ALTERATIONS IN CARDIAC RESPONSES TO ADRENERGIC AGONISTS

AND IN CIRCULATING THYROID HORMONES

IN ALLOXAN INDUCED DIABETIC RATS

by

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ABSTRACT

The effects of adrenergic agonists in the heart were investigated in isolated cardiac tissues and in the whole heart in 8 week diabetic and age-matched control rats by measuring contractile force following administration of PE in presence of timolol, or ISO. Plasma thyroid hormones as well as insulin, glucose and triglyceride was measured. The contractile force of the isolated cardiac tissues was measured in tissue baths under physiological conditions and results expressed by construction of concentration response curves to adrenoceptors agonists, 10^{-9} to 10^{-4}M for ISO and 10^{-7} to 10^{-3}M for PE. The contractile force of the whole heart was measured as left ventricular systolic pressure, without pharmacological intervention and following administration of 10^{-6}M PE or 10^{-5}M ISO in the perfusate. The hearts were clamp-frozen at 0, 15 and 30 seconds, and 1, 4 minutes following adrenoceptor agonists for measurement of cAMP levels. Cardiac cAMP was measured in the frozen ventricles by employing Amersham commercial SPA kit while protein was measured by using Bio-Rad Dye method. The tissue content of cAMP was expressed as pmol cAMP/mg protein.

It was found that the contractile response to PE was increased whereas that to ISO was decreased significantly in diabetic papillary muscle but not the left atria from the same group of animals. The chronotropic effect of PE was increased and that of ISO was decreased significantly in diabetic right atria. The contractile force of the whole heart preparation was increased in diabetic hearts following both adrenoceptor agonists and the increased positive inotropic effect of ISO was accompanied by an increase in cAMP levels. In all of the diabetic animals, the thyroid hormone levels were significantly lower than those of the controls. These findings confirmed that the cardiac alterations and the low thyroid state in ALX-induced diabetic rats are similar to those that occur in STZ-induced diabetic rats although further studies would be needed to reveal the interrelation between the diabetes-associated low thyroid state and diabetic cardiomyopathy.

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α	alpha
AC	adenylyle cyclase
ADP	adenosine 5'-diphosphate
ADR	adrenergic receptor
ALX	alloxan
ATP	adenosine trisphosphate
BW	body weight
β	beta
CA	catecholamine
Ca^{2+}	calcium
cAMP	cyclic adenosine 3',5' monophosphate
CAT	catalase
CEC	chlorethylclonidine
CI	cardiac index
CPM	counts per minute
DAG	diacylglycerol
Db-cAMP	Dibutyryl cyclic adenosine monophosphate
dl	decilitre
E	epinephrine
EC_{50}	concentration at which a drug produces half of the maximal effect
E_{MAX}	maximal effect
DNA	deoxyribonucleic acid
Fig.	figure
FOR	free oxygen radicals
FSK	forskolin
g	gram

G	GTP activated protein(s)
IP$_3$	inositol-1,4,5-trisphosphate
ISO	isoproterenol
IL	interleukin
I.V.	intravenous
Kg	kilogram
LA	left atria
LPL	lipoprotein lipase
LVP/LVSP	left ventricular (systolic) pressure
min	minute
mg	milligram
ml	millilitre
mm	millimetre
mmHg	millimetre of mercury
mM	millimolar
M	molar concentration, mole/L
ms	millisecond
N	number of the observations
NE	norepinephrine
nm	nanometre
P	probability
PE	phenylephrine
pD$_2$	agonist affinity constant
PK	protein kinase
PKA	cAMP dependent protein kinase
PM	papillary muscle
pmol	picomole
%	percentage

PX	peroxidase
RA	right atria
RIA	radioimmunoassay
rT_3	3,3,5'-triiodothyronine, reverse T_3
sec	second
SEM	standard error of mean
SOD	superoxide dismutase
SR	sarcoplasmic reticulum
STZ	streptozotocin
SZL-49	4-amino-6,7-dimethoxy-2-quinazolinyl-4-(2-bicyclo[2,2,2]octa-2,5 dienylcarbonyl-2-piperazine
TCA	trichloroacetic acid
TG	triglyceride
THs	thyroid hormones
T_3	3,5,3'-triiodothyronine
T_4	thyroxine
TRH	thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone, thyrotropin
μCi	microcurie
μl	microlitter
μM	micromolar
VW	ventricular weight
Wb4101	2-(2,6-dimethoxyphenoxyethyl)-amino-methyl-1,4-benzodioxane
<	smaller than
[ ]	concentration
[ ]p	plasma concentration

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DEDICATION

I wish to dedicate this thesis to my parents.

INTRODUCTION

1. CARDIAC ADRENERGIC RECEPTORS AND THEIR SIGNALLING CASCADES

The heart is under the regulation of the sympathetic and parasympathetic nervous systems. The former exerts its regulation by activating cardiac adrenoceptors (ADRs), including α- and β-ADRs via the sympathetic neurotransmitters norepinephrine (NE); the latter exerts its regulatory function in the heart via muscarinic cholinergic receptors (M). The activation of cardiac ADRs leads to positive chronotropic and inotropic responses; on the other hand, activation of cardiac M receptors is negatively linked to cardiac function. The predominant autonomic cardiac receptors mediating the inotropic responses are ADR while the predominant control of chronotropic effect is cholinergic under physiological conditions (Guyton 1990).

1.1 α and β-ADR in Regulation of Cardiac Function

ADRs consist of two types of receptors, α and β, based on their pharmacological characteristics (Alhquist 1948). Each type of ADR can be further divided into at least two subtypes, α_1 and α_2 (Berthelson and Pettinger 1977, Hoffman et al 1979) and β_1 and β_2 (Lands et al 1967). α_2 and β_2 also known as autoreceptors, mainly exist presynaptically, modulating the release of catecholamine (CA) from the nerve terminals negatively (α_2) or positively (β_2). The cardiac α_1- and β_1- ADRs are positively linked to the contractility and chronotropic response when the receptors are occupied. α_1-ADRs mediate positive inotropic effects of NE (Bruchner et al 1985) and as well are linked to the development of cardiac hypertrophy (Simpson et al 1985, Lee et al 1988). β_1-ADR mediate the positive inotropic and chronotropic responses.

1.2   Signalling Pathways of Cardiac ADRs

1.2.A   β-ADRs

The different types of ADRs exert their regulatory roles through biochemical pathways via cellular membrane products, known as "second messengers". All of the β subtypes are linked to cyclic adenosine 3',5' monophosphate (cAMP) formation via activation of a catalytic enzyme - adenylyl cyclase (AC) (Lands 1967). The binding of the β-ADRs leads to activation of AC via a guanine trisphosphate (GTP) binding protein - G_S. The activation of G_S results in dissociation of its α-subunit from its beta-gamma units and the α-subunit couples with the catalytic subunit of AC thus resulting activation of AC (). Conversely, α_2-ADRs are negatively linked to AC by coupling of the receptor to a G_i protein (reviewed by Berridge 1984). Cyclic AMP passes the signal on by activation of a cAMP-dependent kinase, protein kinase A (PKA). Increases in cAMP and PKA by CA are associated with increased contractility in rat and human heart (England and Shahid 1987, Murray et al 1987, Kaumann et al 1989).

1.2.B   α-ADRs

The α_1-ADRs in various tissues including the cardiomyocytes, can be further subdivided into at least two distinct subtypes, α_{1A} and α_{1B}. These subtypes can be distinguished on the basis of their sensitivity toward selective antagonists only since no absolutely selective agonists for these subtypes have been found. The α_{1A}-subtype has a higher affinity than the α_{1B}-subtype for the antagonists 5-methyl-urapidil, WB-4101 and (+)-niguldipine or the novel prazosin derivative, SZL-49. The α_{1B}-subtype is characterized

by its irreversible alkylation by CEC. Based on their respective sensitivity toward selective α_{1A}-antagonists, it was found that 20% of cardiac α_1-ADR belong to the α_{1A}-subtype and 80% of the binding sites correspond to the α_{1B}-subtype in rat (Groß and Hanft 1988; Groß et al 1988a). Evidence indicates at least two subtypes of pharmacologically distinct cardiac α_1-ADRs exist in other species as well (rabbit: Takanashi et al 1991; canine: del Balzo et al 1990). Furthermore, molecular cloning confirmed the existence of at least two subtypes of cardiac α_1-adrenoceptors encoded by two different genes (Lomasney et al 1991).

The nature of the α_1-ADR subtypes responsible for the positive inotropic effect is a matter of current investigation. In rabbit papillary muscle, the involvement of α_1B-subtype was suggested based on the concentration-dependent inhibition by CEC of the α_1-ADR-mediated positive inotropic effects (Takanashi et al 1991). The α_{1A} subtype may also participate in this effect, although to a smaller extent than the α_{1B}-subtype, since WB-4101, an α_{1A}-selective antagonist, was reported to shift the concentration-response curve of the positive inotropic effect of PE (Endoh et al 1992). However, in rat heart, the α_{1A} appears to be responsible for the α_1-ADR-mediated positive inotropic effects, as it was reported that only SZL-49 and WB4101, but not CEC inhibited the positive inotropic effect of NE in both papillary muscle (Rokosh and Sulakhe 1991) and isolated cells (Gambassi et al 1991).

The α_1-ADRs are coupled to at least two cellular mechanisms: the α_{1A}-subtype is linked to an increase in Ca^{2+} influx via its action on calcium channels (McGrath and O'Brien 1987, Han et al 1987, Tsujimoto 1989). On the other hand, α_{1B}-ADR is linked to hydrolysis of phosphoinositides by activation of phospholipase C (PLC), resulting in the production of two second messengers - a water soluble inositol 1,4,5-trisphosphate (IP3) and a hydrophobic product 1,2-diacylglycerol (DAG) from the cleavage of phosphotidylinositol 4,5 bisphosphate (PIP2) (Berridge 1982). The occupancy of the cardiac α_1-ADR involves several cellular events, such as modulation of ionic conductance (reviewed by Endoh 1991), intracellular alkalinization accounting for a shift of 0.1 pH unit at 30 μM PE or 100 μM NE

(Astarie et al 1991, Fuller et al 1991, Terzic et al 1992, Wallert and Frohlich 1992), regulation of glycogen and glucose metabolism (reviewed by Osnes et al 1985), stimulation of protein synthesis (Fuller et al 1990), and phosphorylation of cytosol proteins. It appears that the intracellular alkalinization of the cardiomyocyte via stimulation of Na^+/H^+ antiporter is closely related to α_1-ADR-mediated positive inotropic effect, probably mainly by phosphorylation of myosin light chain kinase (Pucéat et al 1990, Khalil et al 1992) or increasing the sensitivity of contractile proteins to Ca^{2+} rather than increasing $[Ca^{2+}]_i$ (Endoh and blinks 1988, Pucéat et al 1990, Gambassi et al 1992).

1.3   Adrenoceptor-Coupled Second Messengers in the Heart

1.3.A α_1-ADR-linked IP$_3$/DAG

The role of IP$_3$ as a second messenger in initiation of α_1-mediated contraction in smooth muscles has been generally recognized, and it may play the same role in the cardiomyocyte. Enhanced IP$_3$ production following α_1-ADR agonists has been shown in isolated rat ventricle cells (Brown 1985), cultured neonatal cardiomyocytes (Steinberg et al 1987), rat papillary muscle (Poggioli 1986) and many other cardiac tissue/cell preparations (Xiang and McNeill 1990). Studies on rat left atria showed that α_1-ADR stimulation increased the force of contraction and IP$_3$ formation to approximately the same extent (Scholz et al 1987). A later study demonstrated that this increased IP$_3$ precedes the increase in force of contraction in cardiac muscle (Schmitz et al 1987). The selective α_1-ADR agonist, phenylephrine (PE), in the presence of propranolol, has been shown to lead to rapid inositol phosphate formation and a triphasic inotropic response in a concentration-dependent manner in rat left ventricular papillary muscles. The α_1-receptor antagonist, prazosin or the PLC inhibitor neomycin blocked both PIP$_2$ hydrolysis and the triphasic inotropic response to

PE-increased IP$_3$ in the same tissue (Otani et al 1988). These findings suggest a role for IP$_3$ as a second messenger in the contraction of cardiac muscle; although some studies have argued against the role of IP$_3$ in releasing Ca^{2+} (Movsesian et al 1985). A study on saponin-skinned rat ventricular trabeculae showed laser photolysis caused a rapid increase in Ins(1,4,5)P$_3$ ("caged IP$_3$") and Ca^{2+}. The photolysis produced a small increase in the intracellular concentration of Ca^{2+} and a large and rapid force response. The source of the Ca^{2+} responsible for the increase in force of contraction was likely to be SR since the photo-induced contraction was blocked by ryanodine and caffeine. The IP$_3$-induced Ca^{2+} release from sarcoplasmic reticulum (SR) was smaller, slower and less consistent compared to Ca^{2+} required for contractile force, suggesting that IP$_3$ may modulate rather than directly stimulate Ca^{2+} release from SR (Kentish et al 1990). However, reports are consistent for a functional role of IP$_3$ in releasing calcium from SR in the heart via a direct effect on Ca^{2+} release and/or modulation of the release of Ca^{2+} from the SR.

Meanwhile, the co-product of IP$_3$ from hydrolysis of PIP$_2$, DAG, has been assessed in the heart. Okamura's group measured the cardiac content of DAG by employing thin layer chromatography and a flame ionization method following the activation by NE in the presence of various adrenergic antagonists, including phentolamine (α_1, α_2 blocker), prazosin (α_1 blocker), yohimbine (α_2 blocker) and propranolol (β blocker). It was found that DAG was significantly increased over 10-60 min following the application of NE (reaching 80% increase at 60 min). Meanwhile, NE did not increase other neutral phospholipids, such as cholesterol, cardiolipin, phosphatidylethanolamine, phosphatidylcholine or sphingomyosin. The NE-induced DAG accumulation was antagonized by phentolamine or prazosin and was partially antagonized by yohimbine, but not by propranolol. These findings provided the first direct evidence of an association of NE-induced DAG formation in rat heart with α_1-ADR. Besides its role in the activation of PKC (with Ca^{2+} as a co-activator), an increase in tissue content of DAG also releases fatty acids

including arachidonate, a precursor of prostaglandins, and other eicosanoids (Berridge 1984, Nishizuka 1984). However, the functional role of DAG in the heart is still unclear.

Early findings of Endoh and Blinks (1988) on the relation between the force developed and the amplitude of the Ca^{2+} transient induced by α_1-ADR agonists or other positive inotropic interventions, implied that the α_1-ADR agonists may increase the myofibrillar Ca^{2+} sensitivity. The increase in myofibrillar sensitivity to Ca^{2+} following the administration of α_1-ADR was later confirmed in single cardiomyocyte preparation (Capogrossi et al 1988). The definite evidence that α_1-ADR agonists produce a myofibrillar sensitization to Ca^{2+} ions was provided by Pucéat et al (1990) in isolated chemically skinned cardiomyocytes. It was found that PE increased the Ca^{2+} sensitivity of myofilaments independently of intracellular Ca^{2+}, thus α_1-ADR agonists are "sensitizing" cardiotonic agents (Pucéat et al 1990, 1992; Terzic et al 1992).

Pucéat et al (1990) demonstrated in skinned rat myocardial cells that the application of PKC cell-permeant activators prior to skinning increases the myofibrillar responsiveness to Ca^{2+}, as indicated by a leftward shift of the tension-pCa^{2+} relationship. Their finding implied the activation of PKC causes a phosphorylation of the contractile proteins, resulting in increase in sensitivity of myofibrils. The activation of PKC may not lead to phosphorylation of the contractile protein via a direct action but via activation of MLC kinase which specifically phosphorylates MLC-2 (Barany and Barany 1980) resulting in an increase in Ca^{2+} sensitivity of cardiac myofilaments (Morano et al 1985, Clément et al 1992).

In addition to phosphorylation of the contractile proteins, α_1-ADR agonists also could augment myofibrillar responsiveness to Ca^{2+} through their action of intracellular alkalinization. Based on the finding of the activation of the Na^+/H^+ antiporter by α_1-ADR agonists, several lines of evidence suggest that the activation of Na^+/H^+ antiporter participates in the positive inotropic effects of α_1-ADR agonists. First, inhibition of Na^+/H^+ exchange by selective blockers such as hexamethylamiloride and ethylisopropylamiloride

inhibit the increase in contractile force produced by PE in multicellular preparations (Terzic and Vogel 1990, 1991; Otani et al 1990) and in single cells (Gambassi et al 1992) by at least 50%. Second, the time course and magnitude of the α_1-ADR-mediated alkalinization closely correlates that of the positive inotropic effects (Terzic et al 1991, 1992; Gambassi et al 1992). Third, the degree of alkalinization caused by α_1-ADR agonists, 0.1 pH unit (Terzic et al 1992), is known to increase contractile force by several-fold in cardiac tissue (Vaughan-Jones et al 1987; Bountra and Vaughan-Jones 1989; Lagadic-Gossmann and Feuvray 1990). The precise role of PKC/DAG in α_1-ADR-mediated alkalinization and/or phosphorylation of contractile proteins is still a matter of current debate.

1.3.B β-ADR-linked cAMP

Since the original observation of Rall and Sutherland (1958) that the formation of cAMP in the heart is catalyzed by adrenaline, numerous studies have shown that cAMP plays an important role in the regulation of metabolism and function in the heart. It is now generally accepted that cAMP is the second messenger for the positive inotropic effects induced by β-ADR stimulation (Tsien 1977, Scholz 1980). The role of cAMP in β-ADR-induced positive inotropic effects has been established in human heart. Meinertz et al (1974) first demonstrated that dibutyryl cAMP, a membrane-penetrating cAMP analogue, causes positive inotropic effects in the isolated human left ventricular myocardium. In ventricular membrane preparations obtained from nonfailing hearts, isoproterenol (ISO) and noradrenaline activated AC (Bristow et al 1982, Kaumann et al 1982). Subsequently, it was shown that ISO caused an increased formation of cAMP not only in broken cell preparations but also in intact human right atria. In isolated, electrically driven right atrial strips, a submaximal positive inotropic concentration of ISO (300 nM) produced a rapid increase in intracellular cAMP. The peak of the cAMP increase was observed at 60 sec whereas the

increase in contractile force reached its maximum after 120-240 s (Ikezono et al 1987). In the presence of the phosphodiesterase (PDE) inhibitor, papaverine, both the increase in cAMP and the positive inotropic effect of ISO were enhanced and both responses were attenuated by propranolol. Thus, the criteria of Sutherland et al (1968) for a second messenger role of cAMP in the positive inotropic effects of β-ADR agonists were fulfilled on human heart.

It should be noted that there is still some controversy as to whether intracellular cAMP accounts for all the β-ADR-mediated effects in the heart. Besides PKA, the phosphorylation of at least four other proteins, such as troponin I (England 1983, Garvey et al 1988), C-protein (Garvey et al 1988, Schlender et al 1988), phospholamban (Garvey et al 1988, Demaille and Pechere 1988) and the L type Ca^{2+}-channel (Catterall et al 1988, Sperelakis 1988), have been found to be cAMP-dependent. The functional relevance of the phosphorylation of these proteins to the positive inotropic effect of β-ADR induced cAMP is not yet clear. However, it is too early to conclude that these proteins are part of cAMP signalling system in the heart.

2.   ALTERATIONS OF ADRENOCEPTOR AND THEIR SIGNALLING PATHWAYS IN DIABETIC CARDIOMYOPATHY

Cardiovascular diseases have been a major cause of death in human diabetics in North America (Kessler 1971) and mortality due to cardiovascular diseases in the diabetic population is two to three fold higher than in the non-diabetic population (Kannel et al 1984). Among the cardiovascular diseases related to diabetes are autonomic neuropathy, cardiomyopathy and micro- and/or macro-angiopathy.

It has been found that 17% to 40% of diabetics manifested various degrees of cardiac autonomic neuropathy (Hulper and Wilms 1980, Dyrberg et al 1981, Hilsted and Jensen

1979) which has been related to cardiac disease in diabetes (Ewing et al 1974, Allison et al 1986). It has been demonstrated that 50% of diabetics diagnosed with autonomic neuropathy die within 2.5 years (Ewing et al 1980). ADR binding studies using $(-)^3$H-dihydroalprenolol showed a significant 28% reduction in the number of ventricular β-receptor binding sites in STZ-induced diabetic rats. The reduced diabetic ventricular β-receptor maximal binding, which was 39 fmol/mg tissue versus that of the control of 54 fmol/mg, occurred without changes in affinity (Savarese and Berkowitz 1979). A reduced number of cardiac β-adrenoceptors is supported by similar findings in STZ-induced diabetic rats (Heyliger et al 1982, Williams et al 1983, Sundaresan et al 1984, Atkins et al 1985, Ramandham and Turner 1986, Wald et al 1989) and spontaneously diabetic Bio-Breeding rats (Durante et al 1989). These early reports led to intensive studies in the cardiac alterations in pharmacological responses in diabetes as a manifestations of diabetic cardiomyopathy related to the sympathetic neuropathy.

The existence of a diabetic cardiomyopathy was first suggested by Rubler et al in 1972 based on postmortem findings in four diabetic adults who suffered from congestive heart failure in the absence of atherosclerotic, valvular, congenital, hypertensive, or alcoholic heart diseases. Further support was provided by Hamby et al (1974), who noted that the incidence of diabetes in patients with idiopathic cardiomyopathy was twice as high as in the age- and sex-matched cohort without diabetes. An up-to-date definition of diabetic cardiomyopathy refers to it as a myocardial failure in diabetes independent of atherosclerotic coronary artery disease, valvular disease, or hypertension (reviewed by Lebet et al 1979, Jackson et al 1985, Zarich et al 1989).

Intensive studies have demonstrated certain functional and ultrastructural abnormalities in diabetic hearts. The ultrastructural abnormalities include increased lipid deposition, a loss of contractile protein, vacuolization (swollen sarcoplasmic reticulum), myosin formations, myo-cytolysis and contracture bands. Functional alterations are

characterized by a mechanical alteration which manifests as a decreased rate of contraction and delayed relaxation (Fein et al 1980), altered ability to respond to an autonomic stimulus which includes a decreased response to β-ADR agonists, hypersensitivity to α-ADR agonists (Sararese and Berkowitz 1979, Heyliger et al 1982) and a reduced high affinity form of cholinergic receptors (Williams et al 1983).

2.1   Alteration of β-Adrenergic Responses

The study of Foy and Lucas (1979) in pithed alloxan (ALX) or streptozotocin (STZ)-induced diabetic rats was the first which indicated a reduced sensitivity to the pressor effects of norepinephrine and to the positive inotropic and chronotropic effects of isoproterenol (ISO) in diabetic animals. This decreased response to β-ADR agonist can be attributed to the reduced number of receptors, uncoupling of G_s which mediates AC activation and a decrease in activity of AC which results in a decreased cAMP formation (Atkins et al 1985, Ramanadham and Tenner 1985). Receptor binding studies revealed a significant 28% reduction in the number of ventricular β-receptor sites which correlated well with a 24% decrease in in vivo heart rate. A later report clearly demonstrated a simultaneous reduction in the number of ventricular β-receptor and a reduced responsiveness to isoproterenol (Ramanadham and Tenner, 1987). The activity of cardiac adenylate cyclase (AC) was determined by measuring both basal accumulation of cAMP in cardiac tissues and activity of the enzyme in the presence of NaF, forskolin, GTP, or Gpp(NH)p (Atkins et al 1985, Wald et al 1989, Nishio et al 1988, Vadlamudi and McNeill 1983). It was found that cAMP formation in diabetic animals did not differ from that in the control animals, indicating an unaltered AC in diabetes. Another report of measurement of cardiac AC in isolated cell particles from 8 week diabetes rat using the same agents showed similar results, in addition, a depression of epinpherine-induced AC in diabetic heart (Smith et al 1984) supporting an

earlier report of decreased AC in diabetic heart (Menahan et al 1977, Gotzsche 1983). It has been postulated the reduced β-ADR response may be due to an uncoupling of the receptor from G_S protein (Wald et al 1989, Michel-Reher et al 1993). However, the underlying mechanism of the reduced β-responsiveness is not clear yet.

2.2 Alteration of α_1-Adrenergic Responses

To further investigate cardiac adrenergic impairment in diabetes, the α-ADR system has also been studied. In the hearts of STZ-induced diabetic rats, an increased contractile response to an α_1-receptor agonist has been observed (Jackson et al 1986, Yu and McNeill, 1990). The increase in the α_1-mediated contractile response might be attributed to an increase in the number and/or the increase in binding affinity of the receptor, but this does not seem to be the case. Binding studies on cardiac tissues of rats with a duration of diabetes from 72 hours up to six months demonstrated a reduced number of α-ADR starting as early as 72 hours (Heyliger et al 1982). However, studies have indicated that α-ADR responsiveness in the diabetic heart is enhanced. Left atria of STZ-induced diabetic rats were supersensitive and hyperresponsive to α_1-receptor stimulation by phenylephrine (Jackson et al 1986). This finding of supersensitivity to α-receptor stimulation has been supported by several other studies (Downing et al 1983, Canga and Sterin-Borda 1986, Wald et al 1988). The observations of reduced numbers of α-adrenoceptors and an increased sensitivity to α-ADR stimulation in diabetic heart were postulated to be due to an increased affinity of α-adrenoceptors (Wald et al 1988). However, most studies have demonstrated a decreased or unchanged affinity of the α-ADR (Heyliger et al 1982, Williams et al 1983, Durante et al 1989, Latifpour and McNeill 1984). The increased responsiveness to α-ADR agonists in the absence of affinity changes suggest that the supersensitivity provoked by α-ADR agonists may be due to post-receptor mechanisms in diabetic heart, resulting in an increase in α-

receptor linked second messenger or other actions, such as effects on the calcium channel or on the phosphorylation of cytosolic contractile proteins.

