protein Kinase G Activation ↓↓

G_p Inhibition ↓↓ → PtdIns(4,5)P₂ Hydrolysis ↑↑ → Ins(1,4,5)P₃ ↑↑
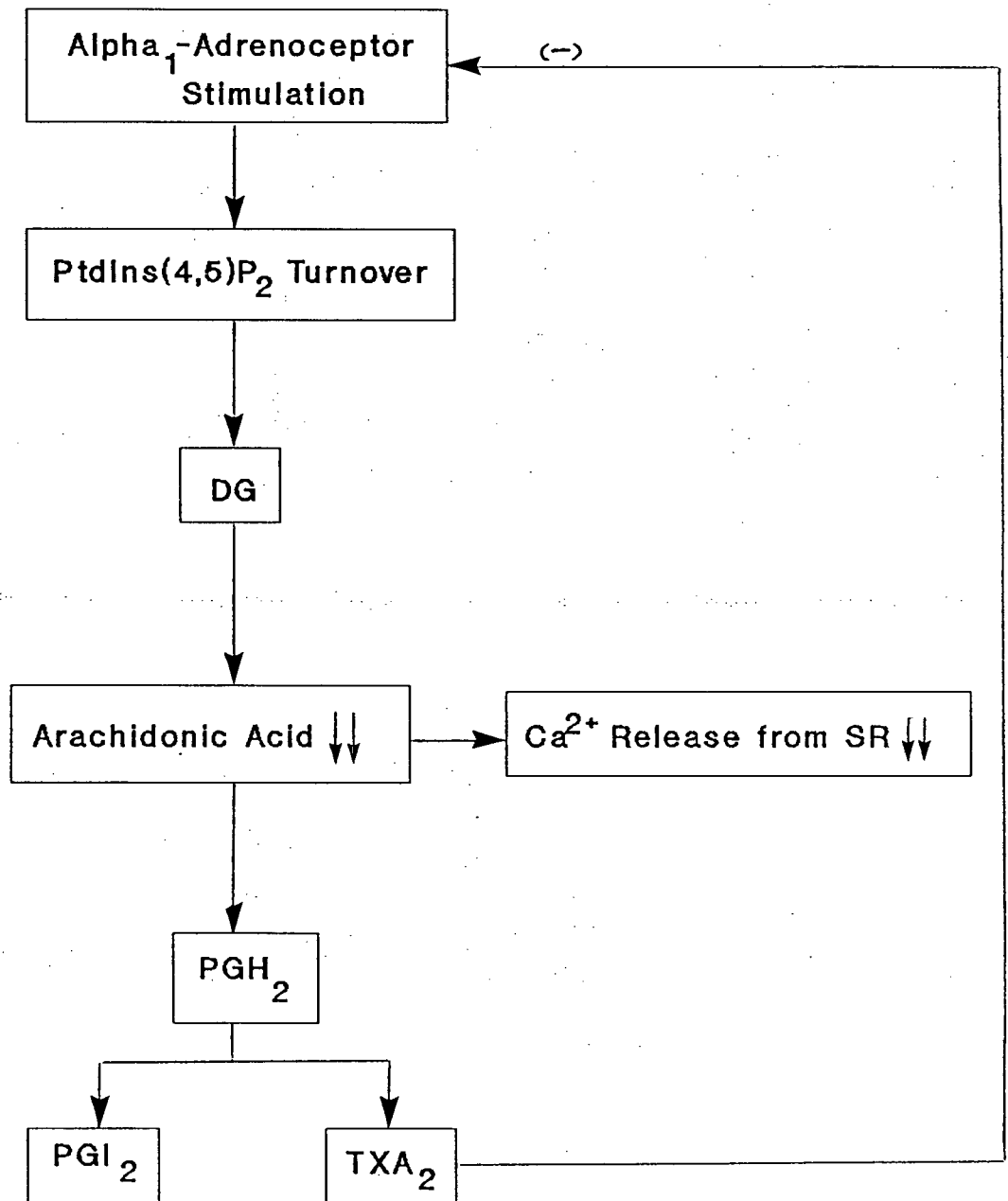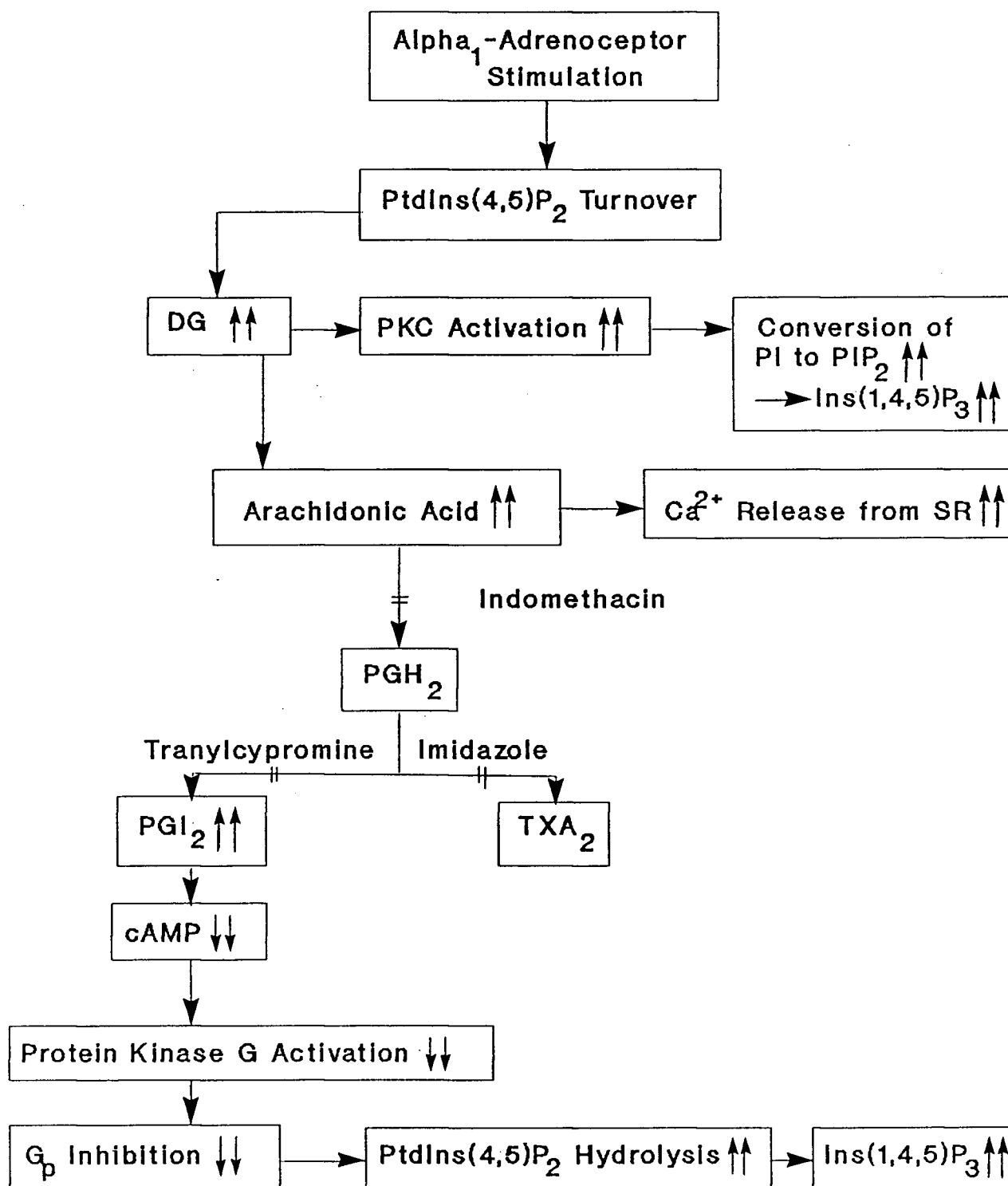
With the addition of indomethacin or imidazole, the inhibition was withdrawn and $alpha_1$-adrenoceptor-mediated positive inotropic effect was therefore enhanced. Further, decreased breakdown of arachidonic acid with the addition of indomethacin or imidazole might increase DG levels which might increase $PtdIns(4,5)P_2$ formation and its turnover. $Ins(1,4,5)P_3$ might thus be increased. However, the exact mechanism involved in the effect of indomethacin or imidazole on the control heart with regard to both function and $Ins(1,4,5)P_3$ formation is still not clear. Previously, indomethacin has been shown to enhance the effect of isoproterenol, activating phosphorylase in diabetic but not in control hearts (Vadlamudi and McNeill, 1983b). There is also no interpretation for this observation.

In the diabetic heart, however, indomethacin and imidazole did not have any significant effect on either function or $Ins(1,4,5)P_3$ formation. This could suggest that thromboxane formation did not play an important role in $alpha_1$-inotropic-effect in the diabetic heart. In fact, recent finding showed that *in vivo* TXA_2 biosynthesis is not altered in diabetes (Alessandrini et al., 1988).

The effect of tranylcypromine may indicate the participation of prostaglandins in mediating the enhanced $alpha_1$-effect of norepinephrine in the diabetic state. In previous studies by Sterin-Borda et al. (1979, 1980), a positive inotropic action of prostacyclin (PGI_2) in rat isolated atria has been reported. Further, there are

reports of an enhanced release of prostacyclin from the perfused heart of diabetic rats (Rosen and Schror, 1980) and from coronary arteries of acutely diabetic dogs (Sterin-Borda et al., 1981). Stimulation of prostaglandin synthesis by ischemia and reperfusion has been demonstrated in isolated rat hearts (Karmazyn, 1986). An increase in the concentration of prostaglandins (PGE_2 and $PGF_{2\alpha}$) in coronary sinus blood has been shown to accompany a decrease in the ventricular multiple response threshold following coronary artery occlusion and release in dogs (Miyazake et al., 1982). In addition, according to Koltai et al. (1988), the diabetic coronaries are more sensitive to the contractile effect of $PGF_{2\alpha}$ than controls. However, it must be mentioned that prostaglandins produce a wide variety of actions on the heart. The effects are quite complex and are dependent on such factors as type of prostaglandin, animal species and experimental format.

Prostaglandins are able to modify transmembrane ionic permeabilities. Prostaglandins have been shown to act as Ca^{2+} ionophores in cardiac as well as other tissues (Karmazyn, 1986) which may, in part, explain the effect of prostaglandins on the diabetic heart. PGE_2 has been shown to inhibit sympathetic neurotransmission in the perfused rabbit heart (Hedqvist et al., 1970). This may suggest that the positive inotropic effect of norepinephrine in the diabetic heart is mainly due to prostaglandins rather than to norepinephrine itself.

Until now the mechanism(s) of the enhanced positive inotropic effect mediated by alpha$_1$-adrenoceptors in the diabetic heart has been unclear. The metabolic pathway of AA or distribution of metabolites from AA may be altered in the diabetic heart, favouring the production of prostacyclins. The supersensitivity and enhanced maximum response to norepinephrine of the diabetic heart could be partly explained by this increase in production of prostaglandins.

It is also possible that arachidonic acid level is relatively high in the diabetic state, which could result in an increase in Ca^{2+} release from SR and therefore an increase in contractility.