An alteration of α-receptor mediated second messengers might be responsible for the increased contractile response in diabetic heart. An increase in IP_3 formation was observed concomitantly with the increase in contractile response to α_1-adrenoceptor stimulation in diabetic ventricle (Xiang and McNeill 1991); on the other hand, an elevation in myocardial DAG was observed in rat at 4 weeks but not at 8 weeks of diabetes and it was also suggested that insulin increased DAG in the heart (Okumar et al 1988). The elevated DAG in diabetic heart may activate PKC which is known to phosphorylate proteins within the myocyte involved in intracellular calcium metabolism (Movsesian et al 1984), resulting in a greater contractile response to α-ADR. The increase in α_1-ADR mediated signalling also suggests that phospholipase C activity may be changed in diabetic heart, possibly as a result of the known supersensitivity to cardiac α-ADR agonists. The effect of an elevated cardiac diacylglycerol content is unknown but conceivably myocardial proteins regulated by PKC-mediated phosphorylation would be affected, which could ultimately affect cardiac function or responsiveness to α-ADR stimulation.

3. ALTERATION OF CARDIAC ADRENERGIC RESPONSES AND THYROID HORMONES

3.1 Cardiac Adrenergic Responses and Thyroid State

The sensitivity to catecholamine in various tissues, including rat heart, can be altered in hyper- or hypothyroidism. It has been known that β-ADR mediated cardiac responses are reduced while α-ADR responses are increased in hypothyroidism. Stiles et al (1984) reported that the number of β-ADR is decreased in rat heart from hypothyroid animals. This change in the number of β-ADR was associated with decreased isoproterenol-induced

contractility and a decrease in cAMP accumulation without any alteration in calcium-stimulated contractility. In addition, the basal, isoproterenol-induced, and sodium fluoride-induced AC activities were all significantly lower in membranes obtained from hypothyroid animals (Brodde et al 1980). Since NaF activates AC through the G_S protein without affecting the receptor itself, these findings suggest that the reduced number of β-receptors may not be the only impairment underlying the reduced cardiac contractile response to ISO in hypothyroid animals. This notion was not supported by the finding that there was no alteration of coupling of β-ADR to AC in the heart of hypothyroid rats; although, a similar significant decrease in the number of β-ADR was observed as that seen by the other groups (Stiles and Lefkowitz 1981). Studies on ventricular slices from hyperthyroid rats with protein synthesis inhibitors led to the postulation that hyperthyroidism can increase the number of β-ADR by permitting the expression of spare-receptors on the surface of the cell, and by stimulating the synthesis of the new receptors (Bilezikian et al 1983). Reports on the association of β-ADR and hypothyroidism seem to be less conclusive. The underlying mechanism of the concomitant reduced β-ADR number and hypothyroidism may involve G_S protein since a functional impairment of G_S protein has been found in hyperthyroidism and a 40% reduction of G_S protein in hypothyroid tissue (Stiles et al 1981).

3.2   Low Thyroid Hormones in Diabetes

Decreased thyroid hormones were found in ALX- or STZ-induced diabetic rats (Serif & Sihotang 1962, Kumaresan & Turner 1966, Pericas 1977, Jolin & Gonzalez 1978). Decreased circulating thyroid hormone levels have also been reported in the diabetic human (Saunder et al 1978). It was found that in diabetic patients, the circulating thyroxine (T_4) and triiodothyronine (T_3) were low, but reversed triiodothyronine (T_3) was elevated and all three thyroid hormones returned to within normal range following insulin treatment. The T_3 level

was directly related to glucose utilization and metabolic clearance rate; moreover, it was inversely related to the plasma ketone body concentration. A later study in humans suggested a decreased de-iodonization of T_4, as well as a decrease in rT_3 clearance rate, may contribute to a decreased T_3 and increased rT_3 in diabetes (Garvin et al 1989, Kabadi 1986).

The hypothalamic-pituitary-thyroid axis was studied in the STZ-induced diabetic rat by measuring plasma binding iodine (PBI), plasma and pituitary thyrotropin (TSH) and hypothalamic thyrotropin-releasing hormone (TRH) and compared with thyroidectomized rats. It was found that 10 days after STZ injection, all of these parameters, including TRH, plasma TSH and pituitary TSH, were reduced in the diabetic group, implying that mechanisms other than a decrease in peripheral deiodonization of T_4 may play a role in low thyroid state in diabetes. It was also found that in thyroidectomized diabetic rats, circulating TSH was lower as compared to thyroidectomized controls, suggesting that damage at higher sites of the hypothalamic pituitary-thyroid axis might also occur. Since the effect of STZ on the tissue content of TSH and TRH was dose-dependent, the possibility of a direct effect of STZ on the hypothalamus and pituitary gland cannot be excluded, although these alterations in hypothalamic-pituitary-thyroid axis were suggested to be related to insulin deficiency and/or hyperglycaemia because insulin administration restored these abnormalities to normal (Gonzalex et al 1980). The multi-site damage to the hypothalamic-pituitary-thyroid axis in STZ-induced diabetes was supported by at least one other group (Mitsuma & Gogimori 1982), who demonstrated that in long term STZ-induced diabetic rats, plasma levels of TRH, TSH, T_4 and T_3 were significantly decreased while rT_3 remained unchanged and in addition, insulin restored these alterations partially at a dose which controlled plasma glucose to normal. These findings indicate that a decreased deiodonization of T_4 may not be the major causative factor underlying the changes in the hypothalamic-pituitary-thyroid axis in diabetes.

3.3   Low Thyroid State and Cardiac β-Adrenoceptor Signalling in Diabetes

To reveal the mechanisms underlying the decrease in the β-adrenergic response in relation to the low thyroid state found in diabetics, the effect of STZ-induced diabetes on the β-adrenoceptor-coupled AC was studied in rat heart particulate fractions. It was found that in diabetic heart, the number of β-ADR was reduced by 34% with no change in affinity for dihydroalprenolol and the maximal ISO-activated cAMP was decreased by 10%. Plasma thyroid hormones were also decreased in the diabetic rat. Insulin administration to diabetic rats restored the number of β-ADR and thyroid hormones to near or above normal (Sundaresan et al 1984). It was hypothesized that the decreased β-ADR response in diabetes may be caused by low circulating thyroid hormones.

In an attempt to restore the decreased β-receptor mediated contractile response in diabetic heart, thyroid hormones and cardiac contractile force were measured in the STZ-induced diabetic rat with or without T_3 administration. The results indicated that the treatment with T_3 alone did not improve cardiac function in diabetic rats although the T_3 level was restored to normal or above normal (Tahiliani and McNeill 1984, Xiang et al 1988). In addition to the fact that T_3 replacement failed to improve cardiac function in diabetes, the role of low thyroid state was challenged by a later study comparing cardiac adrenergic responses in diabetic rats and hypothyroid rats (Sato et al 1988). It was found that in diabetic atria, the maximal effect (E_{max}) of norepinephrine (NE) was decreased without any significant alteration in EC_{50} and a decrease in E_{max} to Ca^{2+} occurred in a similar fashion in spite of a small decrease in number of the β-adrenoceptors. The most convincing evidence was that T_4 administration which was sufficient to restore the contractile responses to transmural nerve stimulation and to NE in hypothyroid atria, did not improve the contractile responses in diabetic tissue. These results are in agreement with reports (Tahiliani and McNeill 1984, Xiang et al 1988) that the contractile responses to ISO did not improve

with T3 administration in the diabetic rats. Another study of T3 replacement in the STZ-induced diabetic rats demonstrated that T3 treatment led to decreases in plasma glucose and lipids in diabetic rats, as well as to tachycardia. It was found that the positive chronotropic and inotropic effects of methoxamine were reduced in diabetic hearts and these reductions were prevented by T3 replacement. In the same study, it was also demonstrated that T3 administration decreased the responsiveness to ISO in control rats, and T3-treated diabetic rats showed a further reduction in the cardiac response to ISO (Goyal et al 1986). The findings in the study of Goyal (Goyal et al 1986) indicated that some of the diabetes-induced alterations in adrenergic activity can be restored by thyroid hormone replacement, but not the response to ISO.

As described above, the relation of low thyroid state in diabetes to the alterations in the cardiac ADR-mediated responses in diabetes remains to be clarified.

4. CHEMICAL-INDUCED DIABETES AND FREE RADICALS

It has been known that numbers of structurally diverse diabetogenic chemicals exist which selectively damage pancreatic β-cells in various species. These chemicals include ALX, STZ and others, such as chlorozotocin, vacor and cyproheptadine. The underlying mechanisms for the destruction of β-cells by these diabetogenic chemicals include (a) generation of free oxygen radicals (FOR) and alteration of the endogenous scavengers of these reactive species, (b) breakage of DNA and consequent increase in the activity of poly-ADP ribose synthetase, an enzyme depleting nicotinamide adenine dinucleotide in β-cells and (c) inhibition of active calcium transport and calmodulin-activated protein kinase activity (Yoon et al 1987).

In a biological system, FOR can be formed in the respiratory burst of phagocytic cells and released into the extracellular space; they can also be formed during mitochondrial

oxidative metabolism, ischemia, and irradiation (Halliwell 1991). In aerobic organisms, there are various defense mechanisms against extra- and intracellular FOR. Superoxide dismutase (SOD), catalase (CAT) and peroxidases (PXs) are important components of these defense mechanisms, which catalyse FOR to their less harmful form or to water (Grankvist et al 1979, Del Maestro 1980). In general, when the intracellular concentration of FOR exceeds the endogenous scavenger system, they may have deleterious effects on the cell. In mammalian cells, the tissue contents of the defensive enzymatic systems vary, which results in a great variation in susceptibilities of the tissues to the free radicals. Furthermore, the activity of SOD, PXs and CAT can be 2-10 times lower in pancreatic β-cells compared to other tissues such as liver or kidney (Malaisse et al 1982, Asayama et al 1986, Godin et al 1987 & 1988). Thus, the β-cells are more susceptible to the deleterious effects of FOR, resulting in diabetes when exposed to excess FOR. It has been demonstrated that the activities of these scavenging enzymes are relatively lower in the heart (Godin et al 1987, 1988) than in other organs.

4.1   Diabetogenic Effects of Alloxan

Alloxan (ALX), also called mesoxalyl urea, mesoxalyl carbamide, 2,4,5,6-tetraoxohexahydropyrimidine or pyrimidinetetrone, is an unstable and reactive chemical substance that exists in several tautomeric forms. ALX was demonstrated to cause selective damage to the pancreatic β-cells, resulting in diabetes in the rat (Dunn & McLetchie 1943), the rabbit (Bailey & Bailey 1943) and the dog (Goldner & Gomori 1943). ALX-induced diabetes has the classical manifestations of type I diabetes, characterized by hypoinsulinemia, hyperglycemia, glucosuria, polydipsia, polyuria, loss in body weight despite polyphagia, hyperlipidemia, ketonuria and acidosis.

In addition to its inhibition of insulin secretion, ALX also directly inhibits both calcium uptake by the islet cell endoplasmic reticulum (Cola et al 1982) and calmodulin-activated protein kinase activity, resulting in immediate and irreversible alteration of β-cell function (Pershadsingh et al 1980, Landt et al 1982). Once taken into the cell, ALX is reduced to form dialuric acid and the autooxidation of dialuric acid yields hydrogen peroxide (H_2O_2), superoxide anion (O_2.) and hydroxyl free radicals ($OH^{\cdot}$) (Cohen & Heikkila, 1974, Malaisse 1982). These oxygen species are highly reactive and interact with a variety of intracellular molecules including the bases of DNA forming hydroxyl adducts. Chemiluminescence in the presence of luminol and ALX has been measured in isolated pancreatic islet cells (Asayame et al 1984). ALX-induced chemiluminescence in islets appeared rapidly and lasted more than five minutes while STZ failed to produced chemiluminescence. SOD and/or CAT markedly suppressed ALX-induced chemiluminescence. ALX-induced chemiluminescence was greater in islets than in red blood cells and in hepatic cells, suggesting the islet cell produced more radicals in the presence of ALX (Asayama et al 1984b). The measurement of luminescence as an index of toxicity of ALX demonstrated that reduced glutathione (GSH) and sulphate ion were needed for maximum luminescence. It was also demonstrated that various metal chelators and sugars and NAD, NADH, NADP and NADPH prevented chemiluminescence (Grankvist 1981). These results suggested that ALX may act via a thiol group and the chemiluminescence effect of ALX was mediated by $OH^{\cdot}$.

In vitro studies in isolated islet cells measuring rubidium accumulation and Trypan blue exclusion support a role of FOR in ALX-induced diabetes. Diethylenetriamine-pentaacetic acid (DETA-PAC) protected islet cells from the cytotoxicity of ALX (Grankvist et al 1979a). Copper(II) which catalyses production of $OH^{\cdot}$ from the Haber-Weisse reaction, mimicked the effect of ALX and the effect was additive with ALX (Fisher and Hamburger 1980a). Hydroxyl scavengers but not scavengers of singlet oxygen protected the islet cells

from ALX attack (Grankvist et al 1979b). SOD and/or CAT suppressed ALX-induced chemiluminescence and inhibition of rubidium uptake and Trypan blue exclusion (Fisher and Hamburger 1980b). These observations suggested that the cytotoxic effect of ALX on islet cells may occur via the Haber-Weisse reaction.

In vivo studies also support a role of FOR in the diabetogenecity of ALX. It was shown that hydroxyl radical scavengers prevented diabetes in mice injected with ALX and the protective action of these agents correlated with their hydroxyl radical scavenging ability (Heikkila et al 1976; Tibaldi et al 1979; Heikkila and Cabbat 1978 and 1980; Mechkstroth et al 1980). Exogenous SOD also blocked diabetogenic action of ALX (Grankvist et al 1981) and the morphological damage produce by ALX (Thaete et al 1985). Administration of DETA-PAC prior to ALX prevented the diabetes in mice but another metal chelating agent, EDTA which does not block the hydroxyl production provided no protection against ALX (Heikkila and Cabbat 1982).

Thus, it is generally accepted that ALX induces diabetes via production of FOR by the Haber-Weisse reaction in the pancreatic β-cells.

4.2   Diabetogenic Effect of STZ

STZ, 2-deoxy-2-(3-methyl-3-nitrosoureido)-D-glucopyranose, is a broad-spectrum antibiotic isolated from Streptomyces achronogenes that has been preferably used over ALX as diabetogenic drug in the experimental study of diabetes due to its higher selectivity for β-cells (Junod et al 1969) and lower rate of mortality when used to induce diabetes in rodents (Hoftiezer and Carpenter 1973). As a cytotoxic nitrosourea compound, STZ has carcinogenic and diabetogenic properties. The glucose moiety (2-deoxy d-glucose) present in the STZ molecule is essential for its diabetogenic action and selectivity for the pancreatic β-cells. When injected in single dose of >60 mg/kg, STZ destroyed pancreatic β-cells selectively with

loss of secretory granules and aggregation of nuclear chromatin within hours (Lazarus and Shapiro 1972). Its diabetogenic action has been shown in various species including rats, dogs, mice, guinea pigs, hamsters, monkeys (Rakieten et al 1963, Brodsky & Logothetopoules 1968, Little 1966, Schien et al 1967).

4.2.A Non-free radical mechanisms

It has been proposed that the cytotoxicity of STZ is due to depression of intracellular levels of NAD and NADP (Anderson et al 1976, Okamoto 1981). The N-nitrosoureado moiety of STZ breaks down into the methyl radical (CH_3.) (Uchigata et al 1982) which can alkalize and cross-link DNA and inhibit incorporation of precursors into DNA (Calabresi and Parks 1980, Weiss 1982). STZ may deplete NAD by at least two mechanisms. The methyl radical was demonstrated to methylate nicotinamide, which is the precursor of NAD and NADP moieties (Chu and Lasley 1974 and 1975), reducing the intracellular nicotinamide levels. A study in mouse islets using 5-methylnicotinamide, a nicotinamide analogue which inhibits NAD degradation but can not produce NAD, suggested an increase in degradation of NAD following STZ exposure (Hinz et al 1973). A second mechanism of the NAD-depleting effect of STZ involves DNA damage. STZ causes DNA cross-linking and strand scission (Okamoto et al 1982, Uchigata et al 1982, Yamamoto 1981), which is not blocked by SOD or CAT. Poly(ADP)-ribose.

The DNA damage activates poly(ADP-ribose) synthetase, which requires NAD as a cofactor in DNA repair. The inhibitors of this enzyme blocked the depression of NAD by STZ in vitro (Yamamoto et al 1981). The depression of cytosolic NAD has been shown to lead to an inhibition of proinsulin synthesis, but to allow for repair for DNA damage, while inhibition of poly(ADP-ribose) synthetase inhibited DNA repair but allowed proinsulin synthesis to continue (Yamamoto et al 1981). A later study demonstrated that poly(ADP-

ribose) synthetase inhibitors prevented diabetes by STZ or ALX without affecting DNA damage in the islet cells; one year later, diabetes had not occurred but β-cells tumors were detected (Okamoto and Yamamoto 1983).

4.2.B Free radical mechanism

Studies suggested that STZ may exert its diabetogenic action via production of free radicals. The glucose moiety of STZ allows it to be selectively taken into the pancreatic β-cells. Once internalized into the β-cells, STZ undergoes spontaneous breakdown to yield methyl radical (CH3.) which is very reactive. Among other interactions, CH3. attacks DNA by alkylating its purine and pyrimidine bases to form adducts. The measurement in islets incubated with STZ, showed that GSH levels were decreased 33%, oxidized glutathione (GSSG) increased 95%. In support to this finding, it was found in STZ-incubated isolated islets, there is 21% reduction of GSH and 74% reduction of GSSG when compared to that of the control cells (Slonim et al 1979). Nicotinamide administration prevented the decrease in GSH in erythrocytes exposed to STZ (Slonim et al 1976). Thus, these findings suggested an increased activity of glutathione reductase recycling system which leads to improvement of GSH levels. A variety of free radical scavengers has also been shown to block the diabetogenic action of STZ in vivo, including SOD (Grandy et al 1982, Robbins et al 1980) and the hydroxyl radical scavenger (Sandler and Anderson 1982, Sandler 1984) and Vitamin E (Slonim et al 1983).

Thus, through different pathways ALX and STZ alter the DNA of β-cells and cause cell damage by inducing strand breaks. Such DNA damage is repaired by a group of enzymes including poly(ADP)-ribose synthetase, which is responsible in DNA repairing process and requires NAD as a cofactor. Its sustained activation during DNA repair depletes the intracellular pool of NAD to levels that lead to β-cell damage and necrosis (Okamoto 1982,

1985). This notion is supported by the observation that SOD (Grankvist et al 1979) and inhibitors of poly(ADP)-ribose synthetase such as nicotinamide prevented the depletion of NAD and death of β-cells as well as onset of diabetes mellitus (Uchigata et al 1983). Moreover, direct evidence supporting the suggestion that ALX or STZ cause DNA fragmentation via free radicals was provided. The generation of H_2O_2 was greatly increased, as was fragmented DNA along with a reduced normal DNA, in isolated rat pancreatic islets when ALX or STZ were added into the incubation media. H_2O_2 mimicked the effects of ALX and STZ on DNA breakdown in the same preparation. These effects of ALX or STZ on the H_2O_2 and DNA fragmentation were concentration and time-dependent, with a Emax > 1mM and taking 10-15 min to generate a significant increase in H_2O_2 (Takasu et al 1991).

Taken together, studies suggest that ALX and probably STZ, exert their free radical diabetogenic action via the following mechanisms (Fig.1): (1) peroxidation of polyunsaturated fatty acid in the plasma membrane, (2) FOR flux induced lysosomal membrane disruption with autophagocytic vacuole formation, (3) DNA degradation, (4) alteration in activity of membrane phospholipase resulting in release of prostaglandins and various endoperoxides, (5) release of lipid peroxidation products to the extracellular space resulting in increased vascular permeability and leukocyte chemotaxis (Del Maestro 1980). The precise radicals mediating the diabetogenecity of these chemicals remain to be confirmed. However, the general belief is that ALX-induced diabetes in rats is more severe in degree and mortality is higher when compared with that of STZ. However, the alterations in cardiac function in ALX-induced diabetes are not as well characterized as those found in the STZ-induced diabetes.

Recently, Kawada proposed a hypothesis on the mechanisms underlying the diabetogenicity of ALX and STZ (Kawada 1992), suggesting that STZ inhibits xanthine oxidase and ALX exerts its diabetogenic action mainly via activation of the cytochrome P-450 system. STZ is transported into pancreatic β-cells through the glucose transporter on the

cell membranes and attacks mitochondria; thus, mitochondrial ATP generation is inhibited. The resulting high concentration of intracellular ADP causes its degradation, which provides hypoxanthine, a substrate of xanthine oxidase (XOD). The activity of XOD is intrinsically high in β-cells; then, the XOD-catalyzed reaction proceeds as shown by the increased formation of uric acid and O_2. free radical. The low activity of SOD in β-cells make them inefficient in scavenging FOR. Besides its action on mitochondria, STZ also directly activates XOD to enhance O_2- generation; therefore, pancreatic β-cells suffer from a double attack by O_2. and hydroxyl radicals derived from the former when exposed to STZ (Barker et al 1984, Nukatsuka et al 1988). Allopurinol, an inhibitor of XOD, can protect animals from the diabetogenic effect of STZ (Hashimoto 1974, Nukatsuka et al 1990). The diabetogenicity by ALX anion radicals, generated from ALX reduction, is probably mediated by the action of the microsomal cytochrome p-450 system. These radicals have a long half-life and directly damage DNA in vitro (Nukatsuka et al 1989, Kawada 1992). This hypothesis excluded the widely accepted hypothesis that the cause of ALX-induced diabetes is attributed to O_2-radicals formed by reduction of ALX because ALX anion radicals appears to be very potent scavengers of O_2. radical. However, confirmation is needed for this hypothesis.

5. CARDIAC ALTERATIONS IN ALX-INDUCED DIABETES

5.1 Early Observations

Foy and Lucas (Foy and Lucas 1976) reported the first in vivo observation of reduced positive chronotropic and inotropic effects of isoproterenol in ALX-induced diabetic rats. This was thought to be related to hyperglycemia since fasting increased the ISO reponses in the diabetic animals significantly. Studies employing the isolated working heart demonstrated numerous cardiac functional alterations in ALX-induced diabetes, including an impaired

cardiac output in 3 day ALX diabetic hearts without apparent effect on coronary flow (Miller 1979) and depressed rate of contraction (Ingebretsen et al 1980). It was also found that ISO infusion increased coronary flow and left ventricular pressure to the same extent in both ALX-induced diabetic and the control hearts (Ingebretsen et al 1981).

5.2   Autonomic Neuropathy in Diabetic Heart

Examination of the innervation of the atria from long-term ALX-induced diabetic rats revealed that contractile responses to sympathetic nerve stimulation and that to exogenous NE in diabetic RA were unaltered compared to the control tissue. Diabetic LA showed supersensitivity to both NE and acetylcholine and an unchanged positive inotropic response to sympathetic nerve stimulation. Observations using the electron microscope (EM) revealed degeneration of noradrenergic profiles in the diabetic atria (Tomlinson and Yusof 1983). These degenerated NE nerve ending may be involved in the suppressed cardiac function in ALX-induced diabetes.

5.3   Diabetic Cardiomyopathy and Possible Causative Factors

5.3.A   Diabetic cardiomyopathy - Functional and Pharmacological alterations

Cardiac performance has been studied at various time points following ALX or STZ injections. It was found that at 30, 100 and 240 days following ALX injection, LVP, +dP/dt and -dP/dt were depressed at higher atrial filling pressures. The same alterations were observed in hearts from STZ-treated rats after 100 days of diabetes (Vadlamudi et al 1982).

In contrast to the early in vivo observations of a decreased ISO response in ALX-induced diabetic rats by Foy and Lucas (1976), a later study using the isolated perfused

working rat heart demonstrated that the sensitivity and responsiveness of the diabetic myocardium to ISO did not differ from that of the controls (Vadlamudi and McNeill 1984). On the other hand, ALX-induced diabetes was shown to be associated with enhanced positive chronotropic and inotropic cardiac responses to exogenous catecholamines (Neshcheret 1985). However, studies on diabetic rabbits suggested a reduced chronotropic response to ISO in ALX-induced diabetic hearts (Zola et al 1988) in agreement with the findings of a reduced basal heart rate and reduced cardiotoxic potential of ISO in diabetic mice (El-Hage et al 1985). The isolated left atria from ALX-induced diabetic rabbits also demonstrated a decreased sensitivity to β-adrenergic stimulation as a rightward shift of the concentration-response curves following administration of ISO and NE although the circulating thyroid hormones were not reduced (Grassby and McNeill 1988). In the same study, it was showed that the concentration-response curves obtained by addition of phenylephrine were unchanged.

5.3.B β-ADR mediated intracellular signalling cascade

In ALX-induced diabetic hearts, there was a reduced activity of cAMP phosphodiesterase (PDE) but cGMP PDE was increased in the same preparation (Perez de Gracia et al 1980). Neither the basal cAMP or cGMP content nor protein kinase A or phosphorylase activities were altered in ALX-induced diabetic hearts. Following administration of ISO, the cAMP/PKA ratio was decreased in diabetic hearts (ALX), but not the LVP or phosphorylase activity. The linear relation between cAMP and PKA was unaltered in diabetic hearts, but the slope of PKA and activation of phosphorylase was found to increase in diabetic hearts. These findings suggested an increased gain in the amplification cascade to PKA in diabetic hearts. Insulin replacement diminished these alterations observed in diabetic hearts (Ingebretsen et al 1981).

5.3.C Alterations in circulating THs and related changes in cardiac ATPase

Neely's group observed that chronic but not acute ALX-induced diabetes resulted in a decreased heart rate, in peak systolic pressure and left ventricular +dP/dt; these cardiac functional alterations were accompanied by reduced thyroid hormones and a decreased activity of ventricular Ca^{2+}-activated myosin ATPase (Garber and Neely 1983). V_1 isoform of ventricular Ca^{2+}-activated myosin ATPase was found to be decreased while both V_2 and V_3 isozyme increased in ALX-induced diabetic rats. T_3 replacement improved the alterations in cardiac function and myosin isozymes only partially although the dosage given maintained serum T_3 at above physiological levels and restored the Ca^{2+}-activated myosin ATPase as well as the heart rate. The cardiac functional measurements were corrected completely when serum T_3 was raised to four times the physiological level (Garber et al 1983). These findings implied a relation between the low thyroid hormones and suppressed cardiac function in diabetic animals.

5.3.D Calcium handling and ultrastructural alterations

An electrophysiological study demonstrated a prolonged action potential (AP) duration in papillary muscles from ALX-induced diabetic hearts while the resting potential did not differ from that of the control tissues. The prolonged AP was a result of a longer plateau and of a slower rate of repolarization and the AP duration was further prolonged with the progress of diabetes; a calcium channel blocker shortened the AP duration in diabetic tissue (Sauviat and Feuvray 1986). These findings suggested that possibly via Ca^{2+} influx, an increased intracellular Ca^{2+} occurs in diabetic heart which may underlie the cardiac functional alterations. Ten weeks after ALX injection, left ventricular pressure, heart rate

and +dP/dt were decreased in rabbits and these depressed cardiac functions were accompanied by a slight elevation in myocardial calcium and a significant decrease in magnesium levels (Bhimji et al 1985). It was revealed using subcellular fractionation of diabetic hearts that alterations in myofibrillar and sarcoplasmic reticulum marker enzymes, had occurred. Study of isolated working rat hearts demonstrated a reduced ATP-dependent Ca^{2+} transport in the SR from ALX-diabetic hearts, accompanied by an increase in SR long chain acylcarnitines which were shown to produce greater inhibition of the SR from diabetic hearts compared to that of control hearts (Lopaschuk et al 1983).

By employing EM, numerous ultrastructural changes were observed in 10 week ALX-induced diabetic rabbits, including myofibrillar damage and various degrees of contraction and mitochondrial damage including swollen and fragmented mitochondria containing amorphous dense bodies and distorted or lysed cristae in mitochondria (Bhimji et al 1986). In the same study, it was also observed that lipid droplets and glycogen granules were increased and the sarcoplasmic reticulum was dilated. These ultrastructural changes may be responsible for the diabetic cardiomyopathy.

5.3.E Metabolic alterations

An in vivo study showed that the cardiac ATP was reduced by 45% in ALX-diabetic rats and insulin administration normalized ATP production in the diabetic hearts (Allison et al 1976). An isolated perfused working heart study demonstrated an impaired cardiac output accompanied by a 40% reduction of glucose uptake and 20% reduction in tissue ATP concentration (Miller 1979). Study in acute ALX-induced diabetes using the working heart preparation showed that the decrease in ventricular performance was accompanied by a 40% reduction in glucose uptake and a 20% reduction in tissue ATP concentrations when the glucose concentration in the perfusate was maintained constant (Miller 1979). An in vivo

study using the same model showed that phosphocreatine and ATP were reduced by 58% and 45% respectively (Allison et al 1976). These findings indicate that metabolic changes exist in the diabetic heart and these changes may play a role in the decrease cardiac function in diabetes. The flux of glucose was decreased by 70% in the diabetic rat heart in parallel with a similar decreased flux through the glycolytic route and cardiac hexokinase was decreased by 50% in both soluble and particulate fractions indicating a shift of the pathways in glucose metabolism (Sochor et al 1984).

As discussed above, a decreased cardiac response to NE and ISO have been reported in ALX-diabetic cardiac tissues although the reported alterations in cardiac function in ALX-induced diabetic hearts varied, as unchanged, increase or decreased depending on the technique used. Various alterations such as altered Ca^{2+} metabolism, ultrastructural changes, low circulating thyroid hormones and alterations of activity of cardiac Ca^{2+}-sensitive ATPase may be responsible for these altered cardiac functions in ALX-induced diabetes. However, clarifications are needed for the diverse results reported for cardiac functions in ALX-induced diabetes.

6.   RATIONALE

The cardiac responsiveness to α- and β-adrenoceptor activation represents the ability of the heart to receive sympathetic regulation. In the diabetic state, the cardiac content of catecholamines varies depending on the methodology used and on the duration of diabetes; nonetheless, the cardiac responsiveness to adrenoceptor activation has been found to be altered in diabetes. It is important to evaluate the significance of the pathological impact of the low thyroid state in relation to the alterations of the cardiac function in the diabetic state.

It has been found that the decreased β-ADR effects in diabetic heart may be attributed to the reduced number of ADRs which cannot be directly related to the low circulating THs.

In contrast, the increase in the positive inotropic effect of α-ADR may be a result of the low circulating THs in diabetes. However, since the reported alterations in the cardiac responses have not been always consistent, it would be good guidance for future study to first clarify the alterations of cardiac effects in adrenoceptor activation in more than one preparation in relation to the measurement of circulating THs.

The alterations in the responsiveness to ADR activation in diabetic hearts may play a role in the cardiac functional changes and these alterations may have therapeutic impact on the management of pathological cardiovascular conditions in diabetic patients. It is of interest to clarify the different observations in isolated cardiac tissues and the whole heart preparations with regard to the response to adrenergic stimulation and to evaluate the functional relevance of the reported changes in diabetic hearts. In addition, the intracellular signalling system underlying the altered cardiac functional response to adrenergic stimulation was also of interest. The present study is designed to clarify the cardiac responsiveness to adrenergic stimulation and the circulating thyroid hormone levels in ALX-induced diabetes.

In the present study the following abnormalities in ALX-induced diabetic rats were investigated:

1.   Alterations in plasma concentration of thyroid hormones.

2.   Alterations in adrenoceptor-mediated responses in isolated cardiac tissues and in the whole heart preparation.

3.   Alteration in the cardiac adrenoceptor-mediated cAMP formation following the adminstration of ISO, in correlation with adrenoceptor-mediated contractile responses.

METHODOLOGY

1. INDUCTION OF DIABETES

Experimental diabetes was induced in male Wistar rats (weighing 200 ± 10 g, UBC Animal Care Centre) by a single dose of ALX (65 mg/kg body weight) injected into the tail vein. Three days after injection, blood glucose was monitored using a Glucometer to determine the success of diabetes induction. Only ALX injected animals which had plasma glucose in excess of 13 mM were kept as the diabetic group. Control animals received a saline injection, in matching volume, into the tail vein. For the following eight weeks, animals were maintained in light-controlled housing with tap water and rat chow provided ad libitum; no pharmacological intervention was introduced during these 8 weeks. The experimental design and the grouping of animals are illustrated in Figure 2.

2. TISSUE BATH PREPARATION

2.1 Tissue Preparation

Seven to eight weeks after the injection, non-fasted animals were weighed and sacrificed by a lethal dose of pentobarbitol (65mg/kg) via intraperitoneal (i.p.) injection. Cardiac tissues were dissected, mounted and dose-response curves were determined as described previously (Ramanadham and Tenner 1983). In brief, the heart was rapidly excised and immediately placed in ice cold Chenoweth-Koelle (CK) buffer solution (in mM: NaCl 120, KCl 5.6, $CaCl_2$ 2.2, $MgCl_2$ 2.1, $NaHCO_3$ 25 and glucose 10) which was continuously oxygenated with 95% O_2 and 5% of CO_2. Both atria were dissected from the ventricles, and 1-2 papillary muscle preparations (PM) were dissected from the left ventricle. Each tissue

had one end mounted on a platinum electrode and with the other end attached by a thread to a Grass FT.03 force-displacement transducer connected to a Grass Polygraph (Model 79D) eight channel recorder (Quincy, MA) which was calibrated before each experiment. Right atria were allowed to beat spontaneously while left atria and papillary muscles were driven by electrical stimulation (1 Hz, 2-4 mV and 5 ms) during the entire experiment. All tissues were maintained in an oxygenated tissue bath containing 20 ml of CK buffer, at 37°C. The CK buffer was changed every 20 minutes during the study.

2.2   Concentration-Response Curves to Adrenergic Agonists

After adjusting the tension to a standard load (atria at 0.5g of resting tension, PM at 1.2 g) the tissues were equilibrated for 60 minutes in oxygenated tissue baths containing CK buffer which was changed every 15 min. At the end of the equilibration period, the basal contractile force was determined. A cumulative dose-response curve to the α_1-agonist, phenylephrine (PE) was obtained in the CK buffer containing 1 μM timolol, and that to the β -adrenergic agonist, isoproterenol (ISO) was measured in CK buffer without timolol. Three minutes were allowed after administration of each concentration of agonist to reach maximal response before adding the next concentration of agonist. The chronotropic response of RA was expressed in beats/min and the contractile responses in left atria and in PM were expressed in grams.

The maximal effect (E_{max}) was calculated as the difference between the agonist stimulated measurement and the basal measurement. The estimated affinity constant, pD_2 was calculated as the negative logarithm of the concentration of agonist which produce 50% of the maximal response ($pD_2 = -\log[EC_{50}]$). Percentage of the maximal response was used to construct the dose response curve for calculation of the pD_2 values.

3.   LANGENDORFF PERFUSION

3.1   Dissection of The Heart

Eight weeks after the injection, animals were sacrificed and the hearts were dissected in an identical fashion to that described for the tissue bath preparation. After dissection, the heart was securely attached by its aortic stump to the Langendorff apparatus, and retrograde perfusion was started immediately by using O_2-saturated (with 95% O_2 and 5% CO_2) CK buffer at 35°C (Langendorff 1895, Rossi et al 1990). A latex balloon (securely tied to a PE-50 cannula) was inserted into left ventricle through the orifice of the pulmonary vein and about 0.3-0.5 ml of saline was injected to inflate the balloon in order to record the left ventricular pressure. The volume of inflatant was adjusted to a level which allowed a consistent maximal measurement without changing the diastolic pressure in the left ventricle. The cannula of the balloon was connected to a transducer which was connected to a Grass polygraph through an amplifier, thus left ventricular pressure (LVP) was recorded and read from the recording paper. After the balloon insertion, the heart was paced by electrical stimulation at a rate of 240 beats/min, 5 msec duration and 200% threshold voltage was used to overcome the positive chronotropic effect of β-adrenoceptor stimulation. The perfusion of CK buffer was continued for at least 20 minute to reach a stable LVP recording, then concentration-response curves to α- or β-ADR agonists were constructed.

3.2   Perfusion of Adrenergic Agonists and Tissue Collection

During the equilibrium perfusion, oxygenated CK buffer was perfused through a site-route cannula connected to syringe A which was driven by a peristalic pump at 0.1 ml/min, while solutions of isoproterenol (ISO) or phenylephrine (PE) were prepared in CK buffer to

reach a final concentration of $10^{-6}M$ for ISO, or $10^{-5}M$ for PE. ISO or PE solution was stored in a syringe (B). At the end of the equilibrium perfusion, ISO or PE was perfused via the side cannula by switching the syringes from A to B while the recording of the contraction was monitored. At 0, 30, 60 seconds and at 4 minutes following the administration of adrenergic agonists, the heart was freeze-clamped with a pre-cooled clamp. Both atria and large vessels were removed and the ventricles were placed into liquid nitrogen and stored at -80°C until assay after being weighed.

4. MEASUREMENTS OF CAMP

4.1 Extraction

The frozen ventricle was powdered with a liquid nitrogen pre-cooled tissue grinder; then approximately 100 mg of frozen powder from each heart was homogenized in 6% trichloroacetic acid (TCA) on ice, with a polytron PT-10 homogenizer for 10 sec at setting 5. An aliquot of 10 µl of homogenate was taken for protein assay, then the homogenate was centrifuged at 6,000 g for 15 min at 4°C. The supernatant was collected while the pellet was discarded, and the supernatant was stored at -80°C until assay. Before performing the assay, the supernatant was de-acidified by washing the supernatant with 4 volumes of water saturated diethyl ether 7 times followed by placing the tubes in hot tap water to evaporate the diethyl ether and a 50 µl aliquot of the supernatant was then used for subsequent assay.

4.2 Measurement of cAMP

Ventricular cAMP was measured with a cAMP [^{125}I]scintillation proximity assay (SPA) kit (RPA538 Amersham, Des Plainer IL) with all volumes reduced to half as

instructed by the supplier. The assay system utilizes the novel technique of scintillation proximity assay, which eliminates the need to separate antibody bound from free ligand common to heterogeneous radioimmunoassays; therefore, no separation step is required. The cAMP SPA assay is based on the competition between unlabelled cAMP for a limited number of binding sites on a cAMP-specific antibody. With fixed amounts of antibody and radioactive ligand, the amount of radioactive ligand bound by the antibody is inversely proportional to the concentration of added non-radioactive ligand. In particular, the antibody-bound cAMP was reacted with the SPA reagent, which contains an anti-rabbit second antibody bound to fluoromicrospheres. Any [^{125}I]cAMP that bound to the primary rabbit antibody is immobilized on the fluomicrosphere, which produces light. Measurement in a β-scintillation counter enables the amount of fluoromicrosphere bound labelled cAMP to be calculated. The concentration of unlabelled cAMP in a sample is then determined by interpolation from a standard curve.

In brief, all of the samples in the standard curve were assayed in duplicate, including non-specific binding tubes (NSB). NSB and zero standard was prepared with 100 μl and 50 μl assay buffer. A standard curve, ranging from 0.2 to 12.8 pmol/tube, were constructed from the stock standard solution provided by the supplier. A 50 μl aliquot of the heart extract specimen and the cAMP standard solutions were pipetted into the tubes which were place on ice. 50 μl [^{125}I]cAMP was dispersed into each tube, followed by dispersion of an equal volume of antiserum into each tube except the NSB tubes. After each addition, all of the tubes were mixed with a vortex mixer. Finally, after adding an aliquot of 50 μl of well suspended anti-rabbit reagent and vortex mixing, all the tubes were covered and shaken overnight on an Orbital Shaker at a speed of 200 rpm at room temperature. The amount of [^{125}I]cAMP bound to the fluoromicrospheres was determined by counting for 2 min in a β-scintillation counter.

4.3   Protein Assay

Tissue content of protein was determined using a Bio-Rad protein assay kit. In brief, the homogenate was diluted (10 µl/100 µl) in TCA buffer and the dye reagent was diluted with 4 volumes of distilled water. A protein standard curve was prepared, ranging from 20 to 100 µg/ml in increments of 20 µg/ml. 5 ml of diluted dye reagent was added into each tube which contained the diluted ventricle homogenate and all of the tubes were vortex mixed and incubated for 10 min before absorbance being measured at 596nm. Absorbance was measured within 1 hour after addition of the dye reagent, and the amount of protein per tube was calculated.

4.4   Calculation of Results

The average cpm/tube between duplicates was calculated and percent bound for each standard and sample was calculated using the following equation:

$$\%B/B_0 = \frac{(\text{STANDARD OR SAMPLE cpm - NCB cpm}) \times 100}{(B_0 \text{ cpm - NSB cpm})}$$

The amount of cAMP per tube was calculated using Linterp software (by Mr. R.W, Burton, Pharmaceutical Sci, UBC) and the ventricular content of cAMP was expressed as pmol/mg protein or as pmol/mg frozen tissue. The protein content was calculated as mg protein per mg frozen tissue.

5.   PLASMA PARAMETERS

5.1   Blood Collection

Mixed blood from the chest cavity was collected with a heparinized syringe upon sacrifice, then transferred into centrifuge tubes on ice. The plasma was separated by centrifuge at 4°C (15,000Xg) for 10 minutes and stored at -80°C until assay.

5.2.   Measurements of Glucose, Triglyceride and Insulin

Plasma glucose and triglyceride (TG) were measured with commercial kits (Boehringer Mannheim Corp., New York, NK). In brief, 10 µl of plasma was transferred into test tubes for assay after thawing at room temperature, and the appropriate assay reagent was added into the tubes. Sixty min of reaction time was allowed at room temperature for glucose, 30 min for TG and cholesterol. The reading was taken with a Colorimeter (Brinkman, PC800) using a 500 nM filter and converted into concentration according to the formula in the instructions of the supplier.

Plasma insulin was measured with commercial ^{125}I RIA kit (07-160102, ICN Biomedicals Inc). In brief, 100 µl aliquots of plasma were transferred into test tubes and several known concentrations of insulin were used for constructing a standard curve. An equal volume of ^{125}I-insulin was then added, followed by anti-insulin antibody. After one hour incubation at 37°C, a second antibody was added, then incubated overnight at room temperature on an orbital shaker (Bellco Grass Inc). The protein bound fraction was separated by centrifugation at 4,800 rpm for one hour, at 4°C. The supernatant was removed by aspiration and the radioactivity of the precipitate was counted in a gamma counter. A

standard curve was plotted and the plasma concentration of insulin was calculated from the curve by employing Linterp software (by R.W, Burton, Pharmaceutical Sci, UBC).

5.3   Measurement of Circulating Thyroid Hormones

Thyroid hormones were measured by employing ^{125}I RIA thyroxine (T4) and triiodothyronine (T3) kits (Amerlex-M T3, IM.3001) and T4 (IM.3011, Kodak Clinical Diagnostics Ltd. Amersham, UK). In brief, 50 µl aliquots of the standard T3 and plasma specimens were pipette into glass tubes. Five hundred µl [^{125}I]-T3 solution was dispensed into each tube, followed by an equal volume of specific T3 antibody suspension except for NSB tubes. All the tubes were vortex-mixed, covered with parafilm and incubated at 37°C for 1 hour. At the end of the incubation, the tubes were placed onto a magnetic separator and left for 15 min. After separation, the supernatant was discarded and the tubes were inverted on a pad of absorbent paper. The tubes were allowed to drain for 5 min, and any remaining droplets of liquid on the rims of the tubes were blotted. All the tubes were turned to the upright position and counted in gamma counter for 1 min. T4 assay was performed using a similar protocol except incubation was at room temperature for 45 min, instead of 37°C for 1 hour.

6.   STATISTICAL ANALYSIS

All statistical analyses were performed using the commercial program NCSS. The results of plasma parameters, body weight, heart weight and cardiac index were expressed as mean ± S.E.M. The results from isolated cardiac tissue were calculated as described in section 2.2 and E_{max} and estimated pD_2 of ISO or PE from diabetic tissues were compared to those of the controls. The basal measurements of isolated cardiac tissues and those of the

whole heart preparation were compared. All of the above parameters were analyzed by t-test; a P value of P<0.05 was chosen as significant.

The agonist-stimulated LVP and cardiac cAMP content at various time points were analyzed against their basal levels by two way ANOVA followed by the Newman-Keuls tests. The significant differences were determined by one way ANOVA. The measurements in diabetic hearts and the controls at the same time point were analyzed by t-test, a P value of <0.05 was chosen as significant.

RESULTS

1. DIABETIC PROFILE

The body weight of ALX-induced diabetic rats was significantly lower than control when either the net weight gain, or the weight at sacrifice (Tab.1 and Fig.3) were compared. This observation indicates that over the 8 week experimental period, ALX-induced diabetic rats gained less weight than the age-matched control rats. The diabetic hearts had smaller ventricle weight, but the greater cardiac index (CI, calculated as ventricle weight/body weight, g/Kg) indicated cardiac hypertrophy in diabetic hearts (Table 2 and Figure 4). Statistical analysis showed that the increase in CI and reduction in size of the diabetic heart were significant as compared to that of the control hearts.

As mentioned in the previous section, 72 hours after ALX injection, animals having a plasma glucose in excess of 13 mM were used as the diabetic group; 80% of ALX-injected animals became diabetic. By termination, the measurements of plasma insulin, glucose and triglyceride (TG) in diabetic group revealed the typical characteristic plasma profile of insulin- dependent diabetes as shown in Table 1 and Figure 5. After 8 week of diabetes, all of the diabetic animals in this study manifested hypoinsulinemia, hyperglycemia and hyperlipidemia.

In addition to hyperglycemia, hypoinsulinemia and hyperlipidemia, the level of the circulating thyroid hormones was also found to be lower in the diabetic group. The plasma thyroid hormones levels were shown in Figure 6. Both circulating T_3 and T_4 were reduced in diabetic animals and the reductions were significant. The magnitude of reduction in T_4 (93%) was greater than that of T_3 (40.7% reduction) as demonstrated in Table 3.

2.   ADRENERGIC RECEPTOR MEDIATED CARDIAC CONTRACTILE FORCE

2.1   Tissue Bath Preparation

2.1.A   Basal tension and heart rate

The measurements of basal heart rate in tissues bathed in CK buffer with or without timolol are shown in Figure 7. The basal heart rate in diabetic right atria (RA) was significantly lower than that of the controls in the absence or presence of timolol.

The basal contractile force measured in the tissues bathed in CK buffer with or without 1 μM timolol are illustrated in Figure 8. There was no difference between the basal tension measured in LA with or without timolol; but basal contractile force of PM was significantly reduced in diabetic PM bathed in CK buffer without timolol as compared to the control value.

2.1.B   Concentration-response curves to adrenoceptor agonists

Preliminary studies indicated that the time required to reach the maximal response was 3-4 min for either ISO or PE in both the tissue bath and Langendorff preparations.

The responses were expressed as the differences between the values after each dose of adrenergic agonist and the basal value of the same tissue. The cumulative concentration-response curves to ISO and PE constructed in right and left atria (RA and LA) and papillary muscles were presented in Figure 9. The left three panels demonstrated the concentration-response curves to ISO in cardiac tissues. The response to ISO at various concentrations in both atria in the diabetic tissues was slightly decreased compared to that of the controls. The response to ISO in diabetic papillary muscles are almost two folds of that of the controls. On

the right side of the Figure 9, it was illustrated that the chronotropic response to PE was markedly increased in diabetic RA compared to the controls. The positive inotropic response to PE in diabetic LA was similar to that measured in the control LA and the same parameter in diabetic papillary muscles was increased when compared to the controls (Figure 10). Figure 11 illustrates the positive chronotropic E_{MAX} in right atria. There was an decrease in E_{MAX} of ISO in diabetic RA. On the other hand, E_{MAX} of PE was increased significantly in diabetic tissue.

The positive inotropic E_{MAX} of adrenoceptor agonists in left atria and papillary muscles are illustrated in Figure 12. In left atria, the difference in E_{MAX} of ISO or PE between diabetic and control was not significant although the E_{MAX} of ISO in diabetic left atria was lower than the control. The same parameters measured in papillary muscles demonstrated that there was a significantly decreased E_{MAX} of ISO, and a significantly increase in E_{MAX} of PE in diabetic tissues versus controls. The calculated pD_2 in all the tissues did not differ significantly from the controls (Figure 13 and Figure 14) although there were slight alterations in the diabetic tissues.

2.2   Langendorff Whole Heart Preparation

The left ventricular pressure (LVP) measured in the Langendorff preparation was illustrated in Figures 15-17.

2.2.A   Basal LVP

Figure 15 shows that the basal LVP measured in all the groups. The measurements from diabetic hearts were significantly higher than that of the controls. When basal LVP in

PE- and ISO-perfused control hearts was compared, or that between the diabetic hearts, no significant difference was found.

2.2.B Adrenoceptor stimulated LVP

The LVP was increased at all the time points (15, 30, 60 sec and at 4 min) in both control and diabetic groups when perfused with either ISO or PE. The LVP in diabetic hearts was greater than the time-matched control hearts following the perfusion of ISO and PE (Figure 16 and Figure 17).

Statistical analysis of the absolute values of LVP showed that following the ISO administration, at 15 sec, LVP was significantly increased in both diabetic and control groups compared to the basal levels of LVP and the small difference between control and diabetic hearts was not significant (Figure 16). In the control hearts (empty bars), the ISO stimulated LVP declined gradually over the time (at 30, 60 sec and 4 min) following an initial peak; but LVP was still significantly greater than the basal level up to 60 sec following the administration of ISO. On the other hand, the ISO-stimulated LVP in diabetic hearts (cross-hatched bars in Figure 16) appeared to have a smaller magnitude of decline over time compared to the control hearts and the LVP remained significantly above the basal level from 15 sec up to 4 min following ISO administration. In other words, ISO-stimulated LVP in diabetic hearts seems to be longer lasting than that of the controls. However, when the net increase in LVP following ISO perfusion was analyzed, there was no significant difference between the control and the diabetic hearts although at 1 and 4 min, the differences were noticeable as 13 and 29 mmHg, respectively (Table 7).

As illustrated in Figure 17, following PE administration, the LVP of the control hearts (empty bars) increased to a small extent at time points from 15 sec to 4 min although none of the PE-stimulated LVP measurements differed significantly from the basal levels of LVP

(Figure 17 and Table 8). In Fig.17, the PE-stimulated LVP of diabetic hearts at various time points (cross hatched bars) were all significantly greater than the diabetic basal level; at 15, 30 and 60 sec time points following PE administration. PE-induced increases (maximal LVP - basal LVP) in LVP in diabetic hearts were not significantly higher than those of the control hearts, although the maximal LVP were significantly different between the control and diabetic hearts (Table 8).