Tranylcypromine is well known as a monoamine oxidase inhibitor (Baldessarini, 1985). The resulting increased monoamine level in the central nervous system has been suggested to be the mechanism involved in the use of tranylcypromine as an antidepressant. The importance of monoamine oxidase inhibition in the heart is not clear. The possibility can not be excluded that changes in the monoamine levels may be involved in the effect of tranylcypromine on alpha$_1$-adrenoceptor-mediated inotropic effect. However, we feel this possibility is unlikely.

Reduced cAMP levels are observed in ischemic cardiac tissue after treatment with PGI$_2$ (Roesen et al., 1981). Decreased cAMP levels could result in a decrease in protein kinase G activation, which may result in a decrease in

inhibition of G protein (G_p protein) involved in PtdIns(4,5)P_2 turnover. Ins(1,4,5)P_3 levels may therefore increase. In this regard, the addition of tranylcypromine could decrease Ins(1,4,5)P_3 levels. It also could be that, with the addition of tranylcypromine, arachidonic acid levels increased and DG levels also increased. The resulting activation of protein kinase C by DG may inhibit PtdIns(4,5)P_2 hydrolysis and Ins(1,4,5)P_3 levels may therefore decrease.

Our results regarding inotropic responses of control and diabetic hearts to alpha$_1$-adrenoceptor stimulation in the presence of indomethacin, imidazole and tranylcypromine are different from the data from Canga and Sterin-Borda (1986), which could be due to a number of reasons. Firstly, we used right ventricles of the rats while they employed right atria of the rats. Secondly, in our study, diabetes was induced by a single i.v. injection of STZ at 55 mg/kg and the duration of diabetes was 6 weeks. In contrast, they used 3-day diabetic rats which were made diabetic with a single i.p. injection of STZ at 100 mg/kg. Further, in our study, STZ was dissolved in citrate buffer which had a pH value of 4.5, whereas they dissolved STZ in citrate buffer with pH 4.8. All these variations in species, STZ dose and injection route and duration of diabetes may have some impact on the responses of the heart to alpha$_1$-adrenoceptor stimulation.

An important point with respect to the generalization of this study to intact animals and humans is that the experiments were conducted with isolated right ventricles. Prostaglandin production may be changed due to this intervention. Therefore, the importance of endogeneous prostaglandins in the involvement of the cardiac response to $alpha_1$-adrenoceptor stimulation in the diabetics cannot necessarily be inferred.

SUMMARY AND CONCLUSIONS

1. Wistar rats made diabetic with streptozotocin are characterized by hypoinsulinemia, hyperglycemia and hyperlipidemia.

2. Using a radioisotope method, it was shown that stimulation of $alpha_1$-adrenoceptors by norepinephrine (in the presence of propranolol) in right ventricles resulted in the production of inositol monophosphate (IP_1) in a time- and concentration-dependent manner in both control and diabetic rats. Right ventricles from diabetic rats, however, showed decreased IP_1 formation in response to norepinephrine as compared with controls.

3. Omega-3 fatty acid supplementation had no significant effect on the decrease in norepinephrine-stimulated IP_1 and inositol bisphosphate (IP_2) formation in diabetic rat ventricles. The data indicate that the changes in cardiac $alpha_1$-adrenoceptor-mediated inositol phosphates formation cannot contribute to the previously described improved cardiac function of omega-3 fatty acid-treated STZ-diabetic rats.

4. Right ventricles from diabetic rats were supersensitive (increase in PD_2 values) and showed a greater maximum

inotropic response to alpha$_1$-adrenoceptor stimulation by norepinephrine (in the presence of propranolol) when compared with stimulation of control right ventricles.

5. Using an inositol 1,4,5-trisphosphate [Ins(1,4,5)P$_3$] protein binding assay kit, it was shown that norepinephrine induced a time- and concentration-dependent increase in Ins(1,4,5)P$_3$ contents in right ventricles of both control and diabetic rats. The increase in Ins(1,4,5)P$_3$ levels preceded the increase in the contractility of the heart. Ins(1,4,5)P$_3$ levels in the diabetic right ventricles were significantly higher than those in the controls. These observations suggest that the changes in Ins(1,4,5)P$_3$ levels may be involved in the increased inotropic responsiveness to alpha$_1$-adrenoceptor stimulation in the diabetic heart. The Ca^{2+} overload induced by Ins(1,4,5)P$_3$ could further be involved in the development of diabetic cardiomyopathy.

6. In the presence of the cyclooxygenase inhibitor indomethacin, the positive inotropic effect of norepinephrine was significantly increased in control but not diabetic right ventricles. Similarly, Ins(1,4,5)P$_3$ levels were elevated in control right ventricles treated with indomethacin as compared with untreated controls, and remained elevated in the

diabetic right ventricles treated with indomethacin compared with untreated diabetics.

7. The thromboxane synthetase inhibitor imidazole had similar effects as indomethacin on $alpha_1$-adrenoceptor-mediated positive inotropic effect and $Ins(1,4,5)P_3$ formation in control and diabetic right ventricles. The nature and physiological significance of the enhanced positive inotropic effect and $Ins(1,4,5)P_3$ formation in the control heart with the addition of indomethacin and imidazole are still unclear.

8. Inhibition of prostacyclin synthetase by tranylcypromine reduced the positive inotropic effect of norepinephrine only in diabetic right ventricles and had no effect in the control preparations. Corresponding with the above functional changes, tranylcypromine diminished norepinephrine-induced $Ins(1,4,5)P_3$ formation in diabetic preparations without altering it in the controls. The effect of tranylcypromine may indicate the participation of prostaglandins in mediating the enhanced $alpha_1$-inotropic-effect of norepinephrine in the diabetic heart.

9. Diabetic right ventricles had a higher activated protein kinase C activity in the membrane fraction as

compared with controls and this was accompanied by a decrease in cytosolic protein kinase C activity. The increase in protein kinase C activity associated with the membrane fraction may be involved in the sustained increase in alpha$_1$-adrenoceptor-mediated positive inotropic effect of the diabetic heart.

REFERENCES

Abdel-Latif, A.A., Akhtar, R. and Hawthorne, J.N. 1977. Acetylcholine increases the breakdown of triphosphoinositide of rabbit iris muscle prelabelled with [^{32}P]phosphate. Biochem. J. 162:61-73.

Agarwal, M.K. 1980. Streptozotocin mechanisms of action. FEBS Lett. 120:1-3.

Ahmed, S.S., Jaferi, G.A., Narang, R.M. and Regan, T.J. 1975. Preclinical abnormality of left ventricular function in diabetes mellitus. Am. Heart J. 89:153-158.

Alessandrini, R., McRae, J., Feman, S. and Fitzgerald, G.A. 1988. Thromboxane biosynthesis and platelet function in type I diabetes mellitus. N. Engl. J. Med. 319:208-212.

Arieff, A.J. and Caroll, H.J. 1972. Nonketotic hyperosmolar coma with hyperglycemia: clinical features, pathophysiology, renal function, acid base balance, plasma cerebrospinal fluid equilibria and the effects of therapy in 37 cases. Medicine 51:73-94.

Atkins, F.L., Dowell, R.T. and Love, S. 1985. Beta-adrenergic receptors, adenylate cyclase activity and cardiac dysfunction in the diabetic rat. J. Cardiovasc. Pharmacol. 7:66-70.

Baldessarini, R.J. 1985. Drugs and the treatment of psychiatric disorders. In: Goodman and Gilman's The Pharmacological Basis of Therapeutics. 7th Edition. Gilman, A.G., Goodman, L.S., Rall, T.W. and Murad, F. (eds.), Macmillan Publishing Company, N.Y., pp. 387-445.

Balodimos, M.C. 1971. Diabetic nephropathy. In: Joslin's Diabetes Mellitus. Marble, A., White, P., Bradley, R.F. and Krall, R.F., (eds.), Lea & Febinger, Philadelphia. pp. 526-561.

Bang, H.O. and Dyerberg, J. 1972. Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. Acta Med. Scand. 192:85-94.

Bansal, V.S., Inhorn, R.C. and Majerus, P.W. 1987. The metabolism of inositol 1,3,4-trisphosphate to inositol 1,3-bisphosphate. J. Biol. Chem. 262:9444-9447.

Bar, R.S., Mugges, M., Roth, J., Kahn, C.R., Havrankova, J. and Imperato-McGinley, J. 1978. Insulin resistance, acanthosis nigricans and normal insulin receptors in a

young woman: evidence for a post receptor defect. J. Clin. Endocrinol. Metab. 47:620-625.

Batty, I.R. and Nahorski, S.R. 1985. Differential effects of lithium on muscarinic receptor stimulation of inositol phosphates in rat cerebral cortex slices. J. Neurochem. 45:1514-1521.

Batty, I.R., Nahorski, S.R. and Irvine, R.F. 1985. Rapid formation of inositol 1,3,4,5-tetrakisphosphate following muscarinic receptor stimulation of rat cerebral cortical slices. Biochem. J. 232:211-215.

Baukal, A.J., Guillemette, G., Rubin, R., Spat, A. and Catt, K.J. 1985. Binding sites for inositol trisphosphate in the bovine adrenal cortex. Biochem. Biophys. Res. Commun. 133:532-538.

Benfey, B.G. 1973. Characterization of alpha-addrenoceptors in the myocardium. Br. J. Pharmacol. 48:132-138.

Benfey, B.G. 1980. Cardiac alpha adrenoceptors. Can. J. Physiol. Pharmacol. 58:1145-1157.

Bergh, C.H., Hjalmarson, A., Sjogren, K.G. and Jacobsson, B. 1988. The effect of diabetes on phosphatidylinositol turnover and calcium influx in myocardium. Horm. Metabol. Res. 20:381-386.

Berridge, M.J. 1983. Rapid accumulation of inositol trisphosphate reveals that agonists hydrolyse polyphosphoinositides instead of phosphatidylinositol. Biochem. J. 212:849-858.

Berridge, M.J. 1986. Inositol phosphates as second messengers. In: Phosphoinosidites and Receptor Mechanisms. Putney, J.W., Jr. (ed.), New York, Alan R. Liss, Inc., pp. 25-46.

Berridge, M.J., Dawson, R.M., Downes, C.P., Heslop, J.P. and Irvine, R.F. 1983. Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. Biochem. J. 212:473-482.

Berridge, M.J., Downes, C.P. and Hanley, M.R. 1982. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem. J. 206:587-595.

Berridge, M.J. and Irvine, R.F. 1984. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature (Lond.) 312:315-321.

Besterman, J.M., Duronio, V. and Cuatrescasas, P. 1986. Rapid formation of diacylglycerol from phosphatidylcholine. A pathway for generation of a second messenger. Proc. Natl. Acad. Sci. USA 83:6785-6789.

Bhimji, S., Godin, D.V. and McNeill, J.H. 1985. Biochemical and functional changes in hearts from rabbits with diabetes. Diabetologia 28:452-457.

Black, S.C., Katz, S. and McNeill, J.H. 1988. Effects of oral omega-3 fatty acid treatment on STZ-diabetic rat heart function and plasma lipids. J. Mol. Cell. Cardiol. 20 (Suppl. 3):S9.