3.   CARDIAC CONTENT OF CAMP

Figure 18 demonstrates the appearance of a typical standard curve of an RIA. It was shown that at 0 concentration of cAMP, the percentage binding between the antibody and radio-labelled cAMP was 100%. The percentage binding decreased as the concentration of cAMP increased due to competitive binding of cAMP-specific antibody by the unlabelled versus labelled cAMP.

3.1   Basal Cardiac cAMP

The basal cardiac cAMP when expressed in pmol/mg protein, in the ventricle from control and diabetic hearts perfused with or without timolol, was at similar levels (Figure 19 and Table 9, Table 10). But when the same parameter was expressed in pmol/mg frozen tissue, the basal cardiac cAMP, when perfused without timolol, was at same level in the control and the diabetic hearts (Table 9). In contrast, in timolol perfused hearts, basal cAMP levels in diabetic hearts were twice that of the controls (Table 10).

3.2   ISO-Stimulated cAMP Formation

In control hearts, at 15 sec following administration of ISO, the ventricular cAMP significantly increased over the basal levels (Table 9, Figure 19). By 30 sec, no ISO-stimulated cAMP can be detected, thus cAMP has returned to its basal level, and it remained slightly lower than its basal level up to 4 min. These findings corresponded with the ISO-stimulated LVP in the control hearts. In diabetic ventricle, 15 sec following the administration of ISO, there was a significantly increased cAMP formation over the basal level and this increase was to the same level as that of the control hearts at 15 sec. In contrast to cAMP in the control ventricles, following ISO administration, cAMP remained significantly higher than the basal level at 30, 60 sec and up to 4 min. The measurements of cAMP in diabetic hearts at 30 sec and 60 sec were significantly higher than the control values at the same points.

When the results of cAMP measurements were expressed as pmol/mg frozen tissue as demonstrated in Table 9, the changes in cardiac cAMP were similar to the changes when expressed as pmol/mg protein.

3.3   PE-Stimulated cAMP Formation

Following PE perfusion, cardiac content of cAMP in the control hearts was slightly decreased at 15 sec, slightly increased at 60 sec and returned to basal level by 4 min. The same measurement in diabetic hearts appeared to the opposite of that of the controls: Cardiac cAMP was slightly increase at 15 sec, then slightly decreased at 60 sec and 4 min (Table 10).

Ventricular protein content was calculated and, as shown in Table 11 the diabetic hearts contained significantly less protein than did the control hearts.

Unlike the results in ISO treated hearts, results (expressed as pmol/mg frozen tissue) in PE perfused hearts showed that the basal levels of cAMP were significantly higher in diabetic hearts than the controls. In the control hearts, following the administration of PE, cardiac cAMP was significantly increased and the cAMP level remained high for at least 4 min compared to its basal level. On the other hand, in diabetic hearts, following the administration of PE, cardiac cAMP was lower than its basal level.

Table 1. Measurements of general parameters control rats. All the results are expressed as mean ± SEM, * indicates p<0.05 vs control values.

	Control (N=40)	ALX-diabetic (N=54)
Gain in Body Weight (g)	293 ± 3.7	57.6 ± 9*
$[Insulin]_p$ ($\mu IU/ml$)	42.6 ± 2.06	8.65 ± 0.74*
$[Glucose]_p$ (mM)	7.6 ± 0.2	19.7 ± 0.8*
$[Triglyceride]_p$ (mM)	1.42 ± 0.11	6.37 ± 0.57*

Table 2. The heart weight and the ventricular weight (mg) /body weight (g) ratio (cardiac

index) in 8 week ALX-induced diabetic and control rats. Results are expressed as

mean ± SEM, * indicates p<0.05 vs control values.

	Control (N=40)	ALX-diabetic (N=54)
Heart weight	1.481 ± 0.026	1.047 ± 0.027*
Cardiac Index	2.93 ± 0.054	4.00 ± 0.118*

Table 3.   Plasma thyroid hormones in 8 week ALX treated and control rats. Results are

expressed as mean ± SEM, * indicates p<0.05 vs control values.

	Control (N=40)	ALX-diabetic (N=54)	% Reduction in diabetic rats
$[T3]_p$ (ng/dl)	42.5 ± 1.2	25.2 ± 1.24*	40.7%
$[T4]_p$ (μg/dl)	4.1 ± 0.1	0.28 ± 0.06*	93.2%

Table 4.   Basal beating rate of RA, basal contractile force of LA and PM, maximal effects

(E_{MAX}) of isoproterenol in cardiac tissues from 8 week ALX-induced diabetic and

age-, sex-matched control rats. All the values are expressed as mean ± SEM, *

indicates $p < 0.05$ when compared to the control value.

		RA (beat/min)	LA (g)	PM (g)
Basal	Control	232 ± 5 (N=24)	0.55 ± 0.05 (N=24)	0.69 ± 0.07 (N=29)
	ALX	197 ± 6* (N=22)	0.58 ± 0.06 (N=25)	0.45 ± 0.05* (N=25)
E_{MAX}	Control	206 ± 6	1.2 ± 0.07	0.67 ± 0.06
	ALX	171 ± 6*	0.98 ± 0.10	0.38± 0.05*

Table 5. Basal rate of RA, basal contractile force of LA and PM, maximal effects (E_{MAX}) of phenylephrine in cardiac tissues from 8 week ALX-induced diabetic and age-, sex-matched control rats. All the values are expressed as mean ± SEM, * indicates $p <$ 0.05 when compared to the control value.

		RA (beat/min)	LA (g)	PM (g)
Basal	Control	232 ± 5 (N=22)	0.55 ± 0.05 (N=26)	0.69 ± 0.07 (N=33)
	ALX	197 ± 6* (N=23)	0.58 ± 0.06 (N=25)	0.45 ± 0.05* (N=22)
E_{MAX}	Control	26 ± 3	0.79 ± 0.04	0.17 ± 0.02
	ALX	73 ± 11*	0.78 ± 0.07	0.36 ± 0.04*

Table. 6. Estimated affinity constants (pD$_2$) of ISO or PE in right atria (RA), left atria (LA) and papillary muscle (PM) isolated from 8 week diabetic rats and age-matched controls. N see Table 4 and 5.

		RA	LA	PM
pD$_2$ of ISO	Control	8.22 ± 0.11	7.76 ± 0.12	7.28 ± 0.12
	ALX	8.34 ± 0.09	7.72 ± 0.15	7.06 ± 0.2
pD$_2$ of PE	Control	6.08 ± 0.18	5.32 ± 0.14	5.18 ± 0.18
	ALX	5.66 ± 0.15	5.45 ± 0.11	5.47 ± 0.12

Table 7.   Left ventricular pressure (LVP) measured before and at 15 sec, 30 sec, 60 sec and 4 min following administration of isoproterenol in the whole heart preparation in 8 week alloxan-induced diabetic and control rats. LVP is expressed as mean ± SEM, + indicates p<0.05 vs basal value, * indicates p< 0.05 vs control values at the same time point.

(mmHg)	Basal	15 sec	30 sec	60 sec	4 min
Control[1]	70.4 ± 2.8 (N=27)	124.4 ± 3.4+ (N=18)	111.7 ± 3.8+ (N=15)	99.5 ± 4.6+ (N=10)	84.0 ± 6.5+ (N=5)
Diabetic[1]	77.9 ± 3.3* (N=26)	136.0 ± 3.9+ (N=20)	122.1 ± 4+ (N=19)	119.2 ± 4.8+* (N=13)	113.3 ± 5.9+* (N=7)
Control[2]		53.9 ± 4.8	42.0 ± 5.2	30.5 ± 6.0	13.0 ± 5.8
Diabetic[2]		57.3 ± 4.5	44.0 ± 4.6	39.6 ± 5.3	29.2 ± 5.3

[1] indicates that values are absolute values.

[2] data are expressed as difference from the basal levels.

Table 8.   Left ventricular pressure (LVP) measured before and at 15 sec, 30 sec, 60 sec and 4

min following administration of phenylephrine in the whole heart preparation in 8

week alloxan-induced diabetic and control rats. LVP is expressed as mean ± SEM, +

indicates p<0.05 vs basal value, * indicates p< 0.05 vs control values.

(mmHg)	Basal	7-8 sec	15 sec	60 sec	4 min
Control[1]	69 ± 2.3 (N=15)	78.2 ± 3.4 (N=11)	74.5 ± 2.7 (N=15)	78.2 ± 3.4 (N=7)	81.7 ± 2.75 (N=3)
Diabetic[1]	75.1 ± 1.7* (N=21)	90.1 ± 2+* (N=16)	86.9 ± 1.9+* (N=17)	93.5 ± 2.3+* (N=12)	96.8 ± 2.38+* (N=4)
Control[2]		10.0 ± 3.0	6.4 ± 3.5	11.0 ± 4.7	15.0 ± 6.2
Diabetic[2]		16.5 ± 2.5	12.7 ± 2.81	21.6 ± 3.6	31.0 ± 5.4

[1] indicates that values are absolute values.

[2] data are expressed as difference from the basal levels.

Table 9.   Cardiac cAMP content measured before and at 15 sec, 30 sec, 60 sec and 4

min following administration of isoproterenol in the ventricle from the

whole heart preparation in 8 week alloxan-induced diabetic and control rats.

Results are expressed as mean ± SEM, + indicates p<0.05 vs basal value, *

indicates p< 0.05 vs control values.

	Basal	15 sec	30 sec	60 sec	4 min
Control[1]	9.5 ± 1.23 (N=7)	23.6 ± 1.6+ (N=4)	13 ± 1.6 (N=4)	9.49 ± 1.5 (N=5)	7.8 ± 1.2 (N=7)
Diabetic[1]	9.3 ± 1.7 (N=7)	28.1 ± 2.2+ (N=4)	24.4 ± 2+* (N=5)	19.7 ± 1.8+* (N=6)	15.4 ± 2+* (N=5)
Control[2]	1.6 ± 0.19	3.5 ± 0.35+	2.3 ± 0.29	1.9 ± 0.29	2.4 ± 0.23
Diabetic[2]	1.8 ± 0.21	2.2 ± 0.34	2.9 ± 0.26	3.1 ± 0.27+*	2.8 ± 0.27

[1] indicates that the values are expressed in pmol/mg protein.

[2] indicates that the values are expressed in pmol/mg frozen tissue.

Table 10. Cardiac cAMP content measured before and at 15 sec, 60 sec and 4 min following administration of phenylephrine in the ventricle from the whole heart preparation in 8 week alloxan-induced diabetic and control rats. Results are expressed as mean ± SEM, + indicates $p<0.05$ vs basal value, * indicates $p< 0.05$ vs control values.

	Basal	15 sec	60 sec	4 min
Control[1]	9.4 ± 0.87 (N=4)	7.0 ± 0.87 (N=4)	11.3 ± 0.92 (N=3)	10.0 ± 1.0 (N=3)
Diabetic[1]	9.1 ± 0.28 (N=4)	11.5 ± 0.92* (N=5)	6.5 ± 0.78* (N=7)	6.6 ± 0.29* (N=4)
Control[2]	2.0 ± 0.14	4.1 ± 0.47+	4.2 ± 0.17+	3.7 ± 0.11+
Diabetic[2]	4.0 ± 0.14*	3.1 ± 0.42+	1.42 ± 0.09+/*	1.3 ± 0.09+/*

[1] indicates that the values are expressed in pmol/mg protein.

[2] indicates that the values are expressed in pmol/mg frozen tissue.

Table 11. Cardiac protein content (mg protein/mg tissue) measured in 8 week alloxan-induced diabetic and control rats. * indicates significantly differ from that of the control value.

	Control hearts	Diabetic hearts
Protein content	0.99 ± 0.06	0.88 ± 0.06 *

Table.12. Increases in LVP and cAMP formation in at 15, 30, 60 seconds and 4 minutes following ISO or PE perfusion in whole heart preparation from 8 week alloxan-induced diabetic and control rats. All the values are expressed as percentage of the basal value, basal measurement is 0.

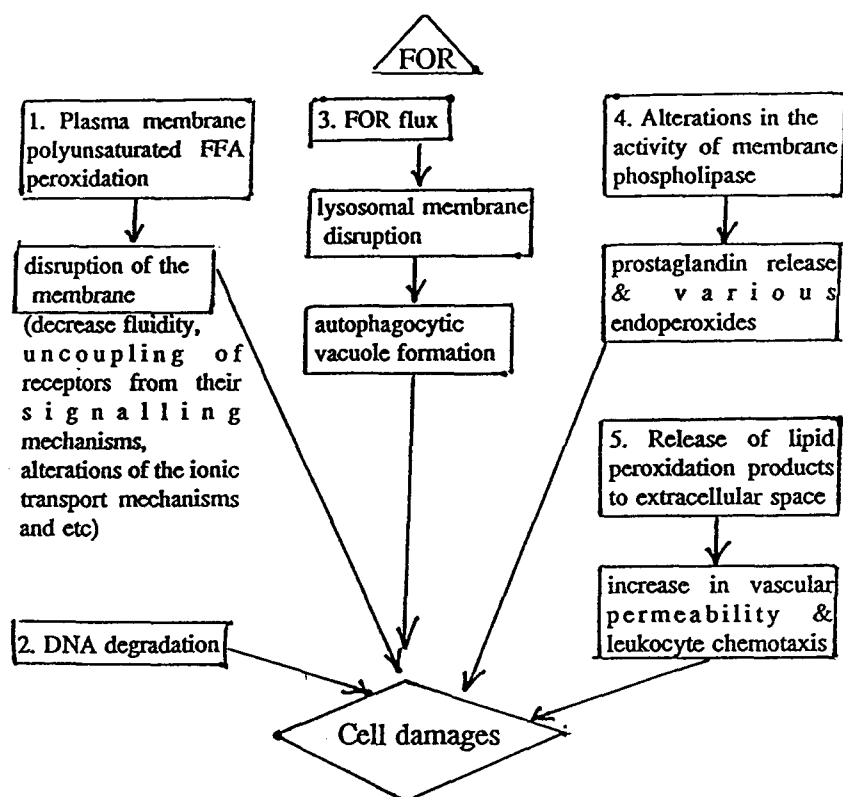|			15 sec	30 sec	60 sec	4 min
LVP	ISO	Control	77.4 %	58.7 %	44.2 %	23.3 %
		Diabetic	65.5 %	60.8 %	61.9 %	57.1 %
	PE	Control	10.2 %	-	18.7 %	22.9 %
		Diabetic	21.4 %	-	31.7 %	49.7 %
cAMP	ISO	Control	148.4 %	36.8 %	0 %	-17.9 %
		Diabetic	202.2 %	162.4 %	111.8 %	65.6 %
	PE	Control	-2.4 %	-	1.9 %	0.6 %
		Diabetic	26.4 %	-	-28.6 %	-27.5 %

Figure 1. Schematic figure of the mechanisms involved in the cellular damage by free oxygen radicals. Abbreviations: FFA, free fatty acid. FOR, free oxygen radicals. ·
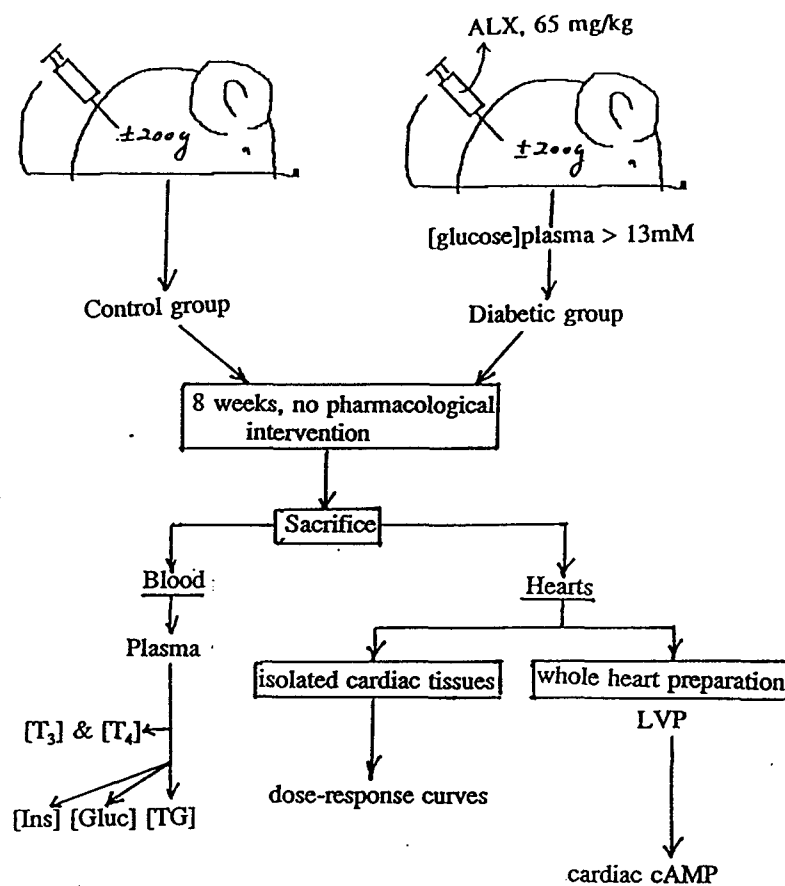
Figure 2. Illustration of experimental design and grouping of the animals. Male Wistar rats from Animal Centre of UBC were injected with either alloxan monophosphate (65 mg/Kg weight) or saline solution in matching volume. Both groups of animals were kept in identical conditions as described in Method section for 8 weeks. Termination of the animals, tissue isolation, and biochemical assays are described in Methods section.
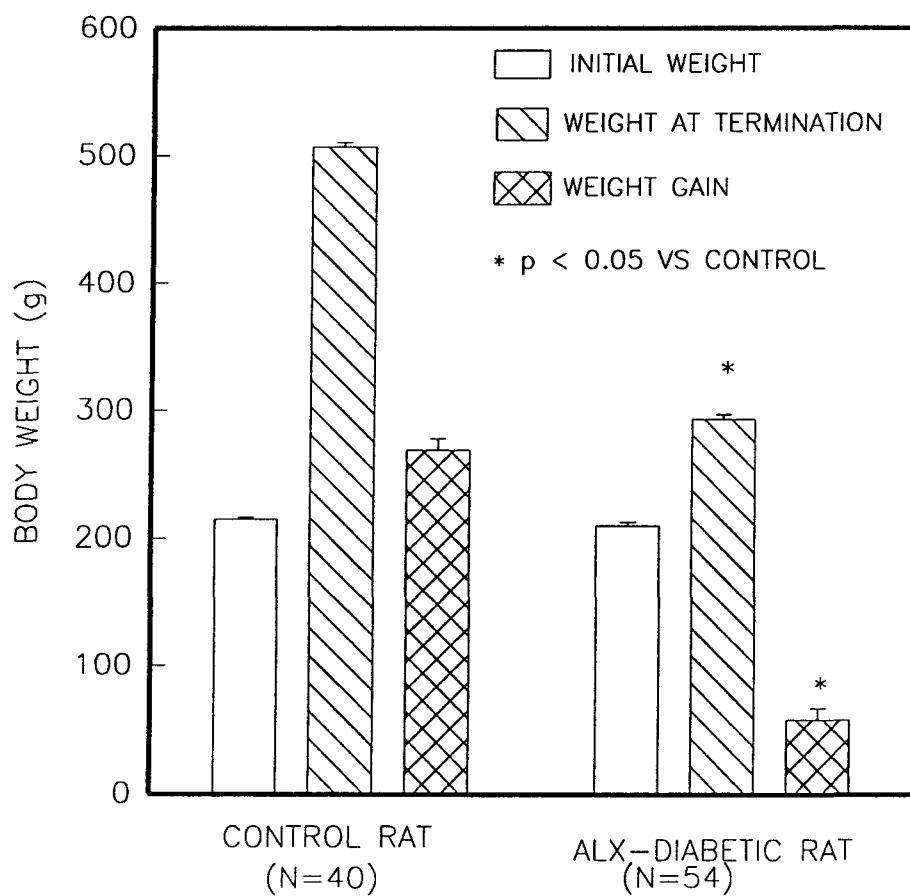
Figure 3. Body weight (BW) of 8 week ALX-induced diabetic and control rats measured before and 8 weeks after a single dose of alloxan injection (65 mg/Kg, i.v.). Weight gain = (BW at termination) - (BW before injection).
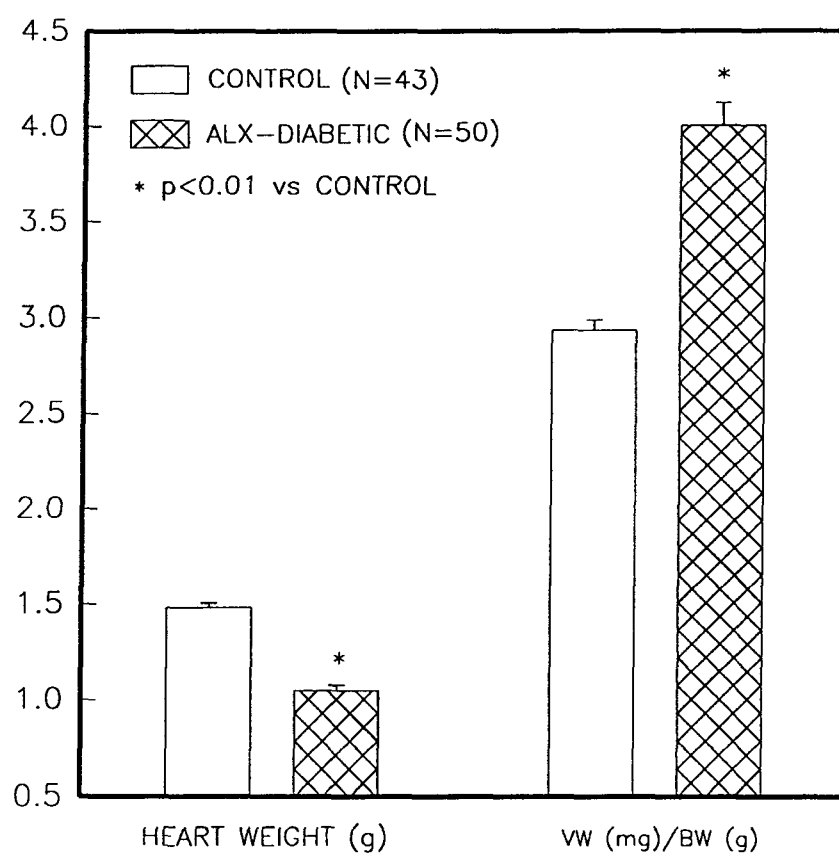
Figure 4. Ventricular weight (as heart weight, g) and cardiac index (ratio of ventricular weight/BW, mg/g) of 8 week diabetic and control rats.
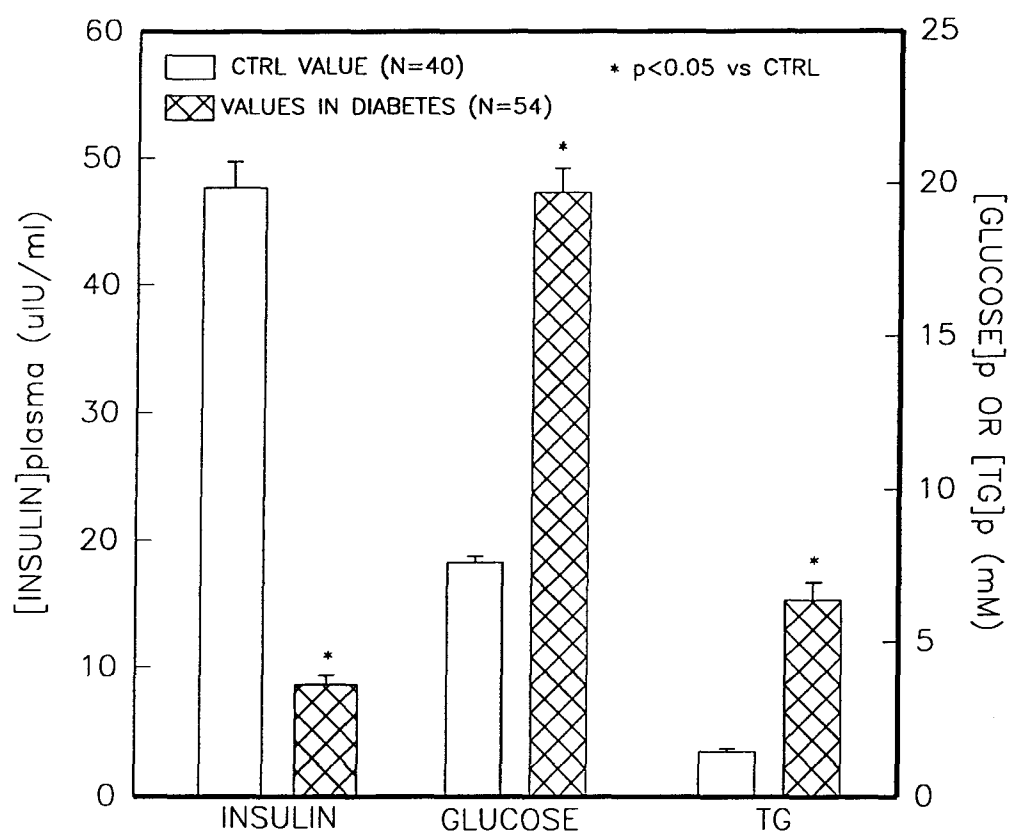
Figure 5.   Plasma levels of insulin, glucose and triglyceride in 8 week ALX-induced diabetic and age-matched control rats.
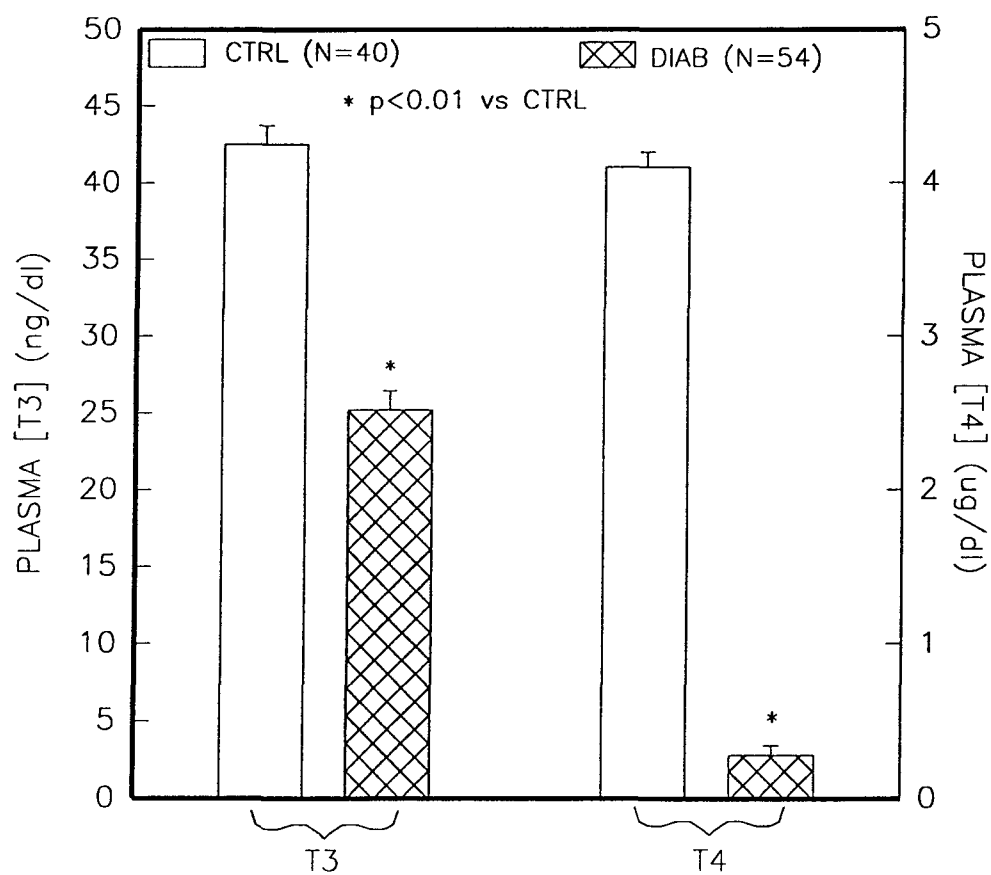
Figure 6. Plasma concentrations of thyroid hormones measured in the mixed blood collected from the chest cavity of 8 week diabetic and control rats. Numbers of animals per group are same as indicated in Fig. 5.
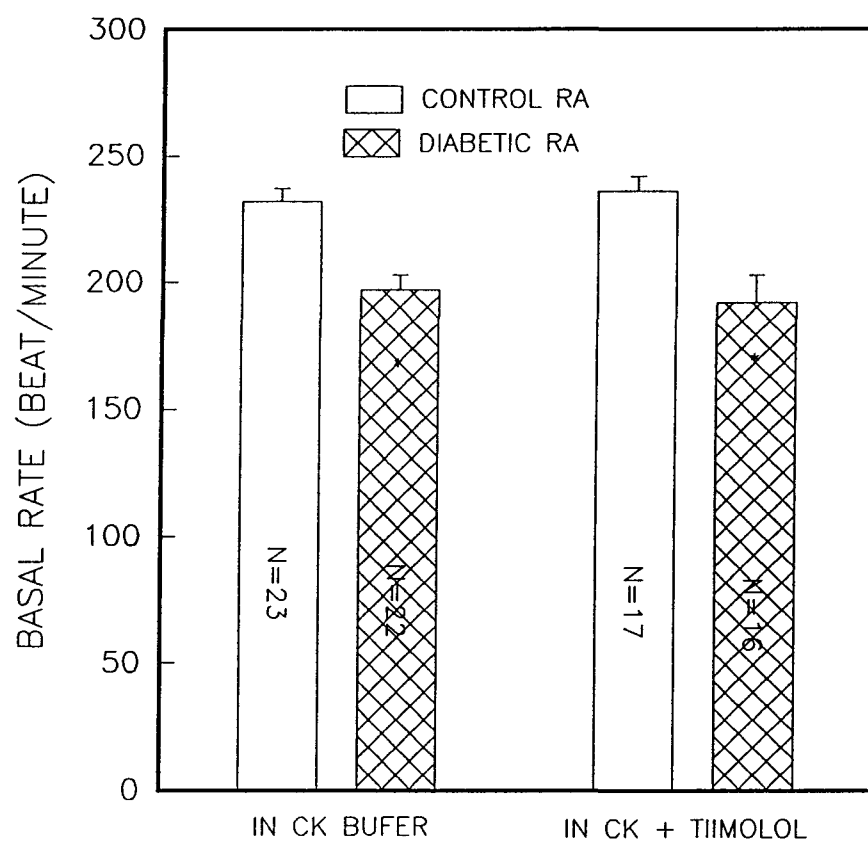
Figure 7. Basal heart rate of the isolated spontaneously beating RA from diabetic and control rats, measured in tissues bathed with or without timolol. * indicates p < 0.05 versus the control values. N=number of rats per group.
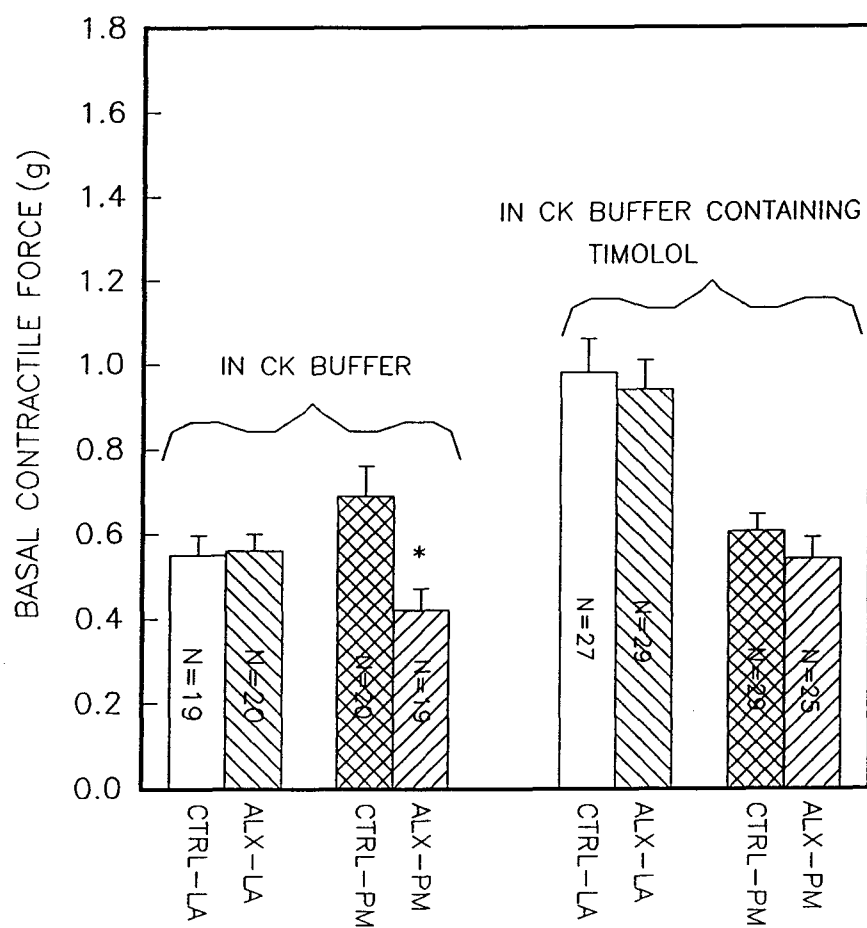
Figure 8.   Basal contractile force of isolated electrically driven (1 Hz, 2-4 mV, 5 ms) LA and PM from diabetic and control rat hearts, measured in tissues bathed with or without timolol. * indicates p < 0.05 versus the control values.
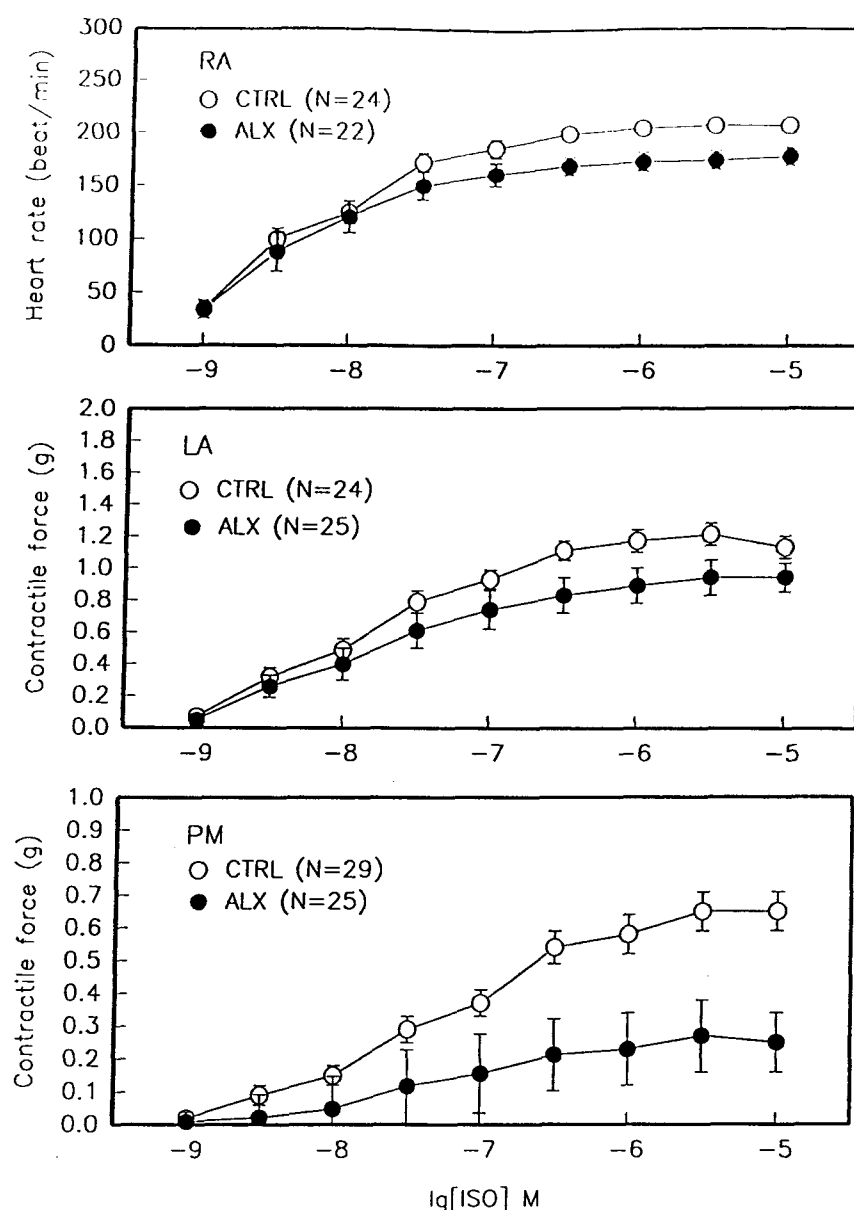
Figure 9. Cumulative concentration-response curves to ISO (10^{-9} to 10^{-5} M) measured in isolated RA, LA and PM from 8 week diabetic and age-matched control rat hearts. All values are the differences between the agonist-stimulated and the basal measurements. Upper panel: Cumulative concentration-response curves to ISO in spontaneous beating RA. Middle panel: concentration-response curves to ISO in LA. Bottom panel: concentration-response curves to ISO in PM.
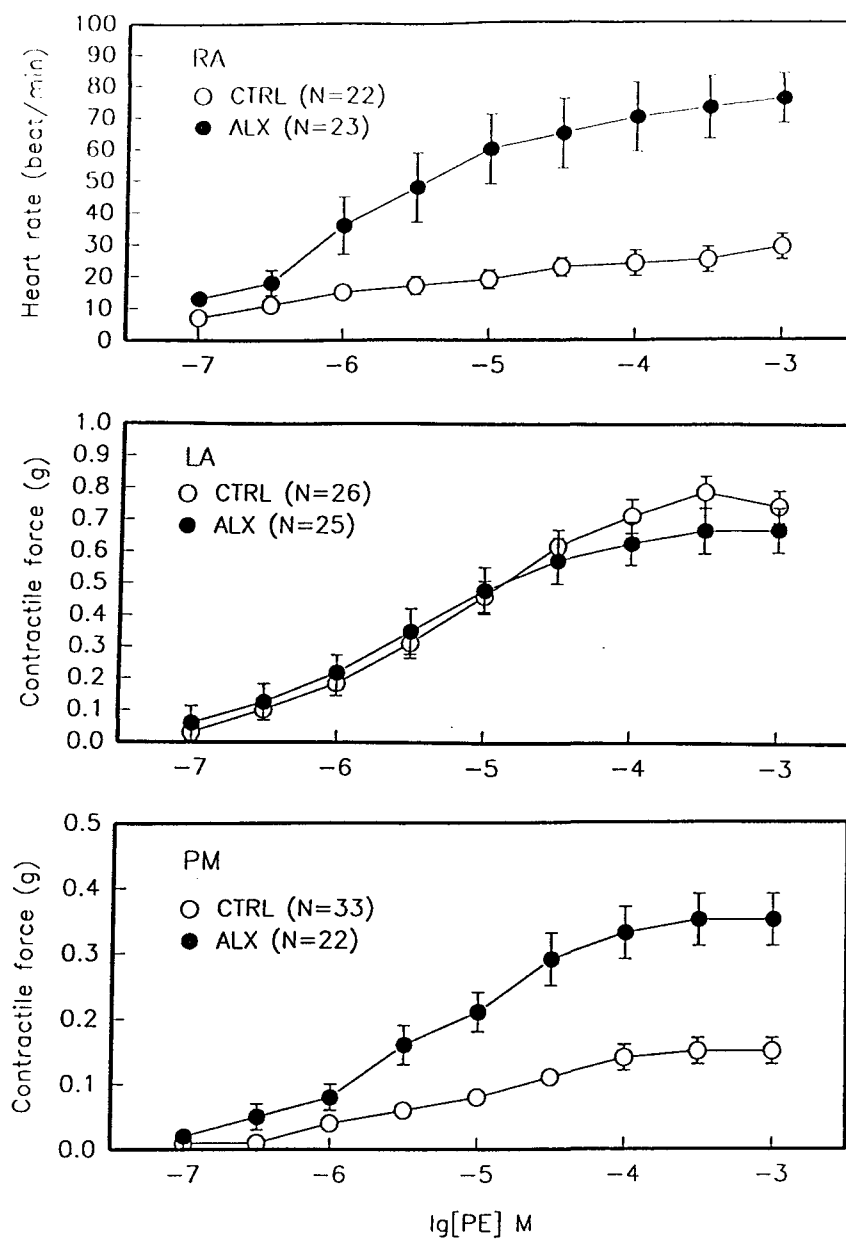
Figure 10. Cumulative concentration-response curves to PE (10^{-7} to 10^{-3}M) measured in isolated RA, LA and PM from 8 week diabetic and age-matched control rat hearts. All values are the differences between the agonist-stimulated and the basal measurements. Upper panel: Cumulative concentration-response curves to PE in spontaneous beating RA. Middle panel: concentration-response curves to PE in LA. Bottom panel: concentration-response curves to PE in PM.
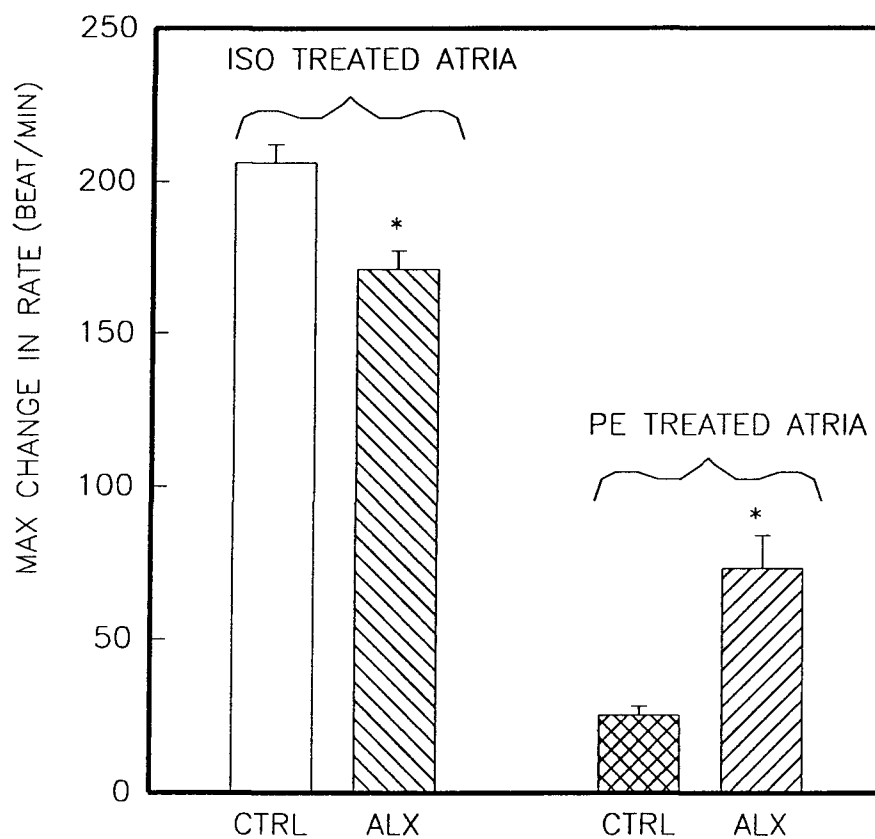
Figure 11. Calculated E_{MAX} in isolated RA to PE or that to ISO. The numbers of tissues are same as that in figure 9. * indicates p < 0.05 versus the control values.
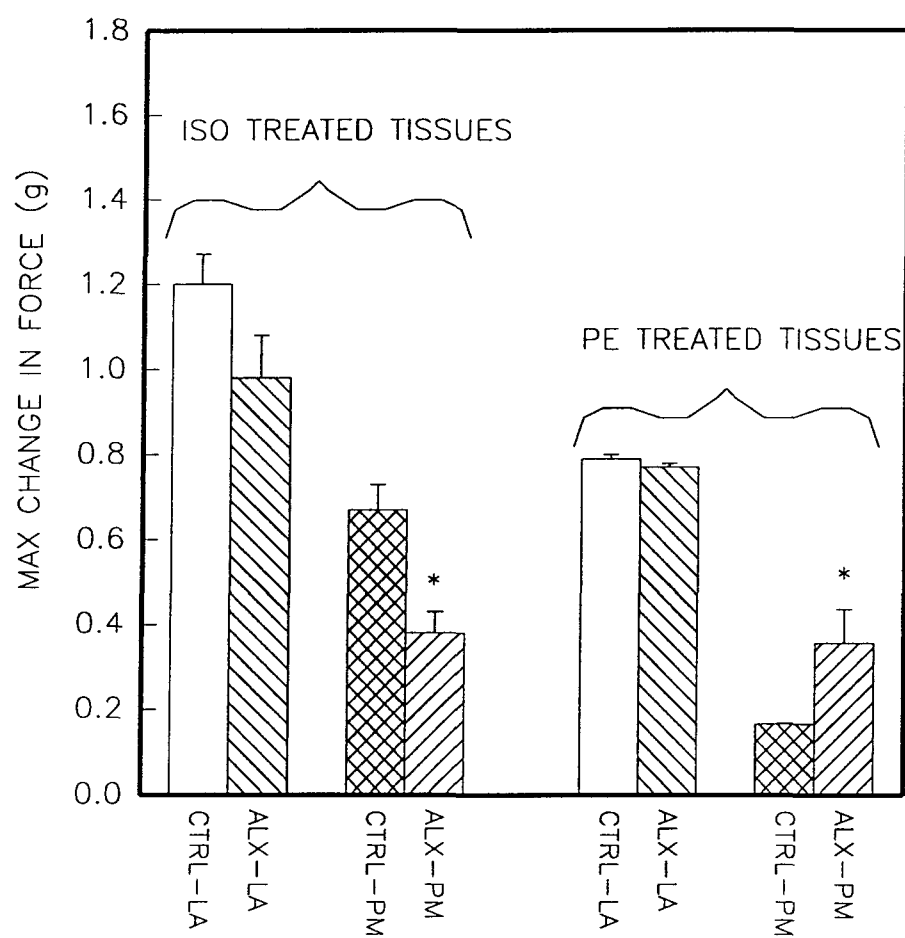
Figure 12. Calculated E_{MAX} in isolated LA and PM to PE or that to ISO. The numbers of tissues are same as that in figure 9. * indicates $p < 0.05$ versus the control values.
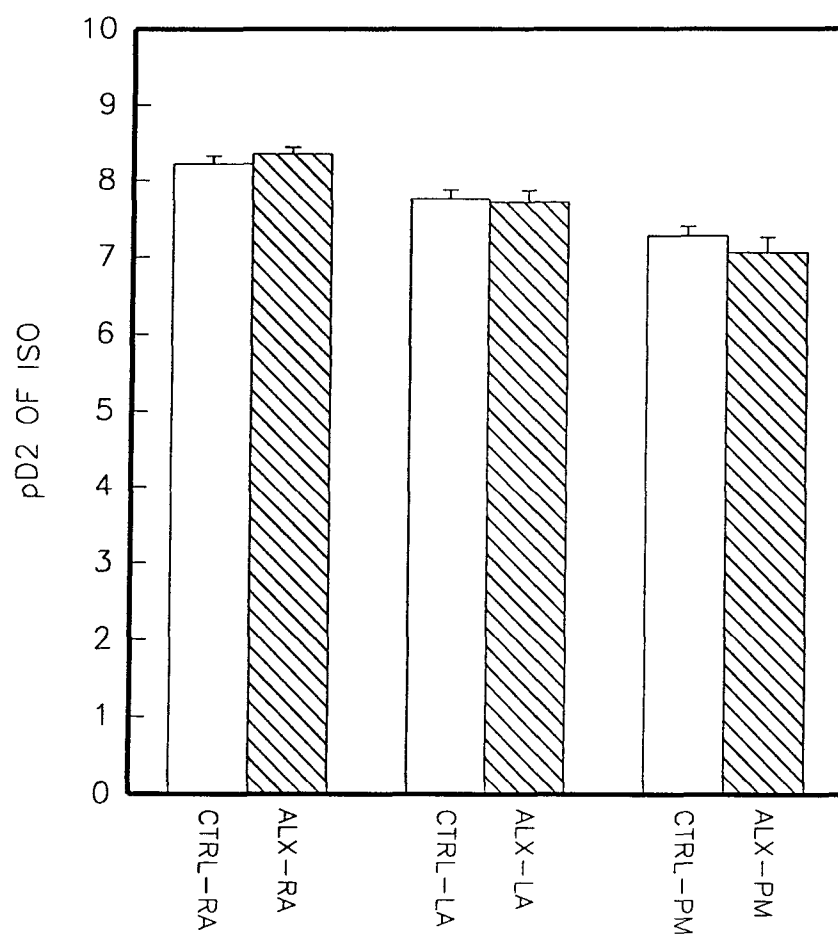
Figure 13. Estimated affinity constant (pD$_2$) of ISO calculated from the results obtained from concentration-response curves.
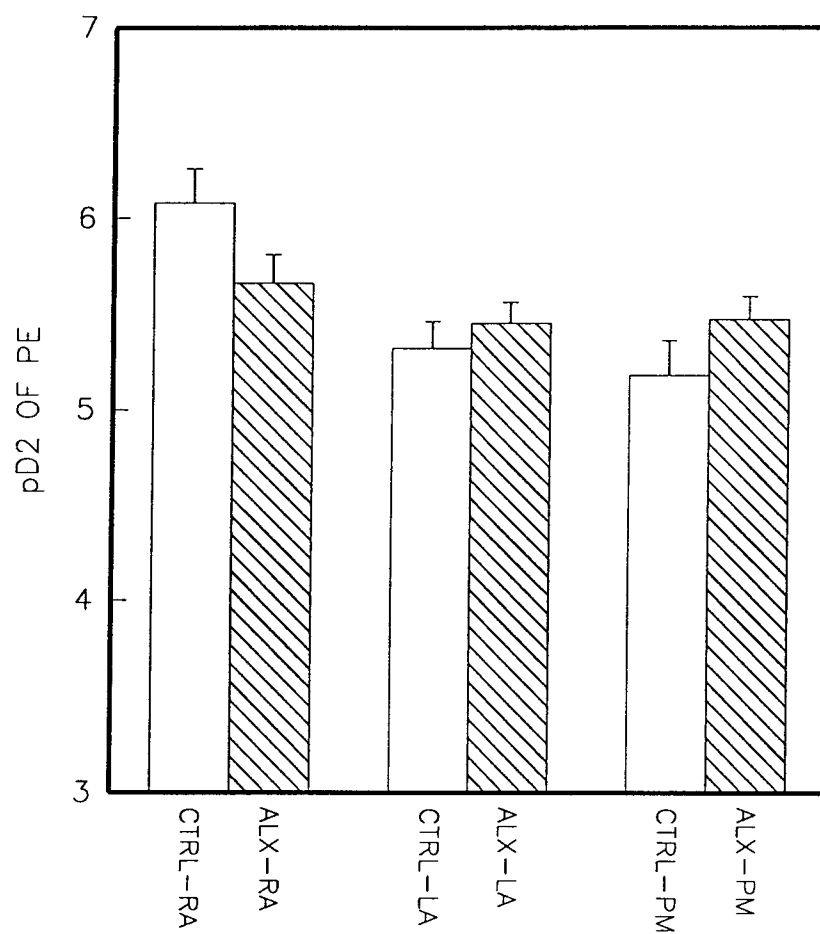
Figure 14. Estimated affinity constant (pD$_2$) of PE in cardiac tissues isolated from 8 week ALX-induced diabetic and age-matched control rats.
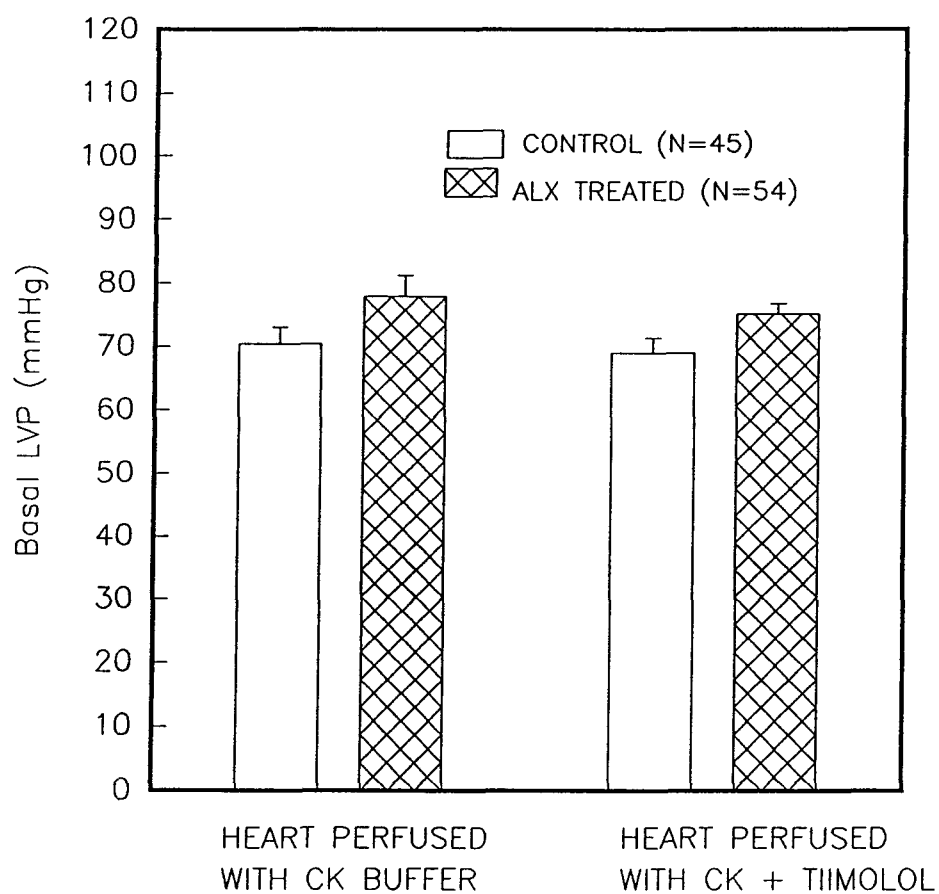
Figure 15. Basal left ventricular systolic pressure (LVP) in 8 week ALX-induced diabetic and age-matched control rats, measured by insertion of a latex balloon in Langendorff whole heart perfusion apparatus.
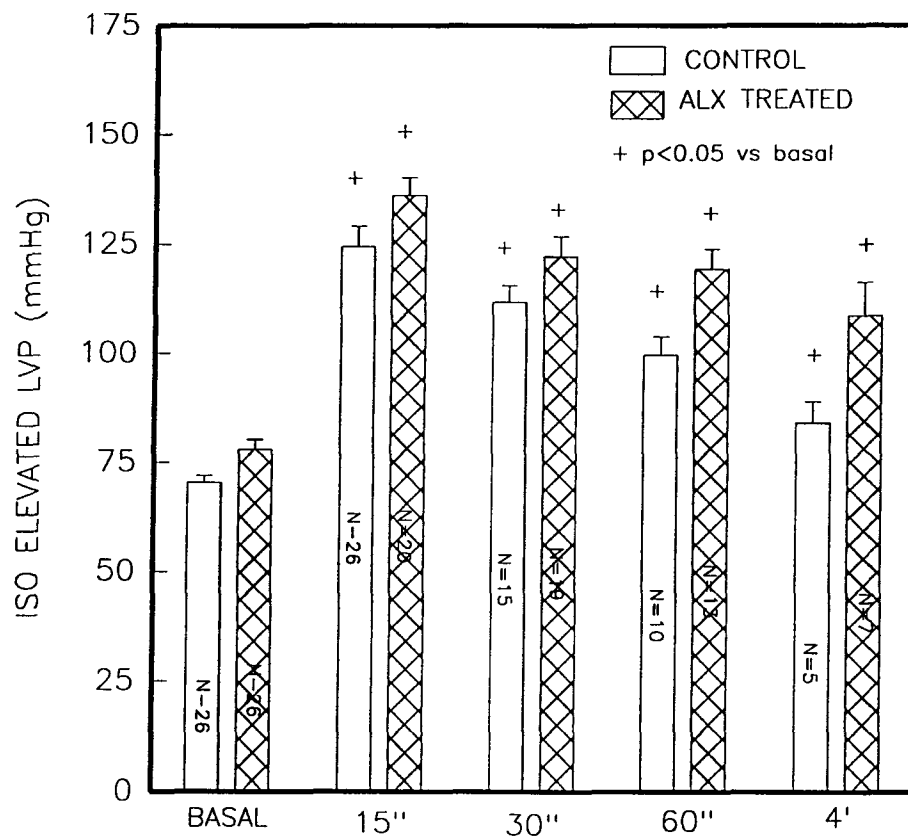
Figure 16. Left ventricular systolic pressure (LVP) in 8 week ALX-induced diabetic and age-matched control rats, measured by insertion of a latex balloon in Langendorff whole heart perfusion apparatus, following administration of ISO (10^{-6}M in perfusate). + indicates $p < 0.05$ versus the basal value.
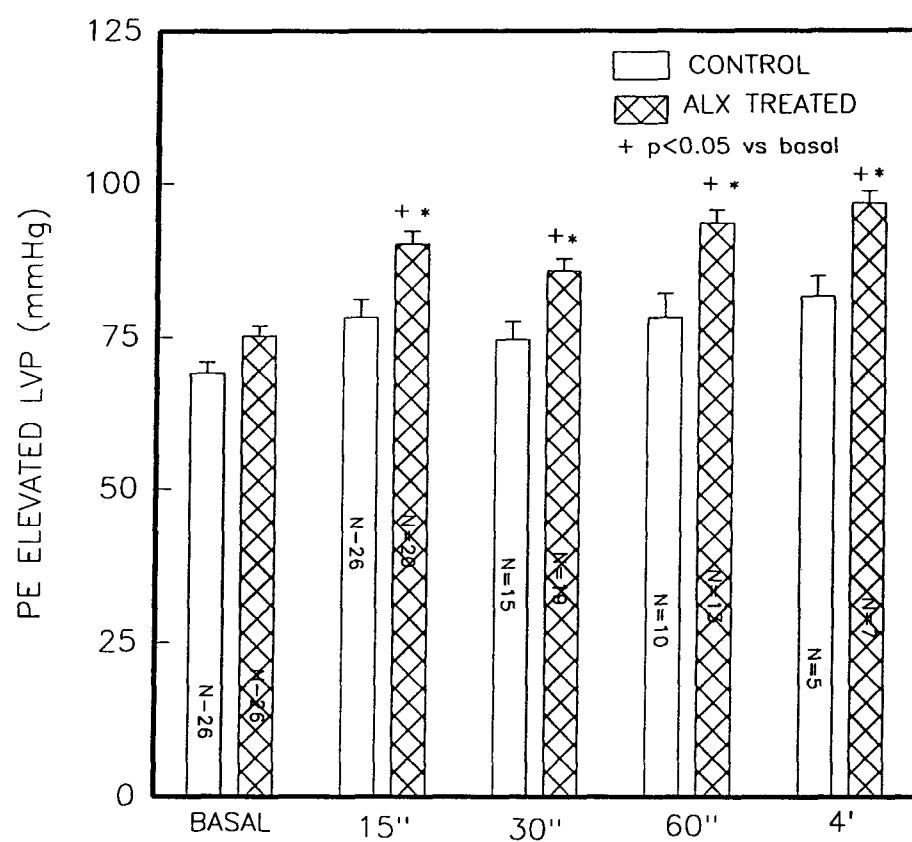
Figure 17. Left ventricular systolic pressure (LVP) of 8 week ALX-induced diabetic and age-matched control rats, measured by insertion of a latex balloon in Langendorff whole heart perfusion apparatus, following administration of PE (10^{-5}M in perfusate containing timolol). + indicates $p < 0.05$ versus the basal value and * indicates $p < 0.05$ versus the control values.
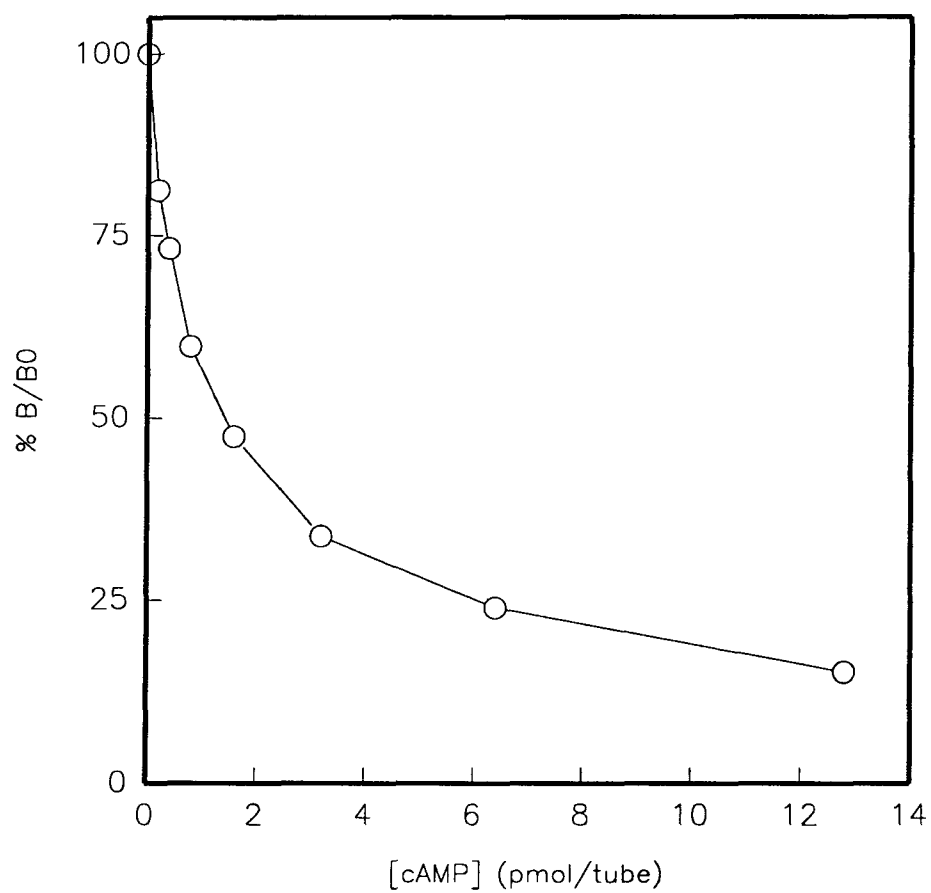
Figure 18. Standard curve of cAMP assay performed with tissue extraction and the volumes of all reagents were half as suggested by the supplier.
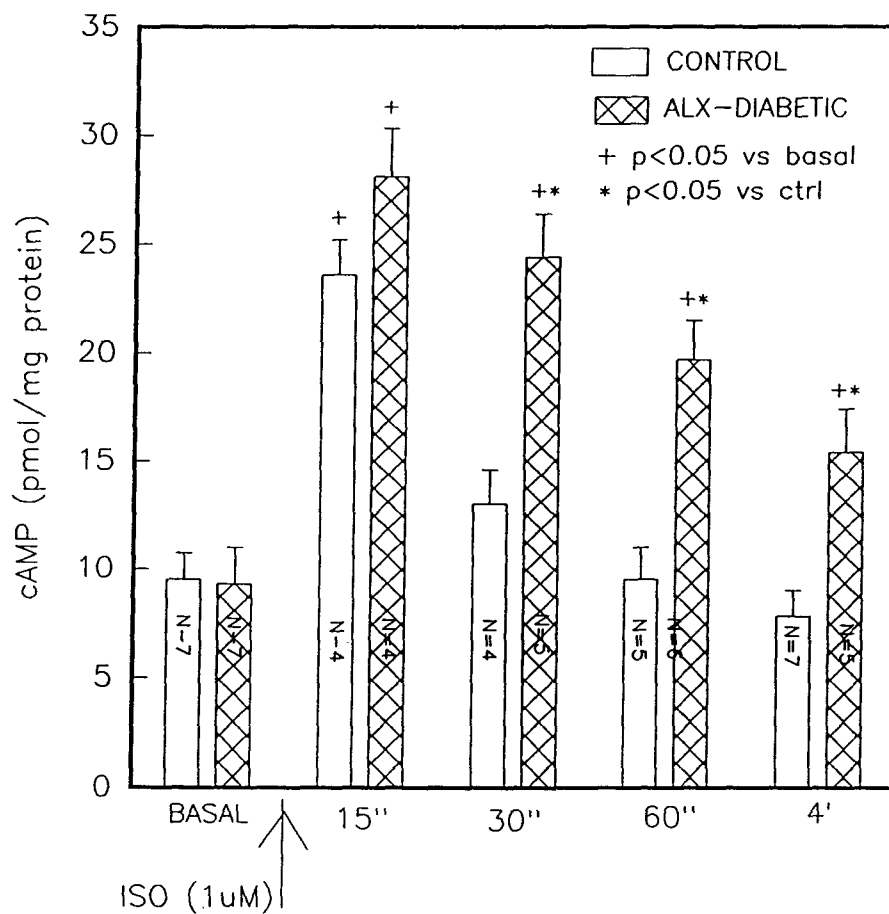
Figure 19. Cardiac content of cAMP measured in the ventricles frozen at 15, 30, 60 sec and 4 min following the administration of ISO in 8 week diabetic and age-matched control rats. All the values are expressed as pmole cAMP/mg protein.
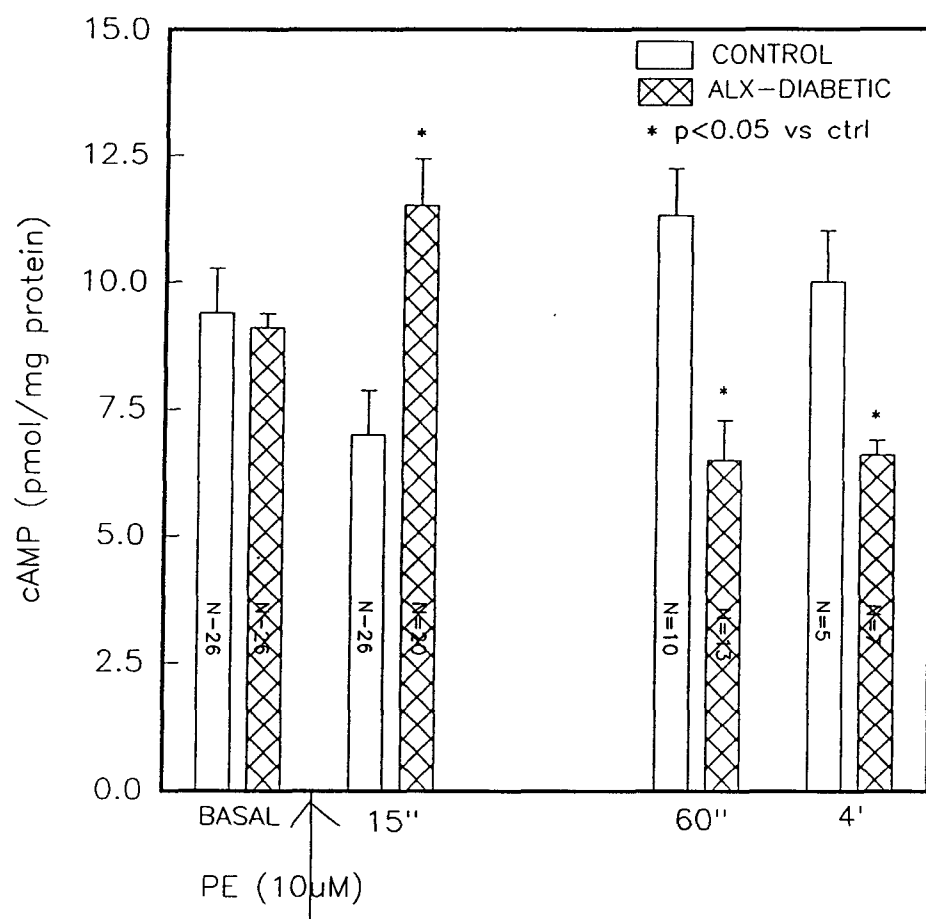
Figure 20. Cardiac content of cAMP measured in the ventricles frozen at 15, 30, 60 sec and 4 min following the administration of PE in 8 week diabetic and age-matched control rats. All the values are expressed as pmole cAMP/mg protein.