Black, S.C., Katz, S. and McNeill, J.H. 1989. Cardiac performance and plasma lipids of omega-3 fatty acid-treated streptozotocin-induced diabetic rats. Diabetes 38:969-974.

Blinks, J.R. and Endoh, M. 1986. Modification of myofibrillar responsiveness to Ca^{2+} as an inotropic mechanism. Circulation 73 (Suppl. III):III85-III98.

Bohm, M., Schmitz, W. and Scholz, H. 1987. Evidence against a role of a pertussis toxin-sensitive guanine nucleotide-binding protein in the alpha$_1$-adrenoceptor-mediated positive inotropic effect in the heart. Naunyn-Schmiedeberg's Arch. Pharmacol. 335:476-479.

Braunwald, E., Sonnenblick, E.H. and Ross, J. 1984. Contraction of the normal heart. In: Heart Disease. Braunwald, E., (ed.), W.B. Saunders Company, Philadelphia, pp. 409-446.

Bristow, M.R., Sandoval, A.B., Gilbert, E.M., Deisher, T., Minobe, W. and Rasmussen, R. 1988. Myocardial alpha- and beta-adrenergic receptors in heart failure: Is cardiac-derived norepinephrine the regulatory signal? Eur. Heart J. 9(H):35-40.

Brown, S.L. and Brown, J.H. 1983. Muscarinic stimulation of phosphatidylinositol metabolism in atria. Mol. Pharmacol. 24:351-356.

Brown, J.H., Buxton, I.L. and Brunton, L.L. 1985. Alpha$_1$-adrenergic and muscarinic cholinergic stimulation of phosphoinositide hydrolysis in adult rat cardiomyocytes. Circ. Res. 57:532-537.

Brown, J.H. and Jones, L.G. 1986. Phosphoinositide metabolism in the heart. In: Phosphoinositides and receptor mechanisms. Putney, J.W. (ed.), Alan R. Liss, Inc., New York, pp. 245-270.

Bruckner, R., Hackbarth, I., Meinertz, T., Schmelzle, B. and Scholz, H. 1978. The positive inotropic effect of phenylephrine in the presence of propranolol. Increase in time to peak force and in relaxation time without increase in c-AMP. Naunyn-Schmiedeberg's Arch. Pharmacol. 303:205-211.

Bruckner, R. and Scholz, H. 1984. Effects of alpha-adrenoceptor stimulation with phenylephrine in the presence of propranolol on force of contraction, slow inward current and cyclic AMP content in the bovine heart. Br. J. Pharmacol. 82:223-232.

Brunner, L.S. and Suddarth, D.S. 1988. Assessment and management of patients with diabetes mellitus. In: Textbook of Medical-Surgical Nursing (Chapter 36), Brunner, L.S. and Suddarth, (eds.), 6th Ed., J.B. Lippinott Company, Philadelphia, pp. 898-941.

Burgess, G.M., Godfrey, P.P., McKinney, J.S., Berridge, M.J., Irvine, R.F. and Putney, J.W. 1984. The second messenger linking receptor activation to internal Ca2+ release in liver. Nature (Lond.) 309:63-66.

Bydlowsk, S.P., Yunker, R.L. and Subbiah, M.T.R. 1985. Delayed effects of experimental maternal diabetes on plasma cholesterol level and vascular prostacyclin synthesis in the offspring. Proc. Soc. Exp. Bio. Med. 179:553-557.

Cahill, G.F. 1971. Physiology of insulin in man. Diabetes 20:785-799.

Canga, L. and Sterin-Borda, L. 1986. Hypersensitivity to methoxamine in atria isolated from streptozotocin-induced diabetic rats. Br. J. Pharmacol. 87:157-165.

Canga, L., Sterin-Borda, L., Borda, E.S., Peredo, H. and Gimeno, A.L. 1985. The positive inotropic effect of sodium arachidonate on auricles from diabetic rats. Eur. J. Pharmacol. 110:47-54.

Carafoli, E. 1985. The homeostasis of calcium in heart cells. J. Mol. Cell. Cardiol. 17:203-212.

Carafoli, E. and Crompton, M. 1978. The regulation of intracellular calcium. In: Current topics in membrane and transport (Vol. 10). Bonner, F., Kleinzeller, A., (eds.), Academic Press, New York, London and Orlando, pp. 151-216.

Caroni, P. and carafoli, E. 1981. The Ca^{2+}-pumping ATPase of heart sarcolemma: characterization, calmodulin

dependence, and partial purification. J. Biol. Chem. 256:3263-3270.

Chamberlain, B.K., Levitsky, D.L. and Fleischer, S. 1983. Isolation and characterization of canine cardiac sarcoplasmic reticulum with improved Ca^{2+} transport properties. J. Biol. Chem. 258:6602-6609.

Charnock, J.S., Abeywardena, M.Y., McMurchie, E.J. and Russel, G.R. 1984. The composition of cardiac phospholipids in rats fed different lipid supplements. Lipids 19:206-213.

Chen, M. and Robertson, R.P. 1979. Effects of prostaglandin synthesis inhibitors on human insulin secretion and carbohydrate tolerance. Prostaglandins 18:557-567.

Chivers, E.R., Barnes, P.J. and Nahorski, S.R. 1988. Muscarinic receptor stimulated turnover of polyphosphoinositides and inositol polyphosphates in bovine tracheal smooth muscle. Br. J. Pharmac. 95:778P.

Cockcroft, S. and Gomperts, B.D. 1985. Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. Nature 314:534-536.

Connolly, T.M., Wilson, D.B., Bross, T.E. and Majerus, P.W. 1986. Isolation and characterization of the inositol cyclic phosphate products of phosphoinositide cleavage by phospholipase C. Metabolism in cell-free extracts. J. Biol. Chem. 261:122-126.

Connolly, T.M., Bansal, V.S., Bross, T.E., Irvine, R.F. and Majerus, P.W. 1987. The metabolism of tris- and tetraphosphates of inositol by 5-phosphomonoesterase and 3-kinase enzymes. J. Biol. Chem. 262:2146-2149.

Craig, J.W. 1980. Clinical implications of the new diabetes classification. Postgrad. Med. 68:122-133.

Craighard, J.E. 1975. The role of viruses in the pathogenesis of pancreatic disease and diabetes mellitus. In: Progress in Medical Virology (Vol. 19). Melnick, J.L., (eds.), Krager, Bassek, Munchea, Paris, London, New York, Sydney, pp. 162-164.

Croft, K.D., Beilin, L.J., Legge, F.M. and Vandongen, R. 1987. Effects of diets enriched in eicosapentaenoic or docosahexaenoic acids on prostanoid metabolism in the rat. Lipids 22:647-650.

Cudworth, A.G. and Woodrow, J.C. 1975. Evidence for HLA-linked genes in "juvenile" diabetes mellitus. Brit. Med. J. 3:133.

Czech, M.P. 1981. Insulin action. In: Diabetes Mellitus. Skyler, J.S. and Cahill, G.F., Jr., (eds.), Yorke Medical Boods, New York, pp. 64-72.

Davey, M.J. and Massingham, R. 1976. A review of the biological effects of prazosin, including recent pharmacological findings. Curr. Med. Res. Opin. 4(suppl. 2):47.

David, H., Meyer, R., Mark, I., Guski, H. and Wenzelides, K. 1979. Morphometric characterization of left ventricular myocardial cells of male rats during postnatal development. J. Mol. Cell. Cardiol. 11:631-638.

Davis, M.D. 1974. Definition, classification and course of diabetic retinopathy. In: Diabetic Retinopathy. Lyn, J.R., Snyder, W.B. and Vassier, A. (eds.), Grune and Stratton, New York, pp. 145-170.

Dawson, A.P. and Irvine, R.F. 1984. Inositol (1,4,5)-trisphosphate promoted Ca2+ release from microsomal fractions of rat liver. Biochem. Biophys. Res. Commun. 120:858-864.

DeDeckere, E.A.M. and Ten Hoor, F. 1980. Influences of dietary fats on coronary flow rate and left ventricular work of the isolated rat heart: sunflower seed oil vs. lard. Nutr. Metab. 24:396-408.

Dhalla, N.S., Das, P.K. and Sharma, G.P. 1978. Subcellular basis of cardiac contractile failure. J. Mol. Cell. Cardiol. 10:363-385.

Dillman, W.H. 1980. Diabetes mellitus induces changes in cardiac myosin of the rat. Diabetes 29:579-582.

Dillman, W.H. 1982. Influence of thyroid hormone administration on myosin ATPase activity and myosin isoenzyme distribution in the heart of diabetic rats. Metabolism 31:199-204.

Docherty, J.R. and McGrath, J.C. 1979. An analysis of some factor influencing alpha-adrenoceptor feedback at the sympathetic junction in the rat heart. Br. J. Pharmacol. 1:189-197.

Downes, C.P., Mussat, M.C. and Michell, R.H. 1982. The inositol trisphosphate phosphomonoesterase of the human erythrocyte membrane. Biochem. J. 203:169-177.

Downing, S.E., Lee, J.C. and Fripp, R.R. 1982. Enhanced sensitivity of diabetic hearts to alpha-adrenoceptor stimulation. Am. J. Physiol. 245 (14):H808-H813.

Drew, G.M. 1980. Presynaptic modulation of heart rate responses to cardiac nerve stimulation in pithed rats. J. Cardiovasc. Pharmacol. 2:843-856.

Dryden, W.F., Charnock, J.S., McMurchie, E.J. and Abejwardena, M.Y. 1982. Dietary lipids as modulators of cardiac responses to inotropic drugs. Br. J. Pharmacol. 76:183P.

Duchen, L.W., Anjorin, A., Watkins, P.J. and MacKay, J.D. 1980. Pathology of autonomic neuropathy in diabetes mellitus. Ann. Intern. Med. 92:301-303.

Dunlop, M.E. and Larkins, R.G. 1984. Activity of endogenous phospholipase C and phospholipase A_2 in glucose stimulated pancreatic islets. Biochem. Biophys. Res. Commun. 120:820-824.

Dunn, J.S. and Mcletchie, N.G.B. 1943. Experimental alloxan diabetes in the rat. Lancet 11:384.

Dyerberg, J. and Bang, H.O. 1978. A hypothesis on the development of acute myocardial infarction in Greenlanders. Scand. J. Clin. Lab. Invest. 42:7-13.

Eberhard, D.A. and Holz, R.W. 1988. Intracellular Ca^{2+} activates phospholipase C. TINS 11(12):517-520.

Endoh, M. 1982. Adrenoceptors and the myocardial inotropic response: Do alpha and beta receptor sites functionally coexist? Trends in autonomic pharmacol. 2:303-322.

Endoh, M. and Blinks, J.R. 1984. Regulation of the intracellular Ca^{2+} transient and Ca^{2+}-sensitivity of myofibrils via alpha- and beta-adrenoceptors in the rabbit papillary muscle. Cell Calcium 5:301.

Fabiato, A. 1983. Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am. J. Physiol. 245:C1-C14.

Fabiato, A. 1986. Inositol (1,4,5)-trisphosphate-induced release of Ca^{2+} from the sarcoplasmic reticulum of skinned cardiac cells. Biophys. J. 49:190a.

Fabiato, A. and Fabiato, F. 1975. Dependence of the caontractile activation of skinned cardiac cells on the sarcomere length. Nature (London) 256:54-56.

Fein, F.S., Kornstein, L.B., Strobeck, J.E., Capasso, T.N. and Sonnenblick, E.H. 1980. Altered myocardial mechanics in diabetic rats. Circ. Res. 47:922-933.

Fein, F.S., Miller-Green, B. and Sonnenblick, E.H. 1985. Altered myocardial mechanics in diabetic rabbits. Am. J. Physiol. 248:H729-H736.

Feldman, J.M. 1981. Diagnosis of cardiovascular autonomic neuropathy in the diabetic patient. Pract. Cardiol. 7:101-116.

Fischer, L.J. 1985. Drugs and chaemicals that produce diabetes. Trends Pharmcol. Sci. 6:72-75.

Flink, I.L., Rader, J.H. and Morkin, E. 1979. Thyroid stimulates synthesis of a cardiac myosin isoenzyme. J. Biol. Chem. 254:3105-3110.

Foy, J.M. and Lucas, P.D. 1978. Comparison between spontaneously beating atria from control and streptozotocin-diabetic rats. J. Pharm. Pharmacol. 30:558-562.

Friday, K.E., Childs, M., Tsunehara, C., Fujimoto, W.Y., Bierman, E.L. and Ensinck, J.W. 1987. Omega 3 fatty acid supplementation has discordant effects on plasma glucose and lipoproteins in type II diabetes. Diabetes 36(Suppl. 1):12A.

Furchgott, R.F. 1972. The classification of adrenoceptors (adrenergic receptors). An evalulation from the standpoint of receptor theory. In: Handbook of Experimental Pharmacology, Vol. 33, Blaschko, H., Muscholl, E. (eds.). Springer-Verlag, Berlin, pp. 283-335.

Ganguly, P.K., Pierce, G.N., Dhalla, K.S. and Dhalla, N.S. 1983. Defective sarcoplasmic reticular calcium transport in diabetic cardiomyopathy. Am. J. Physiol. 244:E528-E535.

Ganguly, P.K., Rice, K.M., Panagia, V. and Dhalla, N.S. 1984. Sarcolemmal phosphatidylethanolamine N-methylation in cardiac cardiomyopathy. Circ. Res. 55:504-512.

Garber, D.W., Everett, A.M. and Neely, J.R. 1983. Cardiac function and myosin ATPase in diabetic rats treated with insulin, T_3 and T_4. Am. J. Physiol. 244:592-598.

Garong, B.R., Loomis, C.R., Hannun, Y.A. and Bell, R.M. 1986. Specificity and mechanism fo protein kinase C

activation by sn-1,2-diacylglycerol. Proc. Natl. Acad. Sci. USA 83:1184-1188.

Gilman, A.G. 1984. G proteins and dual control of adenylate cyclase. Cell 36:577-579.

Glauber, H., Wallace, P., Griver, K. and Brechtel, G. 1988. Adverse metabolic effect of omega 3 fatty acids in non-insulin-dependent diabetes mellitus. Am. Intern. Med. 108:663-668.

Gonzales, R.A. and Crews, F.T. 1985. Guanine nucleotides stimulate production of inositol trisphosphate in rat cortical membranes. Biochem. J. 232:799-804.

Goranson, E.S. and Erulker, S.D. 1949. The effect of insulin on the aerobic phosphorylation of creatine in tissues from alloxan-diabetic rats. Arch. Biochem. 242:40-48.

Goring, G.G., Nayler, W.G. and Spieckermann, P.G. 1977. The release of calcium from cardiac mitochondria: the importance of the calcium-protein ratio. Basic Res. Cardiol. 72:77-81.

Goyal. R.K., Rodrigues, B. and McNeill, J.H. 1987. Effect of triodothyronine on cardiac responses to adrenergic agonists in STZ-induced diabetic rats. Gen. Pharmacol. 18:357-362.

Gudbjarnason, S., El-Hage, A.N., Whitehurst, V.E., Simental, F. and Balazs, T. 1987. Reduced arachidonic acid levels in major phospholipids of heart muscle in the diabetic rat. J. Mol. Cell. Cardiol. 19:1141-1146.

Guillemette, G., Balla, T., Baukal, A.J., Spat, A. and Catt, K.J. 1987. Intracellular receptors for inositol 1,4,5-trisphosphate in angiotensin II target tissues. J. Biol. Chem. 262:1010-1015.

Haider, B., Yeh, C., Thomas, G., Oldewurtel, H.A., Lyons, M.W. and Regan, T.J. 1978. Altered myocardial function and collagen in diabetic rhesus monkeys on atherogenic diet. Trans. Assoc. Am. Physicians 91:197-203.

Haines, A.P., Sauders, T.A., Imeson, J.O., Mahler, R.F., Martin, J., Mistry, M., Vickers, M. and Wallace, P.G. 1986. Effects of a fish oil supplement on platelet function, haemostatic variables and albuminuria in insulin-dependent diabetes. Throm. Res. 43:643-655.

Hamakawa, H., Shimizu, T. and Toda, N. 1973. Interactions of phenylephrine and theophylline in contractility and excitability of isolated rabbit left atria. Jap. J. Pharmacol. 23:373-379.

Hamby, R.I., Zoneraich, S. and Sherman, S. 1974. Diabetic cardiomyopathy. JAMA 229:1749-1754.

Harris, W.S., Connor, W.E. and McMurray, M.P. 1983. The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils. Metabolism 32:179-184.

Haslam, R.J. and Davidson, M.M.L. 1984. Potentiation by thrombin of the secretion of serotonin from permeabilized platelets equilibrated with Ca^{2+} buffers. Relationship to protein phosphorylation and diacylglycerol formation. Biochem. J. 222:351-361.

Haugaard, E.S. and Haugaard, N. 1964. Diabetic metabolism. I: Carbohydrate utilization and high energy phosphate formation in heart homogenate from normal and alloxan-diabetic rats. J. Biol. Chem. 239:705-709.

Hawkins, P.T., Berrie, C.P., Morris, A.J. and Downes, C.P. 1987. Inositol 1,2-cyclic 4,5-trisphosphate is not a product of muscarinic receptor-stimulated phosphatidylinositol 4,5-bisphosphate hydrolysis in rat parotid glands. Biochem. J. 243:211-218.

Hedqvist, P., Stjarner, L. and Wennmalm, A. 1970. Inhibition by prostaglandin E_2 of sympathetic neurotransmission in the rabbit heart. Acta Physiol. Scand. 70:139-141.

Heslop, J.P., Irvine, R.F., Tashjian, A.H. and Berridge, M.J. 1985. Inositol tetrakis and pentakisphosphates in GH4 cells. J. Exp. Biol. 119:395-401.

Heyliger, C.E., Pierce, G.N., Singal, P.K., Beamish, R.E. and Dhalla, N.S. 1982. Cardiac alpha- and beta-adrenergic receptor alterations in diabetic cardiomyopathy. Basic Res. Cardiol. 77:610-618.

Heyliger, C.E., Prakash, A. and McNeill, J.H. 1987. Alteration in cardiac sarcolemmal Ca^{2+} pump activity during diabetes mellitus. Am. J. Physiol. 252:H450-H544.

Hiramoto, T. and Endoh, M. 1988. Role of phosphatidylinositol turnover for myocardial alpha-adrenoceptor-mediated positive inotropic effect: Aspects on species difference in the rabbit, rat and dog myocardium. Abstracts of papers presented at the International Symposium on Biosignaling in Cardiac and Vascular Systems, Kyoto, Japan, in press.

Hirata, F. amd Axelrod, J. 1980. Phospholipid methylation and biological signal transmission. Science 209:1082-1090.

Hirata, M., Suematsu, E., Hashimoto, T., Hamachi, T. and Koga, T. 1984. Release of Ca^{2+} from a non-mitochondrial store site in peritoneal macrophages treated with saponin by inositol 1,4,5-trisphosphate. Biochem. J. 223:229-236.

Ho, A.K., Thomas, T.P., Chik, C.L., Anderson, W.B. and Klein, D.C. 1988. Protein kinase C: subcellular redistribution by increased Ca^{2+} influx. J. Biol. Chem. 263:9292-9297.

Hock, C.E., Holman, M.A. and Reibel, D.K. 1987. Effect of dietary fish oil on myocardial phospholipids and myocardial ischemic damage. Am. J. Physiol. 252:H554-H560.

Hoffman, P., Mentz, P., Blass, K.E. and Forster, W. 1982. Influence of dietary linoleic acid on cardiac function and prostaglandin release and on the effects of isoprenaline in the isolated rat heart. J. Cardiolvasc. Pharmacol. 4:714-720.

Hoftiezer, V. and Carpenter, A.M. 1973. Composition of streptozotocin-induced diabetes in the rat, including volumetric quantitation of the pancreatic islets. Diabetologia 9:178-184.

Hokin, L.E. 1985. Receptors and phosphoinositide-generated second messengers. Annu. Rev. Biochem. 54:205-235.

Hokin, M.R. and Hokin, L.E. 1953. Enzyme secretion and the incorporation of P^{32} into phospholipids of pancreas slices. J. Biol. Chem. 203:967-977.

Holman, R.T., Johnson, S.B., Gerrard, J.M., Mauer, S.M., Kupcho-Sandberg, S. and Brown, D.M. 1983. Arachidonic acid deficiency in streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA 80:2375-2379.

Hughes, A.R., Takemura, H. and Putney, J.W., Jr. 1988. Kinetics of inositol 1,4,5-trisphosphate and inositol cyclic 1:2,4,5-trisphosphate metabolism in intact rat parotid acinar cells: Relationship to calcium signalling. J. Biol. Chem. in press.

Illman, R.J., Trimble, R.P., Storer, G.B., Topping, D.L. and Oliver, J.R. 1986. Time-course of changes in plasma lipids in diabetic rats fed diets high in fish or sunflower oils. Atherosclerosis 59:313-321.

Ingebretsen, C.G., Moreau, P., Hawelu-Johnson, C. and Ingebretsen, W.R. 1980. Performance of diabetic rat hearts: effects of anoxia and increased work. Am. J. Physiol. 239:H614-H620.

Ingebretsen, W.R., Peralta, C., Moucher, M., Wagner, L.K. and Ingebretsen, C.G. 1981. Diabetes alters the myocardial cAMP-protein kinase cascade system. Am. J. Physiol. 240:H375-H382.

Inhorn, R.C., Bansal, V.S. and Majerus, P.W. 1987. Pathway for 1,3,4-trisphosphate and 1,4-bisphosphate metabolism. Proc. Nat. Acad. Sci. USA 84:2170-2174.

Irvine, R.F., Letcher, A.J., Heslop, J.P. and Berridge, M.J. 1986. The inositol tris/tetrakisphosphate pathway--demonstration of Ins(1,4,5)P_3 3-kinase activity in animal tissues. Nature 320:631-634.

Irvine, R.F., Letcher, A.J., Lander, D.J. and Downes, C.P. 1984. Inositol trisphosphates in carbachol-stimulated rat parotid glands. Biochem. J. 223:237-243.