DISCUSSION

1. ALX-INDUCED DIABETES

The plasma profile measured in the present study confirmed that ALX-induced diabetes resembles insulin-dependent diabetes mellitus (Type I) observed in diabetic patients, which is characterized by hypoinsulinemia, hyperglycemia, hyperlipidemia and decreased body weight, indicating a general defect in carbohydrate utilization at the cellular level. All of these alterations were found in the diabetic rats in the present study (Table 1).

The present study also provided confirmation of the low circulating thyroid hormone (TH) levels (Table 2) that co-exist with the altered cardiac responses to adrenoceptor agonists in ALX-induced diabetes, as reported by other groups (Garber and Neely 1983, Karasu et al 1990).

2. DECREASED CIRCULATING THYROID HORMONES IN DIABETES

2.1 Causes of the Low Thyroid State in Diabetes

Previous studies have reported a decreased circulating level of T_3 and T_4 in diabetic animals as well as in diabetic patients (reviewed by Notarbartolo et al 1983). In diabetic patients with ketoacidosis, there were reciprocal decrease in T_3 level with an increase in rT_3 (Naeije et al 1978), and in diabetic animals the deiodonization of T_4 in the peripheral tissues was found to be decreased (Gavin et al 1981, Ortiz-Caro et al 1984). These data led to the hypothesis that the low THs in diabetes are due to decreased peripheral deiodonization of T_4 into T_3 in the adipose and hepatic tissues (Gavin et al 1981). However, there had been a lack of explanation of the concomitant decrease in T_4, TRH and TSH found only in experimental diabetic animals (Gonzalez et al 1980, Mitsuma and Nogimori 1982) but not in diabetic

patients without ketoacidosis (Naeije et al 1978, Tahirovic 1991). If the decreased peripheral conversion of T_4 to T_3 is a causative factor of the "low thyroid state" in diabetes, the circulating T_4 would not decrease to a greater extent than T_3 as found in the present study and in other reports (Garber and Neely 1983). A normal rT_3 has been reported in diabetic rats (Mitsuma and Nogimori 1982), further suggesting that the low circulating thyroid hormones in diabetic animal may not represent that of diabetic patients. Gavin et al (1985) also suggested that low thyroid state in diabetic patients does not reflect hypothyroidism in animals since the activity of lipoprotein lipase is rather increased in diabetic rats. As both T_3 and T_4 participate in the long loop negative feedback to higher centres of the hypothalamic-pituitary-thyroid axis, a reduced total circulating THs due to markedly reduced circulating T_3 will in turn increase TRH and TSH release from the higher centres, thus leading to increases in circulating T_4 and correction of the decreased circulating T_3. But increases in TRH, TSH and T_4 have not been observed after almost one decade of intensive investigation; in contrast, it has been reported that there were lower levels of TRH and TSH present in diabetic animals, in addition to reduced circulating THs (Las and Surks 1981, Mitsuma and Noginori 1983, Rondeel et al 1992). Although we did not measure TRH/TSH, the present finding is consistent with previous studies, confirming that the "low thyroid state" in ALX-induced diabetic rats consists of a decreased circulating T_4 as well as T_3 (Garber and Neely 1983, Karasu et al 1990), suggesting mechanisms other than a reduced peripheral deiodonization of T_4 in diabetes.

2.2 Circulating Thyroid Hormones in Diabetes Patients

All of the investigations of THs levels in human diabetes have invariably demonstrated the decreases in circulating T_4, T_3, and reciprocal increase in rT_3 in Type I and II diabetes. The reduced T_3 level in diabetic patients has been shown to return to normal values when glucose metabolism was brought under good control with insulin (Hall et al

1979, Kabadi et al 1982). Among the altered thyroid hormones levels, only T_3 levels have been shown to have a significant correlation with haemoglobin A1 (HbA1) (r = 0.61) and relative body weight (RBW) (r = 0.48) (Yagura et al 1990). An inverse relation of T_3 with glucose metabolism and free fatty acids in Type I diabetes (Hall et al 1979), and with the plasma zinc concentration have been found in Type II diabetic and in obese humans (Chen et al 1991), implying a role of T_3 in insulin resistance as well as the lipid metabolism. The decreased T_3 has also been shown to be more pronounced in female patients and patients having vascular complications (Munoz Nunez et al 1990). On the other hand, it has been shown that rT_3 and to rT_3/T_3 ratio were significantly increased in both types of diabetic patients. There is a positive correlation of rT_3 to only HbA1c (Type I: r = 0.63, Type II: 0.53) and to rT_3/T_3 ratio (r = 0.53 in Type I, r = 0.37 in Type II) in both type of diabetes, but not to blood glucose, RBW and thyroid hormones (Notarbartolo et al 1983). As discussed above, the T_3 levels in diabetes may reflect the glucose levels thus serving as an indicator for glucose control in Type I diabetic patients, and the reduced T_3 is associated with functional changes in diabetic hearts. It should be noted that none of the studies in the diabetic patients have found significant alterations in TRH or TSH levels, suggesting that the low thyroid state in diabetic patient does not involve the higher centres such as hypothalamus and pituitary gland.

Other studies suggested that decreased THs may not be specific in diabetes since significantly decreased plasma T_3 levels have been found in patients with various non-diabetic, non-thyroidal diseases. Kokei and colleagues (Kokei et al 1986) investigated alterations of circulating levels of thyroid hormones in a large number of patients with various diseases, including 71 cases with hemodialysis, 40 cases with diabetes mellitus, 24 cases with liver cirrhosis, 12 cases with cancers and 10 cases with anorexia nervosa, with 110 healthy individuals serving as control group. Serum total protein, albumin, T_3, T_4, TSH in all the patients and TRH in 10 randomly selected 10 patients from each groups were measured. A lower T_3 level was found in all of the pathological groups versus the control

group, but T_4 in diabetic patients was not significantly lower than the control. However, rT_3 was significantly higher in diabetic and anorexia nervosa groups, suggesting the reciprocal low T_3 and high rT_3 level in diabetics may be the result of poor carbohydrate metabolism (carbohydrate deprivation at cells) (Kokei et al 1986). This finding has been supported by a similar study measuring T_3, T_4 and rT_3 in acute bacterial infection, acute myocardial infarction, diabetes with or without ketoacidosis and protein-calorie undernutrition. It was found that there was no specific relationship between diabetes and low thyroid state since alterations in THs were of a similar pattern in all the groups and returned back to normal following recovery (Gomez et al 1989). A more recent study also demonstrated that as far as plasma T_4, T_3 and rT_3 were concerned, there were no differences between the low T_3, T_4 and high rT_3 levels caused by infantile diarrhoea or diabetic acidosis (Tahirovic 1991). These findings suggested a non-specific low thyroid state in various diseases in humans.

Regardless of the precise role and cause, there does exist a low thyroid state in human diabetes. Cytokines may be one of the factors underlying the low thyroid state in diabetic patients, which may at least partially cause an autoimmune reaction. Cytokines have been known to mediate immune responses and the concomitant cytotoxicity of pancreatic β-cell and thyrocytes which manifest clinically as a low thyroid state with diabetes. It was found that interleukin 1 (IL-1) induces IL-6 in both isolated pancreatic β-cells and thyrocytes, and both IL-1 and IL-6 mediated and/or inhibited secreting function of β-cell and thyrocytes at high concentrations (Bendtzen et al 1989).

2.3 Low Thyroid State in Experimental Diabetes

The low thyroid state in diabetic animals consists of decreases in TRH, TSH, low circulating T_3 and T_4, but not an increase in rT_3 (Mitsuma and Nogimori 1982). Garber's group demonstrated that a single intravenous injection of ALX, at dose ranging from 40 to 60 mg/kg in rats, caused a dose-dependent decrease in T_4 starting from 2 days after injection.