Irvine, R.F. and Moor, R.M. 1986. Micro-injection of inositol 1,3,4,5-tetrakisphosphate activates sea urchin eggs by a mechanism dependent on external Ca^{2+}. Biochem. J. 240:917-920.

Iversen, L.L. 1973. Catecholamine uptake processes. Br. Med. Bull. 29:130-135.

Iwakura, K., Hori, M., Kagiya, T., Watanabe, Y. and Yoshida, H. 1988. Increased $alpha_1$-adrenergic receptors coupled with phosphatidylinositol response augment Ca^{2+} overload in cardiomyopathic hamster. Circulation 78(Suppl. II):II-334.

Jackson, C.V., McGrath, G.M. and McNeill, J.H. 1986. Alterations in $alpha_1$-adrenoceptor stimulation of isolated atria from experimental diabetic rats. Can. J. Physiol. Pharmacol. 64:145-151.

Johnson, E.B. and Tjalve, H. 1978. Studies on the tissue-deposition and fate of [^{14}C]-streptozotocin with special referance to pancreatic islets. Acta Endocrinol. 89:339-351.

Johnson, M. and Harrison, H.E. 1979. Platelet abnormalities in experimental diabetes. Thromb. Haemost. 42:333.

Jones, L.G., Goldstein, D. and Brown, J.H. 1988. Guanine nucleotide-dependent inositol trisphosphate formation in chick heart cells. Circ. Res. 62:299-305.

Jones, L.R., Besch, H.R. and Watanabe, A.M. 1977. Monovalent cation stimulation of Ca^{2+}-uptake by cardiac membrane vesicles. J. Biol. Chem. 252:3315-3323.

Joseph, S.K., Thomas, A.P., Williams, R.J., Irvine, R.F. and Williamson, J.R. 1984. myo-Inositol 1,4,5-trisphosphate. A second messenger for the hormonal mobilization of intracellular Ca^{2+} in liver. J. Biol. Chem. 259:3077-3081.

Junod, A., Lambert, A.E., Stauffacher, W. and Renold, A.E. 1969. Diabetogenic action of streptozotocin: Relationship of dose to metabolic response. J. Clin. Invest. 48:2129-2139.

Kagawa, Y., Nishizawa, M., Suzuki, M., Miyatake, T., Hamamoto, T., Goto, K., Motonaga, E., Izumikawa, H., Hirata, H. and Ebihara, A. 1982. Eicosapolyenoic acids of serum lipids of Japanese Islanders with low incidence of cardiovascular disease. J. Nutr. Sci. Vitaminol. 28:441-453.

Kahn, C.R. 1985. Pathophysiology of diabetes mellitus: An Overview. In: Joslin's Diabetes Mellitus (12th ed.), Marble, A., White, P., Bradley, R.F. and Krall, L.P., (eds.), Lea & Febiger, Philadelphia, pp. 43-50.

Kahn, C.R., Baird, K.L., Flier, J.S., Grumfeld, C., Harmon, J.T., Harrison, L.C., Karlsson, F.A., Kasuga, M., King, G.L., Lang, U.C., Podskalny, J.M. and van Obbergehen, E. 1981. Insulin receptors, receptor antibodies, and the mechanism of insulin action. Recent Prog. Horm. Res. 37:477-538.

Kahn, C.R., Meghesi, K. and Barr, R.S. 1977. Receptors for peptide hormones. Ann. Intern. Med. 86:205-219.

Kahn, C.R. and White, M.F. 1988. The insulin receptor and the molecular mechanism of insulin action. J. Clin. Invest. 82:1151-1156.

Kaku, T., Lakatta, E. and Filburn, C.R. 1986. Effect of alpha1-adrenergic stimulation on phosphoinositide metabolism and protein kinase C (PK-C) in rat cardiomyocytes. Fed. Proc. 45:209.

Kannel, W.B. 1978. Role of diabetes in cardiac disease: conclusions from population studies. In: Diabetes and the Heart. Zoneraich, S. (ed.), Charles C. Thomas, Springfield, pp. 97-112.

Kannel, W.B. and McGee, D.L. 1979. Diabetes and cardiovascular risk factors. The Framingham study. Circulation 59:8-13.

Karliner, J.S., Barnes, P., Hamilton, C.A. and Dollery, C.T. 1979. Alpha1-adrenergic receptors in guinea pig myocardium: Identification by binding of a new radioligand, [^{3}H]prazosin. Biochem. Biophys. Res. Commun. 90:142-149.

Karliner, J.S. and Simpson, P.C. 1986. A guanine nucleotide regulatory protein is involved in alpha$_1$-adrenoceptor signal transduction in neonatal rat cardiomyocytes. Clin. Res. 34:312A.

Karmazyn, M. 1986. Contribution of prostaglandins to reperfusion-induced ventricular failure in isolated rat hearts. Am. J. Physiol. 251:H133-H140.

Kasim, S.E., Stern, B., Khilnani, S., McLin, P., Baciorowski, S. and Jen, K.L.C. 1988. Effects of omega-3 fish oil on lipid metabolism, glycemic control, and blood pressure in type II diabetic patients. J. Clin. Endo. Meta. 67:1-5.

Katoh, N. and Kuo, J.F. 1982. Subcellular distribution of phospholipid-sensitive calcium-dependent protein kinase in guinea pig heart, spleen and cerebral cortex, and inhibition of the enzyme by triton X-100. Biochem. Biophys. Res. Commun. 106:590-595.

Katoh, N., Wise, B.C. and Kuo, J.F. 1983. Phosphorylation of cardiac troponin inhibitory subunit (troponin I) and tropomyosin-binding subunit (troponin T) by cardiac phospholipid-sensitive Ca^{2+}-dependent protein kinase. Biochem. J. 209:189-195.

Katz, A.M. 1977. Excitation-contraction coupling. In: Physiology of the heart. Raven Press, New York, pp. 137-159.

Katz, S. 1980. Mechanism of stimulation of calcium transport in cardiac sarcoplasmic reticulum preparations by calmodulin. Ann. N. Y. Acad. Sci. 356:267-279.

Kikkawa, U., Takai, Y., Minakuchi, R., Inohara, S. and Nishizuka, Y. 1982. Calcium-activated, phospholipid-dependent protein kinase from rat brain. J. Biol. Chem. 257:13341-13348.

Kim, J., Agranoff, B., Ueda, T. and Greene, D.A. 1988. Dietary myo-inositol prevents decreased native protein kinase activity in diabetic rat nerve. Clin. Res. 36:586a. (Abstr.).

Kirk, C.J., Creba, J.A., Downes, C.P. and Michell, R.H. 1981. Hormone-stimulated metabolism of inositol lipids

and its relationship to hepatic receptor function. Biochem. Soc. Trans. 9:377-379.

Kohl, C., Schmitz, W., Scholz, H., Scholz, J., Toth, M., Doring, V. and Kalmar, P. 1989. Evidence for alpha$_1$-adrenoceptor-mediated increase of inositol trisphosphate in the human heart. J. Cardiovasc. Pharmacol. 13:324-327.

Koltai, M.Z., Hadhazy, P., Koszeghy, A., Ballagi-Pordany, G. and Pogatsa, G. 1988. Prostaglandins and altered diabetic vasoregulation. Biomed. Biochim. Acta 9:849-854.

Kromann, N. and Green, A. 1980. Epidemiologic studies in the Upernavik District, Greenland. Acta Med. Scand. 208:401-406.

Kromhout, D., Bosschieter, E.B. and Coulander, C.D.L. 1985. The inverse relation between fish consumption and 20 year mortality from coronary heart disease. N. Engl. J. Med. 312:1205-1209.

Ku, D.D. and Sellers, B.M. 1982. Effects of streptozotocin diabetes and insulin treatment on myocardial sodium pump and contractility of the rat heart. J. Pharmacol. Exp. Ther. 222:395-400.

Kuo, J.F., Andersson, R.G.G., Wise, B.C., MacKerlova, L., Salomonsson, I., Brackett, N.L., Katoh, N., Shoji, M. and Wrenn, R.W. 1980. Calcium-dependent protein kinase: widespread occurrence in various tissues and phyla of the animal kingdom and comparison of effects of phospholipid, calmodulin, and trifluoperazine. Proc. Natl. Acad. Sci. USA 77:7039-7043.

Lab, M.J., Allen, D.G. and Orchard, C.H. 1984. The effects of shortening on myoplasmic calcium concentration and on the action potential in mammalian ventricular muscle. Circ. Res. 55:825-829.

Langer, G.A. 1984. Calcium at the sarcolemma. J. Mol. Cell. Cardiol. 16:147-153.

Langer, S.Z. 1974. Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23:1793-1800.

LaRaia, P.J. and Morkin, E. 1974. Adenosine 3',5'-monophosphate dependent membrane phosphorylation; a possible mechanism for the control of microsomal calcium transport in heart muscle. Circ. Res. 35:298-306.

Latifpour, J. and McNeill, J.H. 1984. Cardiac autonomic receptors: effect of long-term experimental diabetes. J. Pharmacol. Exp. Ther. 230:242-249.

Ledet, B., Neubauer, B., Christenren, N.J. and Lundback, K. 1979. Diabetic cardiopathy. Diabetologia 16:207-209.

Lee, T.S., Macgregor, L.C., Fluharty, S.J. and King, G.L. 1989. Differential regulation of protein kinase C and (Na,K)-adenosine triphosphatase activities by elevated glucose levels in retinal capillary endothelial cells. J. Clin. Invest. 83:90-94.

Lernmark, A., Freedman, Z.R., Hormann, C., Rubenstein, A.H., Steiner, D.F., Jackson, R.L., Winter, R.J. and Fraisman, H.S. 1978. Islet-cell surface antibodies in juvenile diabetes. N. Engl. J. Med. 299:375-380.

Levy, M.N. and Martin, P.J. 1979. Neural control of the heart. In: Handbook of Physiology. The Cardiovascular System. Washington, DC: Am. Physiol. Soc., sect. 2, Vol. I, Chapt. 16, pp. 581-620.

Lim, M.S., Sutherland, C. and Walsh, M.P. 1985. Phosphorylation of bovine cardiac C-protein by protein kinase C. Biochem. Biophys. Res. Commun. 132:1187-1195.

Lindemann, J.P. 1986. Alpha-adrenergic stimulation of sarcolemmal protein phosphorylation and slow responses in intact myocardium. J. Biol. Chem. 261:4860-4867.

Lloyd-Mostyn, R.H. and Watkins, P.J. 1975. Defective innervation of the heart in diabetic autonomic neuropathy. Br. Med. J. 3:15-17.

Lo, W.W.Y. and Hughes, J. 1987. Specific binding of [3H]inositol trisphosphate to rat cerebral cortical microsomal membrane. Neurosci. Lett. 81:331-334.

Lopaschuk, G.D., Katz, S. and McNeill, J.H. 1983a. The effects of alloxan and streptozotocin-induced diabetes on calcium transport in rat cardaic sarcoplasmic reticulum. The possible involvement of long chain acyl-carnitines. Can. J. Physiol. Pharmacol. 61:439-448.

Lopaschuk, G.D., Tahiliani, A.G., Vadlamudi, R.V.S.V., Katz, S., and McNeill, J.H. 1983b. Cardiac sarcoplasmic reticulum function in insulin- or carnitine-treated diabetic rats. Am. J. Physiol. 245:H969-H976.