The fact that the T_4 level did not change with the duration of diabetes from two days to three months implied thyroid damage by ALX (Garber and Neely 1983). Investigations of the activity of lipoprotein lipase (LPL) in diabetic and thyroidectomized diabetic animals revealed that the low thyroid state in diabetes does not always reflect hypothyroidism (Gavin et al 1985). A study using BioBreeding/Worcester (BB/W) rats, a model of spontaneous developed diabetes and lymphocytic thyroiditis (LT), showed that before the onset of LT, the metabolism of iodine was lower and the expression of thyroid peroxidase and thyroglobulin mRNA were decreased in BB/W compared to that of the control Wistars (Fukasawa 1991), suggesting a genetic component. A recent study on the hypothalamic secretion of TRH in vitro by measuring the hypothalamic content and release of TRH and plasma T_4, T_3 and TSH in 1-2 week STZ-induced diabetic rats indicated that reduced TRH may be the primary defect in the hypothalamic-pituitary-thyroid axis (Rondeel 1992). This finding fits in with the general concepts of the negative feedback control of thyroid hormones and is in agreement with the other reports. In conclusion, the low thyroid state in experimental diabetes appears to be part of the overall reduction in release of hormones from multiple sites along the hypothalamic pituitary thyroid axis and differs from the low thyroid state in diabetic patients. The latter appears to be a result of fasting/starvation at the cellular level due to the failure of carbohydrate utilization yielding a decrease in the peripheral deiodonization of T_4.

The updated understanding of the alterations in thyroid hormones in various pathological states does not suggest a therapeutic importance of the low thyroid state in diabetic patients. In contrast, some studies suggested beneficial effects of low thyroid state in diabetes. Thyroid hormones inhibit the second phase of glucose-induced insulin secretion in the isolated pancreas (Lenzen 1978) and the activity of adipose tissue lipoprotein lipase (LPL) was increased in hypothyroidism, suggesting that a low thyroid state may be desirable in diabetes (Gavin et al 1985) in order to counter-balance the insulin deficiency. However, a study comparing measurements of adipose LPL in thyroidectomized rats, STZ-induced diabetic rats and thyroidectomized diabetic rats versus that of control and reduced food

control rats, showed the activity of LPL in thyroidectomized diabetic rats was further reduced than that of hypothyroid rats (Gavin et al 1985) suggesting that the low thyroid state in diabetes does not reflect hypothyroidism.

In the present study, it was found that circulating T_3 and T_4 in diabetes were significantly lower than that of the control group and the percentage reduction of T_4 in diabetes was greater than that of T_3. The present findings are in agreement with the previous reports as a manifestation of the dysfunction of the hypothalamic-pituitary thyroid gland in ALX-induced diabetic rats. To further investigate the low thyroid state in diabetes, the following approach should be pursued. The measurements of the plasma TRH, TSH and rT_3 may provide a further explanation and in vitro studies of the hormonal release from isolated hypothalamus, pituitary gland and thyroid gland would confirm the multiple-site hypothesis. In fact, a recent study on STZ-induced diabetic rats provided these measurements (Rondeel et al 1992). Plasma TRH, TSH and thyroid hormones were measured in 2 week diabetic and the control rats; in addition, the hypothalamic TRH release was determined in vivo and in vitro. It was found that plasma T_3, T_4 and TSH was 40-60% lower in diabetic animals while free T_4 was at similar level as that of the controls. Both in vivo and in vitro studies demonstrated a reduced hypothalamic release of TRH and that of the control hypothalami did not change when the glucose concentration was increased to a range of 10-30 mM. These findings suggested that the reduced hypothalamic TRH release is responsible for the low thyroid state in chemical-induced diabetes and this reduction in TRH release is not a result of hyperglycemia.

3.   THE CARDIAC FUNCTIONAL ALTERATIONS IN DIABETES

The present findings showed significant alterations in E_{MAX} of adrenergic agonists calculated from the concentration-response curves in isolated cardiac tissues from diabetic animals. In whole heart preparations, the responses to adrenergic agonists were increased in

diabetic heart but the differences were not statistically significant compared to those of the controls.

3.1 Alterations of Adrenoceptor-Mediated Contractile Responses in Isolated Cardiac Tissues

3.1.A Reduced cardiac function under basal conditions

The reduced heart rate measured in diabetic right atria and the decreased contractile tension in diabetic PM are consistent with the previous findings in ALX-induced diabetic rabbits (Bhimji et al 1985, Grassby and McNeill 1988) and STZ-induced diabetes in various strains of rats (Dowell et al 1986, Yu and McNeill 1991). An increased intracellular Ca^{2+} following the inotropic stimulation in diabetes (Lopaschuk et al 1983, Makino et al 1987, Yu and McNeill 1992) may lead to a prolongation of the action potential in sinoatrial node which would cause reduction in the heart rate. Metabolic changes in diabetes, such as the reduced high-energy phosphate levels (Allison et al 1976), markedly reduce glucose uptake and reduction in ATP (Miller 1979), decrease in the activity of the myocardial Ca^{2+}-ATPase and the reduced V_1 myosin isoenzyme due to low thyroid state (Garber et al 1983), found in ALX-induced diabetic hearts may contribute to the reduced basal cardiac function.

3.1.B Chronotropic and inotropic responses of ISO in ALX-induced diabetes

The present observation of the significant reduction of the E_{MAX} of ISO without changes in pD_2 in ALX-induced diabetic cardiac tissues has also been found in STZ-induced diabetes: in RA (Yu and McNeill 1991), LA (Foy and Lucas 1978), in left ventricular PM (Heyliger et al 1982) and in right ventricular strips (Ramanadham and Tenner 1987, Yu and McNeill 1991). These reduced responses to ISO in the diabetic hearts can be attributed to the

following: (1) Decreased receptor binding. The observations of decreased number of β-ADR in ALX-induced diabetic ventricles without change in activity of AC (Ingebretsen et al 1983) support findings in STZ-induced diabetic rats which showed a reduced number of β-ADR of STZ-induced diabetic hearts (Heyliger et al 1982) and a duration-dependent reduction of β-ADR in ventricles (Latipour and McNeill 1984). (2) A reduced activity of the β-ADR signalling cascade involving G_S, AC, PKA and phosphorylase system may occur in the heart. The previous report showed that there was no change in the activity of AC (Ingebretsen et al 1983), PKA or phosphorylase in ALX-induced diabetic hearts (Ingebretsen et al 1981) but the cAMP/PKA ratio was increased. A study using isolated perfused working hearts, in which cardiac cAMP content and the activity of phosphorylase were measured showed no differences in cAMP accumulation, PKA and activity of phosphorylase kinase in control versus ALX-induced diabetic hearts (Miller 1983). However, the functional relevance of these alterations in the signaling cascade of β-adrenergic receptors in ALX-induced diabetic heart was not clear. (3) Abnormal handling of Ca^{2+} was reported in diabetic heart as significantly decreased activity of the sarcolemmal Ca2+ transporter and attenuated PKA-induced enhancement of SR Ca2+ transport (Schaffer et al 1991). Previous reports have demonstrated a prolonged plateau of the ventricular AP in ALX-induced diabetic animals (Sauviat and Feuvray 1986) and the subsquent slower repolarization may contribute to a decrease in -dP/dt. Moreover, it has been found that in the SR from long-term ALX-induced diabetic hearts had a significant decrease in the rate of ATP-dependent, tris-oxalate-facilitated Ca^{2+} transport and this was accompanied by a decrease in Ca^{2+} ATPase activity. In addition, the levels of long chain acylcarnitines associated with the microsomal SR were found to be increased in diabetes (Lopaschuk et al 1983), suggesting a role for the inhibition of SR Ca^{2+} transport by long chain acylcarnitines accumulated during long-term diabetes. (4) Ultrastructural and biochemical changes in ALX-induced diabetic hearts. The ultrastructural damage shown in the hearts from ALX-induced diabetic rabbits by application of electron microscopy,includes increases in lipid droplets and glycogen granules (Bhimji et

al 1986), swollen mitochondria with distorted cristae and disrupted Z-lines in the myofibrils (Bhimji and McNeill 1989). Various biochemical alterations have been reported. In ALX-induced diabetic rabbits, increases in cardiac TG, cholesterol and glycogen have been found accompanied with a significantly decreased activity of ATPase in SR and myofibrils (Bhimji et al 1985). A recent study monitoring and evaluating several extracellular matrix constituents in the hearts of ALX-induced diabetic rats using immunochemical techniques found an increase in VI collagen among the matrix components studied (Spiro and Crowley 1993), suggesting the surrounding tissue in addition to the changes in cardiomyocytes, may also have a role in diabetic cardiomyopathy. (5) The low thyroid state in diabetes. It is generally accepted that the decreased positive chronotropic and inotropic effects of β-ADR in diabetes may not be a direct result of the low thyroid state in diabetes although low thyroid state affects the β-ADR-mediated actions by decreasing the number of receptors (Heyliger et al 1982, Williams et al 1983) and NaF or ISO-stimulated activity of AC (Ishac et al 1983) and various other alterations as discussed in chapter one in the diabetic heart. T_3 administration to diabetic animals at a dose sufficient to restore the circulating levels of T_3 did not correct the impaired cardiac functions and strongly supports this hypothesis. Two more recent studies indicated clearly that the low thyroid hormones found in diabetics may not be the major cause of the reduced responsiveness to β-ADR in diabetic rats. The cardiac responses to adrenergic agonists were studied in STZ-induced diabetic or thyroidectomized rats in comparison to that of the same groups of rats treated with either T_4 or insulin. By examining the responsiveness to NE or transmural electrical stimulation in the isolated LA, it was demonstrated that only insulin restored the levels of THs and the cardiac functions in diabetic rats but not the T_4 treatment which restored cardiac responsiveness completely in thyroidectomized rats (Sato et al 1989). A similar observation on LA from ALX-induced diabetic rats was also reported by another group (Karasu et al 1990). On the other hand, alterations in the responsiveness to α-ADR appeared to be related to the low thyroid state.

3.1.C Chronotropic and inotropic responses to PE

The increased E_{MAX} of PE with unchanged pD_2 found in the present study is in agreement with the previous reports in the whole heart perfusion preparations from ALX-induced diabetic rats (Heyliger et al 1982, Vadlamudi et al 1982), rabbit atria (Grassby and McNeill 1988) and various isolated cardiac tissues from several strains of rats injected with STZ (Xiang and McNeill 1991, Yu and McNeill 1991). The increased response to PE in ALX-induced diabetes has not been well documented. According to the findings in STZ-induced diabetes, the increased response may be mediated by postreceptor mechanisms since the number of the α-ADR was found to be decreased without a binding affinity change (Heyliger et al 1982, Williams et al 1983). This enhanced responsiveness to the α-ADR has been suggested to be related to the low thyroid state in diabetes since T_3 administration in STZ-induced diabetic rats restored the cardiac responsiveness to PE to normal (Goyal et la 1986). In theory, hyperresponsiveness at each of the steps along the postreceptor signalling pathway may be involved, such as activity of PLC, hydrolysis of PIP_2 producing IP_3 or other PI metabolites and the DAG/PKC cascade; in addition to increased intracellular Ca^{2+} and/or increases in $[Ca^{2+}]$ close to myofibrils and other contractile components may lead to an increased response. Among these potential mechanisms, only increase in the IP_3 formation in the presence of NE and propranolol has been reported in STZ-induced diabetic ventricles (Xiang and McNeill 1991). This increase in IP_3 may underlie the increased cardiac α-adrenergic response; however, little is known about the post α-ADR alterations in diabetes since the intracellular events between activation of the cardiac α-ADR and increase in intracellular Ca^{2+} are not clear yet although the activation of contractile proteins or increased contractility are probably responsible.

3.2 Increased Adrenoceptor-Mediated Contractile Responses in Diabetic Whole Heart Preparation

3.2.A Basal LVP

In the whole hearts, basal LVP was shown to be increased in the present study (Figure 13 and Tables 5 and 6), in agreement with the findings in STZ-induced diabetic rats (Yu and McNeill 1991) which demonstrated an increased basal contractile force in ventricular strips but not in atria or PM from the same heart. In the ALX-induced diabetic rabbit atria a greater contractile force has been shown (Ozuari et al 1992, Grassby and McNeill 1988); although other reports suggested a decreased basal LVP in diabetic rabbit hearts (Bhimji et al 1985) and in ALX-induced diabetic rat working heart (Ingebrestsen et al 1980, Vadlamudi et al 1982). The discrepancy of this parameter may be due to basal LVP levels, distribution of agonist/receptor through the heart, the duration of diabetes and the technique involved; however, the present study did not intend to test these issues.

3.2.B ADR agonists stimulated LVP

No suppression of the positive inotropic effect of ISO on LVP was observed in the present study; in contrast, the responses to both of the α- and β-adrenoceptor agonists were found slightly increased in diabetic hearts compare to the control hearts. The previous findings indicated an unchanged maximal LVP in response to ISO in 180 day ALX- and STZ-induced diabetic working hearts (Vadlamudi and McNeill 1984), and a supersensitivity to NE in 7-8 month ALX-induced diabetic LA (Tomlinson and Yusof 1983), unchanged response to ISO infusion in ALX-induced diabetic isolated working rat hearts (Ingebretsen et al 1980) and unaltered ISO-stimulated LVP in the same type of animals (Ingebretsen et al 1981). On the other hand, other reports demonstrated an decreased peak systolic pressure

associated with a lower activity of the ventricular Ca^{2+}-activated myosin ATPase in 2 week ALX-induced diabetic rats which was also observed in fasting rats (Garber and Neely 1983). However, T_3 replacement did restore cardiac function at doses which successfully restored circulating T_3 level and ATPase (Garber et al 1983). In 1 to 12 month ALX- or STZ-induced diabetes, declines in the rate of LVP development (+dP/dt) and that of LVP relaxation (-dP/dt) were found in working heart rat hearts perfused at 15 cm H_2O atrial filling pressure (Vadlamudi et al 1982). In terms of maximal LVP which is the parameter measured in the present study, the present finding is in agreement with some of the previous studies but not all of them.

The adrenoceptor-mediated increase in the LVP may be caused by an increased coronary perfusion rate in diabetic hearts which may lead to a relatively larger amount of ISO reached the β-ADR than that of the control hearts. This does not seem to be the case since it has been demonstrated that the coronary flow rate is unchanged in ALX-induced diabetic animals (Miller 1979, Ingebretsen et al 1980) and remained unchanged up to 1 year of diabetes (Vadlamudi et al 1982). An increase in IP_3 formation observed in right ventricle strips from STZ-induced diabetic rats (Xiang and McNeill 1991) may be responsible for the greater positive inotropic responses to NE. Other than this, at present, there is no convincing evidence supporting the post-receptor mechanisms underlying the increased response to α-ADR stimulation.

However, the present study is the first report of the measurement of alterations in the responses to α- and β-ADR agonists in ALX-induced diabetic rat cardiac tissues and the whole heart preparation under identical experimental condition, together with measurement of plasma thyroid hormones. The present findings are in agreement with previous findings in the same preparation, but there is a discrepancy between the results on the positive inotropic effect of ISO obtained from the isolated tissue and the whole heart preparations. As discussed in 3.2.1., the techniques may be one of the reasons for the difference. In addition, the type of the tissue involved may be another cause. Histologically, compare to the atrial

tissue, ventricular SR has a better developed Ca^{2+} transport system (Dhalla et al 1980) and a higher Ca^{2+} binding, uptake and higher activity of Ca^{2+}-ATPase, thus a lower Ca^{2+} content (McDonald and MacLeod 1973). It is well known that physiologically, the ventricle is the source of the contractile force of the whole heart and pharmacologically, the number of the receptors may not be evenly distributed in various types of the cardiac tissues. All these suggest that the observations in the isolated atria or PM do not always represent that of the ventricle and may not reflect the functional or pharmacological aspects of the heart as an organ.

4.   ISOPROTERENOL STIMULATED cAMP FORMATION

The present study demonstrated a measurement of cardiac cAMP content at various time points following the administration of ADR agonists. The present findings showed that ISO stimulated cAMP formation in both the control and diabetic hearts to a similar level. The profile of measurement of ISO in the control hearts suggests the ISO-stimulated cAMP formation is transient because cAMP phosphodiesterase catalyses the degradation of cAMP effectively; in contrast, the ISO-stimulated cAMP in diabetic hearts appear to be longer lasting presumably caused by the decreased activity of cAMP PDE in diabetic hearts (Perez de Gracia et al 1980) due to insulin deficiency (Solomon et al 1989). In response to ISO, the slightly higher maximal increase in cAMP in the diabetic hearts may be a result of the increased amplification cascade to PKA in ALX-induced diabetic hearts as suggested previously (Ingebretsen et al 1981). However, following the administration of ISO, the net increase in the LVP seemed to match that of cAMP expressed as pmol/mg protein. An increased activity of G_s (Hei et al 1992) or alterations in the activity of AC (Ingebretsen et al 1983) has been ruled out in diabetic hearts. The prolonged ISO-stimulated cAMP may not be necessarily be the only mechanism underlying the increase in LVP because of the existence of a compartmental cAMP in the heart (Tsien 1977) and because of alterations ofthe activity

of phosphorylase to phosphorylase kinase in diabetic heart (Miller 1983). To further investigate the role of prolonged cAMP formation in slightly increased ISO-mediated increase in LVP, several approaches may be taken. If cAMP is the major contributor to the greater ISO-stimulated LVP in diabetic heart, either NaF, forskolin, methylxanthines or cAMP analogues must produced the same response in LVP as ISO in diabetic hearts and increases in LVP or cAMP by all of these agents including ISO should be associated with increase in PKA followed by increases in either of troponin I, C-protein, phospholamban or L type Ca^{2+}.

The present study demonstrated an prolonged elevation in LVP and cAMP in diabetic hearts following ISO administration, while in the same group of animals, plasma THs are found to be significantly lower than those of the controls.

5. CARDIAC PROTEIN CONTENT IN DIABETES

A 30% reduction in protein synthesis in addition to a 20% reduction in RNA together with a 10% decrease in efficiency of the protein synthesis have been found in the whole heart preparation of ALX-induced diabetic hearts, and the observation in the isolated cardiac myocytes was similar to that of the whole heart (Williams et al 1980). An increase in proteolysis in diabetic heart was associated with alterations in lysosomes (Chua et al 1983). A smaller heart weight and lower protein content found in the present study may be a result of the decreased protein synthesis and increased proteolysis due to diabetes.

SUMMARY

This is the first study measuring circulating thyroid hormones and the cardiac adrenergic effects at various time points in diabetes.

1.   A low thyroid state co-exists with alterations in the cardiac adrenergic response in ALX-diabetic rats. The present findings are in agreement with previous findings in STZ-diabetic rats, ALX-diabetic rabbit and rats.

2.   The positive inotropic response to α-ADR was significantly increased in diabetic isolated tissues and whole hearts. On the other hand, positive inotropic response to β-ADR agonist was significantly decreased in diabetic papillary muscle but not in atria or whole heart preparation.

3.   Maximal ISO-induced cAMP accumulation was unchanged in diabetic hearts but ISO-induced cardiac cAMP accumulation lasted for a longer period of time in diabetic hearts.

CONCLUSIONS

A low thyroid state and cardiac alterations do co-exist in ALX-diabetes as well as in STZ-diabetic rats. In particular, low thyroid state is accompanied by an increase in the positive inotropic effect of α-ADR agonist, supporting its role in supersensitivity of α-ADR in diabetic heart.

The causes of discrepancy between isolated tissues and whole heart preparation in inotropic responses to β-ADR stimulation are not known at present time.

The prolonged ISO-induced cAMP may be explained by the reports of reduced cAMP PDE in diabetic hearts.

BIBLIOGRAPHY

Alandt M, McDaniel ML, Bry CG et al. Calmodulin-activated protein kinases in children with insulin-dependent diabetes mellitus. Lancet 1:1397-1399, 1982.

Allison TB, Bruttig SP, Crass MF3d et al. Reduced high-energy phosphate levels in rat hearts I. Effects of alloxan diabetes. Am J of Physiol 230(6):1744-50, 1976.

Anderson T, Schein PS, McMenamin MG et al. Streptozotocin diabetes: correlation with extent of depression of pancreatic islet nicotinamide ademine dinucleotide. J Clin Invest 54:672-677, 1976.

Aronstam RS and Carrier GO. Insulin prevention of altered muscarinic receptor-G protein coupling in diabetic rat atria. Diabetes 38:1611, 1989.

Asayama K, English D, Slonim AE et al. Chemiluminescence as an index of drug-induced free radical production in pancreatic islets. Diabetes 33:160-163, 1984.

Asayama K, Kooy NW, Burr IM. Effect of vitamin E deficiency and selenium deficiency on insulin secretory reserve and free radical scavenging systems in islets: decrease of islet manganosuperoxide dismutase. J of Lab & Clin Med 107(5):459-64, 1986.

Asayama K, Nyfeler F, English D and et al. Alloxan-induced free radical productin in isolated cells. Selective effect on islet β-cells. Diabetes 33:1008-1011, 1984b.

Asplund K, Grankvist K, Marklund S et al. Partial protection against streptozotocin-induced hyperglycemia by superoxide dismutase linked to polyethylene glycol. Acta Endocrinol 107:390-4, 1984.

Astarie C, Terzic A and Vogel SM. The endogenous catecholamine, epinephrine increases cytosolic pH in single cardiac cells via stimulation of α_1-adrenoceptors. J Mol Cell Cardiol 23:S3, 1991.

Atkins FL et al. β-Adrenergic receptors,adenylate cyclase activity, and cardiac dysfunction in the diabetic rat. J Cardiovasc Pharmaco 17:66, 1985.

Bailey CC and Bailey OT. The production of diabetes mellitus in rabbits with allxoan. J Am Med Assoc 122:1165-1166, 1943.

Baker MS and Gebicki JM. The effect of pH on the conversion of superoxide to hydroxyl free radicals. Arch Biochem Biophys 234(1):258-64, 1984.

Barany M and Barany K. Phosphorylation of myofibrillar proteins. Annu Rev Physiol 42:275-292, 1980.

Barker CF, Naji A, Silvers WK, Bartlett ST, Francfort J. Immunologic factors in pathogenesis and treatment of human and animal diabetes. World J of Surg 8(2):214-20, 1984.

Bendtzen K, Buschard K, Diamant M, Horn T, and Svenson M. Possible role of IL-1, TNF-alpha, and IL-6 in insulin-dependent diabetes mellitus and autoimmune thyroid disease. Thyroid Cell Group. Lymphokine Research (3):335-40, 1989.

Berridge MJ and Irvine RF. Inositol phophate and cell signalling. Nature 341:197-205, 1989.

Berridge MJ. Inositol trisphosphate and diacylglycerol as second messenger. Bioch J 220:345-360, 1984.

Berthelsen S and Pettinger WA. A functional basis for classification of alpha-adrenergic receptors. Life Sciences 21(5):595-606, 1977.

Bhimji S, Godin DV and McNeill JH. Biochemical and functional changes in hearts from rabbits with diabetes. Diabetologia 28:452-7, 1985.

Bhimji S, Godin DV and McNeill JH. Myocardial ultrastructural changes in Alloxan-induced diabetes in rabbits. Acta Anat 125:195-200, 1986.

Bilezikian JP and Loeb JN. The influence of hyperthyroidism and hypothyroidism on alpha- and beta-adrenergic receptor systems and adrenergic responsiveness. Endocrine Rev. 4:378, 1983.

Black SC and McNeill JH. Functional status of the cardiac sympathetic system in diabetes. In: Catecholamines and Heart Disease. (Ed) P.K. Ganguly, CRC Press. 1991.

Bountra C and Vanghan-Jones RD. Effect of intracellular and extracellular pH on contraction in isolated, mammalian cardiac cells. Biochem 418:162-187, 1989.

Bouvier M, Leeb-Lunberg LMF, Benovic JL, Caron MG and Lefkowitz RJ. Regulation of adrenergic receptor function by phosphorylation: II. Effects of agonist occupancy of phosphorylation of α_1- and β_2-adrenergic receptors by protein kinase C and the cyclic AMP-dependent protein kinase. J Biol Chem 259:9742-9749, 1987.

Brodde OE, Schumann HJ and Wagner J. Decreased responsiveness of the adenylate cyclase system on left atria from hypothyroid rats. Molecular Pharmacology 17(2):180-6, 1980.

Brosky G and Logothetopoulos J. Mitotic activity of islet cells in alloxan and streptozotocin diabetic mice studies by radioautography. Diabetes 17:306, 1968.

Brown JH, Buxton IL and Brunton LL. α_1-Adrenergic and muscarinic cholinergic stimulation of phosphoinositide hydrolysis in adult rat cardiomyocytes. Circ Res 57:532-537, 1985.

Bruchner R, Mugge A and Scholz H. Existence and functional role of α_1-adrenoceptors in the mammalian heart. J Mol Cell Cardiol 17:639-645, 1985.

Calabresi P and Parks RE. Antiproliferative agents and drugs used for immunosuppression. In: Gilmand and Goodman eds. The pharmacologic basis of therapeutics N.Y. 1991.

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

Capogrossi MC, Kachadorian WA, Ferroni C et al. α_1- and β-Adrenergic stimulation have opposite effects on myofibrillar responsiveness to Ca^{2+} in rat cardiac myocytes. Circulation 78:II-561, 1988.

Catteral WA, Seagar MJ, Takahashi M. Molecular properties of dehydropyridine-sensitive calcium channels in skeletal muscle. J Biochem 263:3535-3538, 1988.