Lorenz, R., Spengler, U., Fischer, S., Duhm, J. and Weber, P.C. 1983. Platelet function, thromboxane formation and blood pressure control during supplementation of the

Western diet with cod liver oil. Circulation 67:504-511.

Luckmann, J. and Sorensen, K.C. 1987. Nursing people experiencing endocrine disorders of the pancreas. In: Medical-Surgical Nursing (Chapter 68), Kay, D., (ed.), 3rd Ed., W.B. Saunders Company, Philadelphia, pp. 1404.

MacLean, N. and Oilvie, R.F. 1955. Quantitative estimation of the pancreatic islet tissue in diabetic subjects. Diabete 4:367-376.

MacLennan, D.H. 1970. Purification and properties of an adenosine triphosphatase from sarcoplasmic reticulum. J. Biol. Chem. 245:4508-4518.

Makino, N., Dhalla, K.S., Elimban, V. and Dhalla, N.S. 1987. Sarcolemmal Ca^{2+} transport in streptozotocin-induced diabetic cardiomyopathy in rats. Am. J. Physiol. 253:E202-E207.

Malaisse, W.J. 1982. Alloxan toxicity to the pancreatic beta-cell: a new hypothesis. Biochem. Pharmacol. 31:3527-3534.

Malhotra, A., Penpargkul, S., Fein, F.S., Sonnenblick, E.H. and Scheuer, J. 1981. The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. Circ. Res. 49:1245-1250.

Martin, J.M., Hunter, D.D. and Nathanson, N.M. 1985. Islet activating protein inhibits physiological responses evoked by cardiac muscarinic acetylcholine receptors. Role of guanosine trisphosphate binding proteins in regulation of potassium permeability. Biochemistry 24:7521-7525.

Masters, S.B., Martin, M.W., Harden, T.K. and Brown, J.H. 1985. Pertussis toxin does not inhibit muscarinic-receptor-mediated phosphoinositide hydrolysis or calcium mobilization. Biochem. J. 227:933-937.

May, W.S. Jr., Sahyoun, N., Wolf, M. and Cuatrecasas, P. 1985. Role of intracellular calcium mobilization in the regulation of protein kinase C-mediated membrane processes. Nature (London) 317:549-551.

McGarry, J.D. and Foster, D.W. 1977. Hormonal control of ketogenesis. Arch. Intern. Med. 137:495-501.

Merritt, J.E., Taylor, C.W., Rubin, R.P. and Putney, J.W., Jr. 1986. Evidence suggesting that a noval guanine nucleotide regulatory protein couples receptors to

phospholipase C in exocrine pancreas. Biochem. J. 236:337-343.

Metz, S.A. 1981. Feedback modulation of glucose-induced insulin secretion by arachidonic acid metabolites: possible molecular mechanisms and relevance to diabetes mellitus. Prostaglandins and medicine 7:581-589.

Michell, R.H. 1975. Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta 415:81-147.

Miller, M.E., Anagnostou, A.A., Ley, B., Marshall, P. and Singer, M. 1987. Effect of fish oil concentrates on hemorheological and hemostatic aspects of diabetes mellitus: A preliminary study. Thrombosis Res. 47:201-214.

Miyazaki, Y., Kotaka, K., Ogawa, K., Satake, T., Sugiyama, S. and Ozawa, T. 1982. Mechanisms of reperfusion arrhythmia. Prostaglandin as a washout metabolite. J. Electrocardiol. (San Diego) 15:389-394.

Mori, T.A., Vandongen, R., Maseri, J.R.L., Dunbar, D. and Stanton, K.G. 1988. Serum lipids in insulin-dependent diabetics are markedly altered by dietary fish oils. Clin. Exp. Pharmacol. Physiol. 15:333-337.

Morris, A.P., Gallacher, D.V., Irvine, R.F. and Petersen, O.H. 1987. Synergism of inositol trisphosphate and tetrakisphosphate in activating Ca^{2+}-dependent K^+ channels. Nature 330:653-655.

Movsesian, M.A., Nishikawa, M. and Adelstein, R.S. 1984. Phosphorylation of phospholamban by calcium-activated, phospholipid-dependent protein kinase. J. Biol. Chem. 259:8029-8032.

Movsesian, M.A., Thomas, A.P., Selak, M. and Williamson, J.R. 1985. Inositol trisphosphate does not release Ca^{2+} from permeabilized cardiac myocytes and sarcoplasmic reticulum. FEBS Lett. 185:328-332.

Mugge, A., Reupcke, C. and Scholz, H. 1985. Increased myocardial $alpha_1$-adrenoceptor density in rats chronically treated with propranolol. Europ. J. Pharmacol. 112:249-252.

Mukherjee, A., Haghani, Z., Brady, J., Bush, L., McBride, W., Buja, L.M. and Willerson, J.T. 1983. Differences in myocardial alpha- and beta-adrenergic receptor numbers in different species. Am. J. Physiol 245:H957-H961.

Nahorski, S.R. and Batty, I. 1986. Inositol tetrakisphosphate: Recent developments in

phosphoinositide metabolism and receptor function. Trends Pharm. Sci. 7:83-85.

Nayler, W., Gevers, W. and Opie, L.H. 1984. Heart cells and organelles. In: The Heart. Opie, L.H. (ed.), Grane & Stratton, London, pp. 15-29.

Nerup, J. 1978. HLA studies in diabetes mellitus: a review. Adv. Metab. Disord. 9:263-277.

Nestel, P.J., Connor, W.E., Reardon, M.F., Connor, S., Wong, S. and Boston, R. 1984. Suppression by diets rich in fish oil of very low density lipoprotein production in man. J. Clin. Invest. 74:82-89.

Nilsson, T., Arkhammer, P. and Berggren, P. 1988. dual effects of glucose on cytoplasmic free Ca^{2+} concentration and insulin release reflects the B-cell being deprived of fuel. Biochem. Biophys. Res. Commun. 153:984-991.

Nishizuka, Y. 1983. Calcium, phospholipid turnover and transmembrane signalling. Phil. Trans. R. Soc. Lond. B. 302:101-112.

Nishizuka, Y. 1984a. The role of protein kinase C in cell surface signal transduction and tumor promotion. Nature 308:693-698.

Nishizuka, Y. 1984b. Turnover of inositol phospholipids and signal transduction. Science 225:1365-1370.

Nishizuka, Y. 1986. Studies and perspectives of protein kinase C. Science 233:305-312.

Nosek, T.M., Williams, M.F., Zeigler, S.T. and Godt, R.E. 1986. Inositol trisphosphate enhances calcium release in skinned cardiac and skeletal muscle. Am. J. Physiol. 250:C807-C811.

Okumura, K., Akiyama, N., Hashimoto, H., Okawa, K. and Satake, T. 1988. Alteration of 1,2-diacylglycerol content in myocardium from diabetic rats. Diabetes 37:1168-1172.

Osnes, J.B. 1978. Cyclic AMP dependent and independent inotropic effects of adrenergic amines. Thesis, Institute of Pharmacology, University of Oslo, Oslo.

Osnes, J.B., Aass, H. and Skomedal, T. 1985. On adrenergic regulation of heart function: role of myocardial alpha-adrenoceptors. In: Alpha-adrenoceptor blockers in cardiovascular disease. Refsum, H. and Mjos, O.D. (eds.), Churchill Livingstone, N.Y., pp. 69-102.

Osnes, J.B., Christoffersen, T. and Oye, I. 1973. Mechanisms of the inotropic effect of catecholamines as revealed from experiments on the perfused rat heart. Acta Physiol. Scand. Suppl. 396:6.

Osnes, J.B. and Oye, I. 1975. Relationship between cyclic AMP metabolism and inotropic response of perfused rat heart to phenylephrine and other adrenergic amines. In: Advances in Cyclic Nucleotide Research (Vol. 5), Drummond, G.I., Greengard, P., Robison, G.A., (eds.), Raven Press, New York, pp. 415-433.

Osnes, J.B., Refsum, H., Skomedal, T. and Oye, I. 1978. Qualitative differences between beta-adrenergic and alpha-adrenergic inotropic effects in rat heart muscle. Acta Pharmacol. Toxicol. 42:235-247.

Otani, H., Otani, H. and Das, D.K. 1986. Evidence that phosphoinositide response is mediated by $alpha_1$-adrenoceptor stimulation, but not linked with excitation-contraction coupling in cardiac muscle. Biochem. Biophys. Res. Commun. 136:863-869.

Otani, H., Otani, H. and Das, D.K. 1988a. $Alpha_1$-adrenoceptor-mediated phosphoinositide breakdown and inotropic response in rat left ventricular papillary muscles. Circ.Res. 62:8-17.

Otani, H., Otani, H. and Das, D.K. 1988b. Positive inotropic effect and phosphoinositide breakdown mediated by arachidonic acid and prostaglandin $F_{2\alpha}$. J. Pharmacol. Exp. Ther. 244:844-851.

Palmer, S., Hughes, K.T., Lee, D.Y. and Wakelam, M.J.O. 1989. Development of a novel, $Ins(1,4,5)P_3$-specific binding assay. Its use to determine the intracellular concentration of $Ins(1,4,5)P_3$ in unstimulated and vasopressin-stimulated rat hepatocytes. Cellu. Signalling 1:147-156.

Penpargkul, S.F., Fein, F., Sonnenblick, E. and Scheuer, J. 1981. Depressed cardiac sarcoplasmic reticular function from daibetic rats. J. Mol. Cell. Cardiol. 13:303-309.

Penpargkul, S.F., Schaible, T., Yipintsoi, T. and Scheuer, J. 1980. The effect of diabetes on performance and metabolism of rat hearts. Circ. Res. 47:911-921.

Peter-Riesch, B., Fathi, M., Schlegel, W. and Wolheim, C.B. 1988. Glucose and carbachol generate 1,2-diacylglycerols by different mechanisms in pancreatic islets. J. Clin. Invest. 81:1154-1161.

Philipson, K.D., Bers, D.M., Nishimoto, A.Y. and Langer, G.N. 1980. Binding of Ca^{2+} and Na^+ to sarcolemmal membranes: relation to control of myocardial contractility. Am. J. Physiol. 238:373-378.

Philipson, K.D., Bersohn, M.M. and Nishimoto, A.Y. 1982. Effects of pH on Na^+-Ca^{2+} exchange in canine cardiac sarcolemmal vesicles. Circ. Res. 50:287-293.

Phillipson, B.E., Rothrock, D.W., Connor, W.E., Harris, W.S. and Illingworth, D.R. 1985. Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oil in patients with hypertriglyceridemia. N. Engl. J. Med. 312:1210-1216.

Pieper, G.M. and Murray, W.J. 1987. In vivo and in vitro intervention with L-carnitine prevents abnormal energy metabolism in isolated diabetic rat heart: chemical and phosphorus-31 NMR evidence. Biochem. Med. Metabol. Biol. 38:111-120.

Pierce, G.N. and Dhalla, N.S. 1981. Cardiac myofibrillar ATPase activity in diabetic rats. J. Mol. Cell. Cardiol. 13:1063-1069.

Pierce, G.N. and Dhalla, N.S. 1983. Sarcolemmal Na^+-K^+ ATPase activity in diabetic rat heart. Am. J. Physiol. 245:C241-C247.

Pierce, G.N. and Dhalla, N.S. 1985. Heart mitochondrial function in chronic experimental diabetes in rats. Can. J. Cardiol. 1:48-54.

Pierce, G.N., Kutryk, M.J.B. and Dhalla, N.S. 1983. Alterations in Ca2+ binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes. Proc. Natl. Acad. Sci. USA 80:5412-5416.

Pittman, C.S., Suda, A.K., Chambers, J.B. and Ray, G.Y. 1979. Impaired 3,5,3-triiodothyronine (T_3) production in diabetic patients. Metabolism 28:333-338.

Poggioli, J., Sulpice, J.C. and Vassort, G. 1986. Inositol phosphate production following $alpha_1$-adrenergic, muscarinic or electrical stimulation in isolated rat hearts. FEBS. Lett. 206:292-298.

Popp-Snijders, C., Schouten, J.A., Heine, R.J. and Van der Veen, E.A. 1987. Dietary supplementation of omega-3 polyunsaturated fatty acids improves insulin sensitivity in non-insulin dependent diabetes. Diabetes Res. 4:141-147.

Ramanadham, S. and Tenner, T.E. Jr. 1983. Alterations in cardiac performance in experimentally-induced diabetes. Pharmacology 27:130-139.

Ramanadham, S. and Tenner, T.E. Jr. 1986. Chronic effects of streptozotocin diabetes on myocardial sensitivity in the rat. Diabetologia 29:741-728.

Rana, R.S., Sekar, M.C., Hokin, L.E. and MacDonald, M.J. 1986. A possible role for glucose metabolites in the regulation of inositol-1,4,5-trisphosphate 5-phosphomonoesterase. J. Biol. Chem. 261:5237-5240.

Regan, T.J., Ettinger, P.O., Khan, M.I., Jesrani, M.V., Lyons, M.M., Oldewurtel, H.A. and Weber, M. 1974. Altered myocardial function and metabolism in chronic diabetes mellitus without ischemia in dogs. Circ. Res. 35:222-237.

Regan, T.J., Lyons, M.M., Ahmed, S.S., Levinson, G.E., Oldewurtel, H.A., Ahmed, M.R. and Haider, B. 1977. Evidence for cardiomyopathy in familial diabetes mellitus. J. Clin. Invest. 60:885-899.

Reibel, D.K., Holahan, M.A. and Hock, C.E. 1988. Effects of dietary fish oil on cardiac responsiveness to adrenoceptor stimulation. Am. J. Physiol. 254:H494-H499.

Roesen, R., Roesen, P., Ohlendorf, R. and Schroer, K. 1981. Prostacyclin prevents ischemia-induced increase of lactate and cAMP in ischemic myocardium. Eur. J. Pharmacol. 69:489-491.

Rosen, P. and Schror, K. 1980. Increased prostacyclin release from perfused hearts of acutely diabetic rats. Diabetologia 18:391-394.

Rosen, P., Senger, W., Feuerstein, J., Grote, H., Reinauer, H. and Schror, K. 1983. Influence of streptozotocin diabetes on myocardial lipids and prostaglandin release by the rat heart. Biochem. Med. 30:19-33.

Rugevics, C.U. and Schumann, H.J. 1987. Alpha1-adrenoceptors in guinea-pig heart ventricles. [^{3}H]Prazosin and [^{125}I]IBE 2254 are bound specifically to a receptor population with high affinity and low density. Arch. Int. Pharmacodyn. 289:234-250.

Rundles, R.W. 1945. Diabetic neuropathy. Medicine 24:110-160.

Saltiel, A.R., Fox, J.A., Sherline, P. and Cuatrecasas, P. 1986. Insulin stimulated hydrolysis of a novel

glycolipid generates modulators of cAMP phosphodiesterase. Science (Wash. D.C.) 233:967-972.

Sanders, T.A. and Roshanai, F. 1983. The influence of different types of omega 3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. Clin. Sci. 64:91-99.

Sanders, T.A., Sullivan, D.R., Reeve, J. and Thompson, G.R. 1985. Triglyceride-lowering effect of marine polyunsaturates in patients with hypertriglyceridemia. Artherosclerosis 5:459-465.

Savarese, J.J. and Berkowitz, B.A. 1979. Beta-adrenergic receptor decrease in diabetic rat hearts. Life Sci. 25:2075-2078.

Schectman, G., Kaul, S. and Kissebah, A. 1988. Effect of fish oil concentrate on lipoprotein composition in NIDDM. Diabetes 37:1567-1573.

Scherer, N.M. and Ferguson, J.E. 1985. Inositol 1,4,5-trisphosphate is not effective in releasing calcium from skeletal sarcoplasmic reticulum microsomes. Biochem. Biophys. Res. Commun. 128:1064-1070.

Scherer, R.W. and Sastre, A. 1985. Synergistic effects of alpha1 and muscarinic receptors on phosphatidylinositol breakdown in murine atria. Fed. Proc. 44:509.

Scheuer, J. and Bhan, A.K. 1979. Cardiac contractile proteins. Adenosine triphosphatase activity and physiological function. Circ. Res. 45:1-12.

Schmitz, W., Scholz, H., Scholz, J. and Steinfath, M. 1987a. Increase in IP_3 precedes alpha-adrenoceptor-induced increase in force of contraction in cardiac muscle. Eur. J. Pharmacol. 140:109-111.

Schmitz, W., Scholz, H., Scholz, J., Steinfath, M., Lohse, M., Puurunen, J. and Schwabe, U. 1987b. Pertussis toxin does not inhibit the $alpha_1$-adrenoceptor-mediated effect on inositol phosphate production in the heart. Eur. J. Pharmacol. 134:377-378.

Scholz, H. 1980. Effects of beta- and alpha-adrenoceptor activators and adrenergic transmitter releasing agents on the mechanical activity of the heart. In: Handbook of Experimental Pharmacology, Vol. 54/I: Adrenergic activators and inhibitors. Szekeres, L. (ed.), Springer-Verlag, Berlin, pp. 651-733.

Scholz, J., Schaefer, B., Schmitz, W., Scholz, H., Steinfath, M., Lohse, M., Schwabe, U. and Puurunen, J.

1988. Alpha-1 adrenoceptor-mediated positive inotropic effect and inositol trisphosphate increase in mammalian heart. J. Pharmacol. Exp. Ther. 245(1):327-335.

Scholz, J., Scholz, H. and Steinfath, M. 1987. Effect of alpha₁-adrenoceptor stimulation on phosphoinositide metabolism and force of contraction in rat heart (Abstract). Naunyn-Schmiedebergs Arch. Pharmacol. 335:R58.

Schwartz, A. 1974. Active transport in mammalian myocardium. In: The Mammalian Myocardium. Langer, J.A. and Brady, A.J., (eds.), John Wiley & Sons, pp. 81-104.

Sekar, M.C., Dixon, J.F. and Hokin, L.E. 1987. The formation of inositol 1,2-cyclic 4,5-trisphosphate and inositol 1,2-cyclic 4-bisphosphate on stimulation of mouse pancreatic minilobules with carbamylcholine. J. Biol. Chem. 262:340-344.

Sibley, W.A. 1982. The neuropathies of diabetes mellitus. In: Management of Diabetes Mellitus. Bressfer, R. and Johnson, D.J., (eds.), John Wright, Boston, Bristol, London, pp. 249-269.

Siegl, P.K.S. and McNeill, J.H. 1980. Comparison of the alpha-adrenergic agonists, phenylephrine and methoxamine in rabbit papillary muscles. Res. Commun. Chem. Path. Pharmacol. 30:221-231.

Simons, L.A., Hickie, J.B. and Balasubramaniam, S. 1985. On the effects of dietary n-3 fatty acids (Maxepa) on plasma lipids and lipoproteins in patients with hyperlipidaemia. Atherosclerosis 54:75-88.

Singer, P., Wirth, M., Berger, I., Voigt, S., Gerike, U., Godicke, W., Koberle, U. and Heine, H. 1985. Influence on serum lipids, lipoproteins and blood pressure of mackerel and herring diet in patients with type IV and V hyperlipoproteinemia. Atherosclerosis 56:111-118.

Skomedal, T., Aass, H. and Osnes, J.B. 1984. Specific binding of [³H]-prazosin to myocardial cells isolated from adult rats. Biochem. Pharmacol. 33:1897-1906.

Skomedal, T. and Osnes, J.B. 1983. Qualitative differences between the inotropic responses in rat papillary muscles to alpha-adrenoceptor and beta-adrenoceptor stimulation by both noradrenaline and adrenaline. Acta Pharmacol. Toxicol. 52:57-67.

Skomedal, T., Osnes, J.B. and Oye, I. 1982. Differences between alpha-adrenergic and beta-adrenergic inotropic

effects in rat heart papillary muscles. Acta Pharmacol. Toxicol. 50:1-12.

Smith, C.D., Cox, C.C. and Snyderman, R. 1986. Receptor-coupled activation of phosphoinositide-specific phospholipase C by a N protein. Science 232:97-100.

Spat, A., Bradford, P.G., McKinney, J.S., Rubin, R.P. and Putney, J.W. Jr. 1986. A saturable receptor for ^{32}P-inositol-triphosphate in hepatocytes and neutrophils. Nature (London) 319:514-516.

Stacpoole, P.W., Alig, J., Ammon, L. and Cockett, S. 1988. Dose-response effects of dietary fish oil on carbohydrate and lipid metabolism in hypertriglyceridemia (Abstract). Diabetes 37 (Suppl. 1):12A.

Stadie, W.C., Haugaard, N. and Vaughan, M. 1953. The quantitative relation between insulin and its biological activity. J. Biol. Chem. 200:745-781.

Starke, K. 1981. Alpha-adrenoceptor subclassification. Rev. Physiol. Biochem. Pharmacol. 88:199-236.

Starke, K. and Endoh, T. 1976. Presynaptic alpha-adrenoceptors. Gen. Pharmacol. 7:307-312.

Starke, K., Montel, H. and Wagner, J. 1971. Effect of phentolamine on noradrenaline uptake and release. Naunyn-Schmiedeberg's Arch. Pharmacol. 271:181-192.

Steinberg, D. 1985. The endocrine pancreas. In: Best and Taylor's Physiological Basis of Medical Practice (Chapter 49), West, J.B., (ed.), 11th Ed., Williams & Wilkins, Baltimore, pp. 818-833.

Sterin-Borda, L., Canga, L., Borda, E., Gimeno, M.F. and Gimeno, A.L. 1980. Inotropic effect of PGI$_2$ on isolated atria at different contraction frequencies. Naunyn-Schmiedeberg's Arch. Pharmac. 313:95-100.

Sterin-Borda, L., Canga, L., Gimeno, M.F. and Gimeno, A.L. 1979. An adrenergic participation subserving a positive inotropism and chronotropism of PGI$_2$ on isolated rat atria. Experientia 35:529-530.

Sterin-Borda, L., Gimeno, M.F., Del Castillo, E. and Gimeno, A.L. 1981. Prostacyclin (PGI2) and U-46619 stimulate coronary arteries from diabetic dogs and their action is influenced by inhibitors of prostaglandin biosynthesis. Prostaglandins 22:267-278.

Straub, R.E. and Gershengorn, M.C. 1986. Thyrotropin-releasing hormone and GTP activate inositol trisphosphate formation in membranes isolated from rat pituitary cells. J. Biol. Chem. 261:2712-2717.

Streb, H., Irvine, R.F., Berridge, M.J. and Schulz, I. 1983. Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-triphosphate. Nature 306:67-69.

Strittmatter, W.J., Hirata, F. and Axelrod, J. 1981. Regulation of beta-adrenergic receptor by methylation of membrane phospholipids. In: Advances in Cyclic Nucleotide Research (Vol. 14), Dumont, J.E., Greengard, P. and Robison, G.A., (eds.), Raven Press, New York, pp. 83-91.

Sunagawa, R., Murakami, K., Minura, G. and Sakanashi, M. 1987. Effects of adrenergic drugs on isolated and perfused hearts of streptozotocin-induced diabetic rats. Japan. J. Pharmacol. 44:233-240.

Supattapone, S., Worley, P.F., Baraban, J.M. and Snyder, S.H. 1988. Solubilization, purification, and characterization of an inositol trisphosphate receptor. J. Biol. Chem. 263:1530-1534.

Tahiliani, A.G., Vadlamudi, R.V.S.V. and McNeill, J.H. 1983. Prevention and reversal of altered myocardial function in diabetic rats by insulin treatment. Can. J. Physiol. Pharmacol. 61:516-523.

Tomlinson, D.R. and Yusof, A.P.M. 1981. On the structural and functional integrity of autonomic nerves in rats with long-term diabetes mellitus induced by alloxan. Br. J. Pharmacol. 74:184P.

Turk, J., Wolf, B.A. and McDaniel, M.L. 1986. Glucose-induced accumulation of inositol trisphosphates in isolated pancreatic islets. Predominance of the 1,3,4-isomer. Biochem. J. 237:259-263.

Ui, M. 1986. Pertussis toxin as a probe of receptor coupling to inositol lipid metabolism. In: Phosphoinositides and Receptor Mechanisms. Putney, J.W., Jr., (ed.), New York, Alan R. Liss, Inc., pp.163-196.

Vadlamudi, R.V.S.V. and McNeill, J.H. 1983a. Effect of alloxan and streptozotocin-induced diabetes on isolated rat heart responsiveness to carbachol. J. Pharmacol. Exp. Ther. 225:410-415.

Vadlamudi, R.V.S.V. and McNeill, J.H. 1983b. Effect of experimental diabetes on rat cardiac cAMP,

phosphorylase and inotropy. Am. J. Physiol. 244:H844-H851.

Vadlamudi, R.V.S.V. and McNeill, J.H. 1984. Effect of experimental diabetes on isolated rat heart responsiveness to isoproterenol. Can. J. Physiol. Pharmacol. 62:124-131.

Vadlamudi, R.V.S.V., Rodgers, R.L. and McNeill, J.H. 1982. The effect of chronic alloxan- and streptozotocin-induced diabetes on isolated rat heart performance. Can. J. Physiol. Pharmacol. 60:902-911.

Vergara, J., Tsien, R.Y. and Delay, M. 1985. Inositol 1,4,5-trisphosphate: A possible chemical link in excitation-contraction coupling in muscle. Proc. Natl. Acad. Sci. USA 82:6352-6356.

Volpe, P., Salviati, G., Di Virgilio, F. and Pozzan, T. 1985. Inositol 1,4,5-trisphosphate induces calcium release from sarcoplasmic reticulum of skeletal muscle. Nature (Lond.) 316:347-349.

Wagner, J. and Brodde, O.E. 1978. On the presence and distribution of alpha-adrenoceptors in the heart of various mammalian species. Naunyn-Schmiedeberg's Arch. Pharmacol. 302:239-254.

Wald, M., Borda, E.S. and Sterin-Borda, L. 1988. Alpha-adrenergic supersensitivity and decreased number of alpha-adrenoceptors in heart from acute diabetic rats. Can. J. Physiol. Pharmacol. 66:1154-1160.

Wei, J.W. and Sulakhe, P. 1979. Regional and subcellular distribution of beta- and alpha-adrenergic receptors in the myocardium of different species. Gen. Pharmacol. 10:263-267.

Westermark, P. and Wilander, E. 1978. The influence of amyloid deposits on the islet cell volume in maturity onset diabetes mellitus. Diabetologia 15:417-421.

Wheeler, T. and Watkins, P.J. 1973. Cardiac denervation in diabetes. Br. Med. J. 4:584-586.

WHO Study Group. 1985. Diabetes mellitus. World Health Organization, Geneva, pp. 17-24 (Tech. Rep. Ser. no. 727).

Willcocks, A.L., Cooke, A.M., Potter, B.V.L. and Nahorski, S.R. 1987. Stereospecific recognition sites for [^{3}H]inositol(1,4,5)-triphosphate in particulate preparations of rat cerebellum. Biochem. Biophys. Res. Commun. 146:1071-1078.

Willcocks, A.L., Strupish, J., Irvine, R.F. and Nahorski, S.R. 1989. Inositol 1:2-cyclic,4,5-trisphosphate is only a weak agonist at inositol 1,4,5-trisphosphate receptors. Biochem. J. 257:297-300.

Williams, R.S. and Lefkowitz, R.J. 1978. Alpha-adrenergic receptors in rat myocardium. Identification by binding of [^{3}H]dihydroergocryptine. Circ. Res. 43:721-727.

Wilson, D.B., Connolly, T.M., Bross, T.E., Majerus, P.W., Sherman, W.R., Tyler, A.N., Rubin, L.J. and Brown, J.E. 1985. Isolation and characterization of the inositol cyclic phosphate products of polyphosphoinositide cleavage by phospholipase C. Physiological effects in permeabilized platelets and Limulus photoreceptor cells. J. Biol. Chem. 260:13496-13501.

Wise, B.C., Glass, D.B., Chou, C.H.J., Raynor, R.L., Katoh, N., Schatzman, R.C., Turner, R.S., Kibler, R.F. and Kuo, J.F. 1982a. Phospholipid-sensitive Ca^{2+}-dependent protein kinase from heart. II. Substrate specificity and inhibition by various agents. J. Biol. Chem. 257:8489-8495.

Wise, B.C., Raynor, R.L. and Kuo, J.F. 1982b. Phospholipid-sensitive Ca^{2+}-dependent protein kinase from heart. I. Purification and general properties. J. Biol. Chem. 257:8481-8488.

Woodcock, E.A., Mcleod, J.K., Smith, A.I. and Clark, M.G. 1987. Study of receptor-stimulated phosphatidylinositol hydrolysis in intact, perfused rat hearts. Clin. Exp. Pharmacol. Physiol. 14:209-213.

Woodcock, E.A., Smith, A.I., Wallace, C.A. and White, L.B.S. 1988. An unusual phosphatidylinositol turnover pathway in noradrenaline-perfused rat hearts. Clin. Exp. Pharmacol. Physiol. 15:251-255.

Yamada, S., Yamamura, H.I. and Roeske, W.R. 1980. Characterization of alpha-1 adrenergic receptors in the heart using [^{3}H]WB4101: effect of 6-hydroxydopamine treatment. J. Pharmacol. Exp. Ther. 215:176-185.

Yazaki, V. and Raben, M.S. 1975. Effect of thyroid state on the enzymatic characteristics of cardiac myosin. Circ. Res. 36:208-215.

Yoon, J.W., Austin, M., Onodesa, T. and Notkins, A.L. 1979. Virus-induced diabetes mellitus: isolation of a virus from the pancreas of a child with diabetic ketoacidosis. N. Engl. J. Med. 300:1173-1179.

Yoon, J.W., Eun, H.M., Essani, K., Roncari, D.A.K. and Bryan, L.E. 1987. Possible mechanisms in the pathogenesis of viruses-induced diabetes mellitus. Clin. Inves. Med. 10:450-456.

Yoon, J.W. and Notkins, A.L. 1983. Virus-induced diabetes in mice. Metab. 32:37-40.

Yoshida, Y., Huang, F.L., Nakabayashi, H. and Huang, K.P. 1988. Tissue distribution and developmental expression of protein kinase C isozymes. J. Biol. Chem. 263:9868-9873.

Yuan, S. and Sen, A.K. 1986. Characterization of the membrane-bound protein kinase C and its substrate proteins in canine cardiac sarcolemma. Biochim. Biophys. Acta 886:152-161.

Zeitler, P., Murphy, E. and Handwerger, S. 1986. Arachidonic acid stimulates $^{45}Ca^{2+}$ efflux and hpl release in isolated trophoblast cells. Life Sci. 38:99-107.

PUBLICATIONS

<u>Manuscripts</u>

Xiang, H., Rodrigues, B. and McNeill, J.H. (1988). Prevention of the effects of diabetes on rat cardiac performance and lipid metabolism by L-carnitine. Proc. West. Pharmacol. Soc. 31:157-159.

Rodrigues, B., Xiang, H. and McNeill, J.H. (1988). Effect of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. Diabetes 37(10):1358-1364.

Xiang, H., Heyliger, C.E. and McNeill, J.H. (1988). Effect of myo-inositol and T_3 on myocardial lipids and cardiac function in streptozotocin-induced diabetic rats. Diabetes 37(11):1542-1548.

Xiang, H. and McNeill, J.H. (1989). Calcium uptake activity of cardiac sarcoplasmic reticulum in myo-inositol-treated diabetic rats. Gen. Pharmacol. (in press).

Xiang, H., Black, S. and McNeill, J.H. (1989). Dietary fish oils fail to alter $alpha_1$-adrenergic receptor mediated phosphoinositide turnover in streptozotocin-induced diabetic rats. Gen. Pharmacol. (in press).

Xiang, H. and McNeill, J.H. (1990). $Alpha_1$-adrenoceptor-mediated phosphoinositide breakdown and inotropic responses in diabetic hearts. Am. J. Physiol. (submitted).

<u>Abstracts</u>

Xiang, H., Heyliger, C.E. and McNeill, J.H. (1987). Effect of myo-inositol treatment on myocardial lipid levels and cardiac function in STZ-diabetic rats. Presented at the 30th Annual Meeting of Canadian Federation of Biological Societies. p.150. WE-PO-126.

Xiang, H., Rodrigues, B. and McNeill, J.H. (1988). Effect of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. Presented at the 31st Annual Meeting of Western Pharmacology Society.

Xiang, H., Heyliger, C.E. and McNeill, J.H. (1988). Effect of myo-inositol (inositol) and/or triiodothyronine (T3) treatment on myocardial lipid levels and cardiac function in STZ-diabetic rats. Presented at the 31st Annual Meeting of American Physiological Society/American Society for Pharmacology and Experimental therapeutics (APS/ASPET). p.A152, 107.15.

Xiang, H., Heyliger, C.E. and McNeill, J.H. (1989). Time-
    and concentration-response studies of alpha1-
    adrenoceptor-mediated phosphoinositide breakdown in
    streptozotocin-diabetic rat heart. Presented at the
    32nd Annual Meeting of Canadian Federation of
    Biological Societies. p.98, 277.