Chen MD, Lin WH, Lin PY, Wang JJ and Tsou CT. Investigation on the relationships among blood zinc, copper, insulin and thyroid hormones in non-insulin dependent diabetes mellitus and obesity. Chung Hua i Hsueh Tsa Chih, Chinese Medical Journal 48(6):431-8, 1991.

Chu BCF, Lasley PD. Increased urinary excretion of nucleic acid and nitotinamide derivatives by rats after treatment with alkylating agents. Chem Biol Interact 10:333-8, 1975.

Chu BCF, Lasley PD. Increased urinary excretion of pyrimidine and nitotinamide dereivatives in rats treated with methyl methanesulphonate. Chem Biol Interact 8:65-73, 1974.

Chua BH, Long WM, Lautensack N, Lins JA and Morgan HE. Effects of diabetes on cardiac lysosomes and protein degradation. Am J Physiol 245(1):c91-100, 1983.

Clément O, Pucéate M, Walsh M and Vassort G. Protein kinase C enhances myosin light chain kinase activity in rat single skinned cardiac cells. Biochem J 285:311-317, 1992.

Cohen G, Heikkila RE. The generation of hydrogen peroxide, superoxide radical, and hydroxyl radical by 6-hydroxydopamine, dialuric acid, and related cytotox%w agents. Journal of Biological Chemistry. 249(8):2447-52, 1974.

Cola JR, McDonald JM, Patke C et al. Active calcium uptake by islet-cell endoplasmic reticulum. J Biol Chem 257:7223-7228, 1982.

Cronin MJ and Canonico PL. Tumour promoters enhance basal and growth hormone releasing factor stimulated cAMP levels in anterior pituitary cells. Biochem Biophys Res commun 128:404-410, 1985.

Cros GH, Chanez PO, Michel A et al. Cardiac β-adrenergic receptors in diabetic rats: alteration of guanyl nucleotide regulation. J Pharmacol 17:595, 1986.

Del Balzo U, Rosen MR, Malfatto G et al. Specific α_1-adrenergic receptor subtypres modulate catecholamin-induced increases and decrases in ventricular automaticity. Circ Res 67:1535-1551, 1990.

Del Maestro RF. An approach to free radicals in medicine and biology. Act Physiol Scand [Suppl]492:153-168, 1980.

Demaille JG and Pechere J. The control of contractility by protein phosphorylation. Adv Cyclic Nucleotide Res 15:337-371, 1983.

Dhalla NS, Sulakhe PV, Lee SL, Singal PK, Varley KG and Yates JC. Subcellular Ca2+ transport in different areas of dog heart. Can J of Physiol & Pharmacol 58(4):360-7, 1980.

Dowell RT, Atkins FL and Love S. Integrative nature and time course of cardiovascular alterations in the diabetic rat. J of Cardiovasc Pharmacol 8(2):406-13, 1986.

Downing SE, Lee JC and Fripp RR. Enhanced sensitivity of diabetic heart to alpha-adrenoceptor stimulation. Am J Physiol 245:H808, 1983.

Durante W, Sunahara FA and Sen AK. Alteration in atrial reactivity in a strain of spontaneously diabetic rats. Br J Pharmacol 97:1137, 1989.

Dyrberg T, Benn J, Christiansen JS et al. Prevalence of diabetic autonomic neuropathy measured by simple bedside tests. Diabetologia 20:190-2, 1981.

Edes I, Solaro RJ and Kranias EG. Changes in phosphoinositide turnover in isolated guinea pig hearts stimulated with isoproterenol. Cir Res 65(4):989-996, 1989.

EL-Hage AN, Herman EH, Jordan AW and Ferrans VJ. Influence of the diabetic state on isoproterenol-induced cardiac necrosis. J of Molecular & Cellular Cardiology 17(4):361-9, 1985.

Endoh M and Blinks J. Actions of sympathomimetic amines on the Ca^{2+} transients and contractions of rabbit myocardium: reciprocal changes in myofibrillar responsiveness to Ca^{2+} mediated through α- and β-adrenoceptors. Circ Res 62:247-265, 1988.

Endoh M, Takanashi M and Norota I. Role of α_{1A}-adrenoceptor subtype in production of the positive intropic effect mediated via myocardial α_1-adrenoceptors in the rabbit papillary muscle. Naunyn Schmiedebergs Arch. Pharmacol 345: 578-585, 1992.

England PJ and Shahid M. Effects of forskolin on contractile responses and protein phosphorylation in isolated perfused rat heat. Biochem J 246:687-695, 1987.

Ewing DJ, Campbell IW, Burt AA and Clarke BF. The natural history of diabetic autonomic neuropathy. QJ Med 49:95-8, 1980.

Ewing DJ, Irving JB, Kerr R et al. Cardiovascular responses to sustained handgrip in normal subjects and in patients with diabetes mellitus: a test of autonomic function. Clin Sci Mol Med 46:295-02, 1974.

Fein FS, Kornstein LB, Strobeck JE, Capasso JM and Sonnenblick EH. Altered Myocardial Mechanics in Diabetic Rats. Circ Res 47: 922-933, 1980.

Fisher LJ, Hamberger SA. Demethylurea, a radical scavenger that protects pancreatic islets from the effects of alloxan and dihydroxyfumarate exposure. Life Sci 26:1405-1409, 1980b.

Fisher LJ, Hamberger SA. Inhibition of alloxan action inisolated pancreatic islets by superoxide dismutase, catalase, and a metal chelator. Diabetes 29:213-6, 1980a.

Foy JM and Lucas PD. Effect of experimental diabetes, food deprivation and genetic obesity on the sensitivity of pithed rats to autonomic agents. British Journal of Pharmacology 7(2):229-34, 1976.

Fukuzawa K and Gebicki JM. Oxidation of alpha-tocopherol in micelles and liposomes by the hydroxyl, perhydroxyl, and superoxide free radicals. Arch Biochem Biophys 226(1):242-51, 1983.

Fuller SJ, Gaitanaki CJ and Sugden PH. Effects of catecholamines on protein synthesis in cardiac myocytes and perfused heart isolated from adult rats. Stimulation of translation is mediated through the α_1-adrenergic receptor. Biochem J 266:727-736, 1990.

Fuller SJ, Gaitanaki CJ, Hatchett RJ and Sugden PH. Acute α_1-adrenergic stimulation of cardiac protein synthesis may involve increased intracellular pH and protein kinase activity. Biochen J 273:347-353, 1991.

Gaeije R, Golstein J, Clumeck N, Meinhold H and Wenzel KW. A low T_3 syndrome in diabetic ketoacidosis. Clinical Endocrin 8(6):467-72, 1978.

Gambassi G, Berrenholtz S, Ziman B et al. Opposing effects of α_{1A}- and α_{1B}- receptors on the inotropic response to α_1-adrenergic stimulation in adult rat myocytes. Circulation 84:II-403, 1991.

Gambassi G, Spurgeon HA, Lakatta EG et al. Different effects of α- and β-adrenergic stimulation on cytosolic pH and myofilament responsiveness to Ca^{2+} in cardiac myocytes. Circ Res 71:870-882, 1992.

Gandy SE, Buse MG and Crouch RK. Protective role of superoxide dismutase against diabetogenic drugs. Clin Res 30:392a, 1982.

Gandy SE, Buse MG, Sorenson JG and Crouch RK. Attenuation of streptozotocin diabetes with superoxide dismutase-like copper(II) (3,5-diisopropylsalicylate)$_2$ in the rat. Diabetologia 24:437-40, 1983.

Garber DW and Neely JR. Decreased myocardial function and myosin ATPase in hearts from diabetic rats. Am J Physio 244(4):H586-91, 1983a.

Garber DW, Everett AW and Neely JR. Cardiac function and myosin ATPase in diabetic rats treated with inulin, T_3 and T_4. Am J Physio 244(4):H592-8, 1983b.

Garvey JL, Kranias EG, Solaro RJ. Phosphorylation of C-protein, tropnin I and phospholamban in isolated rabbit hearts. Biochem J 249:709-714, 1988.

Gater S and Belman S. Tumour promoter uncouples β-adrenergic receptor from adenylyl cyclase in mouse epidermis. Nature 284: 171-173, 1980.

Gavin LA, McMahon FA, Moeller M. The mechanism of impaired T3 production from T4 in diabetes. Diabetes 30(8):694-9, 1981.

Godin DV, Ko KM, Qayumi AK and Jamieson WR. A method for monitoring the effectiveness of allopurinol pretreatment in the prevention of ischemic/reperfusion injury. J of Pharmacol Methods. 22(4):289-97, 1989.

Godin DV, Wohaieb SA, Garnett ME and Goumeniouk AD. Antioxidant enzyme alterations in experimental and clinical diabetes. Molec & Cellular Biochem. 84(2):223-31, 1988.

Goldner MG & Gomori G. Alloxan diabetes in the dog. Endocrinology. 33:297-308, 1943.

Gomez JM, Virgili N, Navarro MA, Roca M, Montana E and Soler J. Study of thyroid function parameters and thyrotropin in general non-thyroid diseases. Medicina Clinica 92(1):5-9, 1989.

Gonzalez C, Montoya E and Jolin T. Effect of streptozotocin diabetes on the hypothalamic-pituitary-thyroid axis in the rat. Endocrinology. 107(6):2099-103, 1980.

Gotzsche O. The adrenergic adenylate cyclase system in heart and lymphocytes from strecptozotocin-diabetic rats. Diabetes 32:1110, 1983.

Goyal RK and McNeill JH. Effects of [Na+] and [Ca2+] on the responses to milrinone in rat cardiac preparations. Eur J of Pharmacol. 120(3):267-74, 1986.

Grankvist K, Marklund S and Jaljedal IB. Influence of trace metals on alloxan cytotoxicity in pancreatic islets. FEBS Lett 105:15-18, 1979a.

Grankvist K, Marklund S and Jaljedal IB. Superoxide dismutase is a prophylactic against alloxan diabetes. Nature 294:158-60, 1981b.

Grankvist K, Markolund S, Sehlin J et al. Superoxide dismutase, catalase and scarvengers of hydroxyl radical protect against the toxic actions of alloxan on pancreatic islet cells in vitro. Biochem J 182:17-25, 1979b.

Grankvist K. Alloxan-induced luminol luminescence as a tool for investigation mechanisms of radical-mediated diabetogenicity. Biochem J 20:685-690, 1981a.

Grassby PF and McNeill JH. Sensitivity changes to inotropic agents in rabbit atria after chronic experimental diabetes. Can J Physio & Pharmacol 66:1475-80, 1988.

Grob G and Hanft G. 5-Methyl-urapidil - an antagonist which discriminates between α_1-adrenoceptor subtypes. Br J Pharmacol 95:568-570, 1988.

Grob G, Hanft G and Rugevics CU. 5-Methyl-urapidil discriminates between subtypes of α 1-adrenoceptor. Eur J Pharmacol 151:330-335, 1988a.

Hall SE, Saunders J and Sonksen PH. Glucose and free fatty acid turnover in normal subjects and in diabetic patients before and after insulin treatment. Diabetologia 16(5):297-306, 1979.

Halliwell B. Lipid peroxidation, free-radical reactions and human disease. Current Concept by Upjohn. 1991.

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

Han C, Abel PW, and Minneman KP. α_1-Adrenoceptor linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 329:333-335, 1987.

Hashimoto S. A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. Analytical Biochemistry. [JC:4nk] 62(2):426-35, 1974.

Hei YJ, Diamond J and McNeill JH. Alterations of G protein function in cardiac tissues from streptozotocin-induced chronic diabetic rats. Gen Pharmacol 23(5):889-896, 1992.

Heikkila RE and Cabbat FS. Prevention of alloxan-induced diabetes by amygdalin in aqueous soluation. Biochem Pharmacol 39:3307-9, 1980.

Heikkila RE and Cabbat FS. Protection against alloxan-induced diabetes by the hydroxyl radical scavenger dimethylurea. Eur J Pharmacol 52:57-60, 1978.

Heikkila RE and Cabbat FS. The prevention of alloxan-induced diabetes in mice by the iron chelator DETAPAC: suggestions for a role for iron in the cytotoxic process. Experientia 38:378-9, 1982.

Heikkila RE. The prevention of alloxan-induced diabetes in mice by demethylsulfoxide. Eur J Pharmacol 44:191-3, 1977.

Heikkila RW, Winston GW and Cohen G. Alloxan-induced diabetes - evidence for hydroxyl radical as a cytotoxic intermediate. Biochem Pharmacol 25:1085-92, 1976.

Heyliger CE, Pierce GN, Singal PK, Beamish RE and Dhalla NS. Cardiac alpha- and beta-receptor alterations in diabetic cardiomyopathy. Basic Res Cardiol 77:610,1982.

Hilsted J and Jenson SB. A simple test for autonomic neuropathy in juvenile diabetics. Acta Med Scand 205:385-9 1979.

Hinz M, Katsilambros N, Maier V et al. Significance of streptozotocin induced nocotinamide- adenosine-dinuleotide (NAD) degradation in mouse tissue islets. FEBS Lett 30:225-8, 1973.

Hoffman BB, De Lean A, Wood CL, Schocken DD and Lefkowitz RJ. Alpha-adrenergic receptor subtypes: quantitative assessment by ligand binding. Life Sciences 24(19):1739-45, 1979.

Hoftiezer V and Carpenter AM. Comparison of streptozotocin and alloxan-induced diabetes in the rat, including volumetric quantitation of the pancreatic islets. Diabetologia 9(3):178-84, 1973.

Hohl CM, Wetzel S, Fertel RH, Wimsatt DK, Brierley GP and Altschuld RA. Hyperthyroid adult rat cardiomyocytes. I. Nucleotide content, beta- and alpha-adrenoreceptors, and cAMP production. Am J Physiol 257(5 Pt 1):C948-56, 1989.

Hulper B and Wilms B. Investigations of autonomic diabetic neuropathy of the cardiovascular system. In Aspects of Autonomic Neuropathy in Diabetes, George Thieme Verlag, Stuttgart, 1980, pp77.

Iitaka M, Fukasawa N, Yanagisawa M, Hase K, Miura S, Hara Y, Ishii J, Kawazu S and Komeda K. Effect of cholera toxin on serum levels of thyrotropin and thyroid autoantibodies in biobreeding/Tokyo (BB/TKY) rats. J of Clin & Lab Immunol 36(1):33-8, 1991.

Ikezono K, Michel MC, Zerkowski HR, Beckeringh JJ and Brodde OE. The role of cyclic AMP in the positive inotropic effect mediated by beta 1- and beta 2-adrenoceptors in isolated human right atrium. Naunyn-Schmiedebergs Arch of Pharmacol. 35(5):561-6, 1987.

Ingebretsen CG, Moreau P, Hawelu-Johnson C and Ingebretsen WR Jr. Performance of diabetic rat heart: effects of anoxia and increased work. Am J Physiol 239(5):H614-20, 1980.

Ingebretsen WR Jr, Peralta C, Monsher M et al. Diabetes alters the myocardial cAMP-protein kinase cascade system. Am J Physio 240(3):H375-82, 1981.

Ingebretsen CG, Hawelu-John C and Ingebretsen WR Jr. Alloxan-induced diabetes reduces Beta-adrenergic receptor number without affecting adenylate cyclase in rat ventricular membrane. J. Cardiovasc. Pharmacol. 5(3):454-61, 1983.

Ishac EJ, Pennefather JN and Handberg GM. Effect of changes in thyroid state on atrial α- and β-adrenoceptors, adenylate cyclase activity and catecholamine levels in the rats. J Cardiovasc Pharmacol 5(3):396-405, 1983.

Jackson CV, McGrath GM and McNeill JH. Alterations in α_1-adrenoceptor stimulation of isolated atria from experimental diabetic rats. Can J Physiol Pharmacol 64(2):145-151, 1986.

Jackson CV, McGrath GM, Taliliani AG Vadlamudi RVSV and McNeill JH. A Functional and Ultrastructural Analysis of Experimental Diabetic Rat Myocardium. Diabetes 34(9):876-883, 1985.

Jacobson MK. Levi V. Juarez-Salinas H. Barton RA. Jacobson EL. Effect of carcinogenic N-alkyl-N-nitroso compounds on nicotinamide adenine dinucleotide metabolism. Cancer Research. 40(6):1797-802, 1980.

Jolin T, Gonzalez C and Gonzalez M. Thyroid iodine metabolism in streptozotocin-diabetic rats. Acta Endocrinologica. 88(3):506-16, 1978.

Junod A, Lambert AE, Stauffacher W and Renold AE. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J of Clin Invest 48(11):2129-39, 1969.

Kabadi UM, Premachandra BN and Maayan M. Low serum 3, 5, 3'-triiodothyronine (T3) and raised 3, 3', 5'-triidothyronine (reverse T3 or RT3) in diabetes mellitus: normalization on improvement in hyperglycemia. Acta Diabetologica Latina 19(3):233-42, 1982.

Kahn JK, Zolar B, Juni JE and Vinik AI. Radionuclide assessment of left ventricular diastolic filling in diabetes mellitus with and without cardiac autonomic neuropathy. J Am Coll Cardiol 7:1303-09, 1986.

Kannel WB, Hjortland M & Castelli W. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol 34:29-34, 1984.

Karasu C, Ozturf Y, Altan N, Yildizoglu-Ari N, Ikizler C and Altan VM. Thyroid hormones mediated effect of insulin on alloxan diabetic rat atria. Gen Pharmacol 21(5): 735-740, 1990.

Kassis S, Zaremba T, Patel J and Fishman PH. Phorbol esters and β-adrenergic agonists mediated desensitization of adenylate cyclase in rat glioma C6 cells by distinct mechanisms. J Biol Chem 260:8911-8917, 1985.

Kaumann AJ, Hall JA, Murray KJ, Wells FC, Brown MJ. A comparison of the effects of adrenaline and noradrenaline on human heart: the role of β_1- and β_2-adrenoceptors in the stimulation of adenylate cyclase and contractile force. Eur Heart J, 10(Suppl B):29-37, 1989.

Kawada J. New hypotheses for the mechnisms of streptozotosin and alloxan inducing diabetes melittus. Japanese J of Pharmacol 112(11):773-791, 1992.

Kelleher DJ, Pessin JE, Ruoho AE and Jonhson GL. Phorbol ester induces desensitization of adenylate cyclase and phosphorylation of the β-adrenergic receptor in turkey erythrocyte. Proc Natl Acad Sci USA 81:4316-4320, 1984.

Kentich JC, Barsotti RJ, Lee TJ, Mulligan IP, Patel JR and Ferenczi MA. Calcium release from cardiac sarcoplasmic reticulum induced by photorelease of calcium or Ins(1,4,5)P3. AM J Physiol 258(2pt2):H610-615, 1990.

Kessler I. Mortality experience of diabetic patients. Am J Med 51:715-717, 1971.

Khalil RA, Lajoie CA, Resnich MS and Morgan KG. Heterogeneous distribution and translocation of protein kinase C isozymes in vascular smooth muscle cell. Biophys J 61:A159-163, 1992.

Kokei S, Inoue T and Iino S. Serum free thyroid hormones and response of TSH to TRH in nonthyroidal illnesses. Nippon Naibunpi Gakkai Zasshi - Folia Endocrinologica Japonica 62(11):1231-43, 1986.

Kumaresan P and Turner CW. Effect of alloxan on feed consumption and on replacement therapy with graded levels of insulin in rats. Proceedings of the Society for Experimental Biol & Med 122(2):526-7, 1966.

Kumaresan P and Turner CW. Effect of alloxan on thyroid hormone secretion rate and replacement therapy with insulin in rats. Endocrinology 79(4):828-9, 1966.

Kuo JF, Andersson RGG, Wise BC, MacKerlova L, Salmonsson I, Brackett NL, Katoh N, Shoji M and Wrenn RW. Calcium-dependent protein kinase: widespread occurrence in various tissues and phyla of the animal kingdom and comparison of effects of phospholipid, calmodulin, and triflroperazine. Proc Natl Acad Sci U.S.A. 77:7039, 1980.

Lagadic-Gossmann D and Feuvray D. Decreased sensitivity of contraction to changes of intracellular pH in papillary muscle from diabetic rat hearts. J Physiol 422:482-497, 1990.

Lambert AE, Blondel B, Kanazawa Y, Orci L, Renold AE. Monolayer cell culture of neonatal rat pancreas: light microscopy and evidence for immunoreactive insulin synthesis and release. Endocrinology. 90(1):239-48, 1972.

Landt M, McDaniel ML, Bry CG, Kotagal N, Colca JR, Lacy PE and McDonald JM. Calmodulin-activated protein kinase activity in rat pancreatic islet cell membranes. Arch of Biochem & Biophys. 213(1):148-54, 1982.

Las MS and Surks MI. Dissociation of serum triiodothyronine concentration and hepatic nuclear riiodothyronine-binding capacity in streptozotocin-induced diabetic rats. Endocrinology. 109(4):1259-63, 1981.

Latifpour J and McNeill JH. Cardiac autonomic receptors: effects of long-term experimental diabetes. J Pharmacol Expt Ther 230:242, 1984.

Lazarus SS and Shapiro SH. Serial morphologic changes in rabbit pancreatic islet cells after streptozotocin. Laboratory Investigation 27:174-83, 1972.

Ledet T, Neubauer B, Christensen NJ and Lundbæk K. Diabetic Cardiopathy. Diabetologia 16:207-209, 1979.

Lenzen S. Dose-response studies on the inhibitory effect of thyroid hormones on insulin secretion in the rat. Metabolism: Clin & Experimental 27(1):81-8, 1978.

Limas C and Limas CJ. Influence of thyroid status on intracellular distribution of cardiac adrenoceptors. Circ Res 61(6):824-8, 1987.

Little JA and Arnott JH. Sulfated insulin in mild, moderate, severe and insulin-resistant diabetes mellitus. Diabetes. 15(7):457-65, 1966.

Lopaschuk GD, Katz S and McNeill JH. The effect of alloxan- and streptozotocin-induced diabetes on calcium transport in rat cardiac sarcoplasmic reticulum. The possible involvement of long chain acylcarnitines. Can J of Physiol & Pharmacol 61(5):439-48, 1983.

Lopaschuk GD, Tahiliani AG, Vadlamudi RV, Katz S and McNeill JH. Cardiac sarcoplasmic reticulum function in insulin- or carnitine-treated diabetic rats. Am J of Physiol. 245(6):H969-76, 1983.

Makino N, Dhalla KS, Elimban V and Dhalla NS. Sarcolemmal Ca2+ transport in streptozotocin-induced diabetic cardiomyopathy in rats. Am J of Physiol 253(2 Pt 1):E202-7, 1987.

Malaisse WJ, Malaisse-Lagae F, Sener A and Pipeleers DG. Determinants of the selective toxicity of alloxan to the pancreatic B cell. Proc of Nat Acad of Sc of USA 79(3):927-30, 1982.

McDonald TF and Macleod DP. Anoxic atrial and ventricular muscle electrical activity, cell potassium, and metabolism: a comparative study. J of Molec & Cel Cardiol 5(2):149-59, 1973.

McGrath JC and O'Brien JW. Blockade by nifedipine of responses to intravenous bolus injection or infusion of alpha 1- and alpha 2-adrenoceptor agonists in the pithed rat. Brit J of Pharmacol 91(2):355-65, 1987.

Menahan LA, Chaudhuri SN, Wefer HE and Shipp JC. Lack of epinephrine stimulation of rat heart adenylase cyclase in experimental diabetes. Hormone and Metabolic Res 9(6):52708, 1977.

Michel-Reher MB, Gross G, Jasper JR, Bernstein D, Olbricht T, Brodde OE and Michel MC. Tissue- and subunit-specific regulation of G-protein expression by hypo- and hyperthyroidism. Biochem Pharmacol 45(7):1417-23, 1993.

Miller TB Jr. Cardiac performance of isolated perfused hearts from alloxan diabetic rats. Am J of Physiol 236(6):H808-12,1979.

Miller TB Jr. Effects of prostaglandin E1 in diabetic heart. Am J Physiol 245(6):H1039-42, 1983.

Mitsuma T and Nogimori T. Effectsf of streptozotocin-induced daibetes mellitus on hypothamamic-pituitary-thyroid axis in rats. Endocronologia Japonica 29(6):695-700, 1982.

Miura S, Hara Y, Iitaka M, Fukasawa N, Hase K, Urabe M, Sakatsume Y, Yanagisawa M. Ishii J, Kawazu S et al. Disturbance of thyroidal iodine metabolism in BB/W rat. Endocrinologia Japonica 38(6):647-53, 1991.

Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 65(1-2):55-63, 1983.

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

Movsesian MA, Thomas AP, Selak M and Williamson JR. IP3 does not release Ca2+ from permeabilized cardiac myocytes and sarcoplasmic reticulum. FEBS 185(2):328-332,1985.

Munoz Nunez A, Gonzalez Sarmiento E, Ergueta Martin P, Escorial Miguel C, Crespo Pinilla J and Maranon Cabello A. Thyroid function study in patients with diabetes mellitus. (Estudio de la funcion tiroidea en pacientes afectos de diabetes mellitus.) Anales de Medicina Interna. 7(6):291-3, 1990.

Murray KJ, England PJ, and Reeves ML. Positive inotropic actions of SK & F94120 occur via a cyclic AMP-dependent mechanism: Activation of cAMP-protein kinase (Abst) Eur J Pharmacol 92:755, 1987.

Nabika T, Nara Y, Yamori Y, Lovenberg W and Endo J. Angiotensin II and phorbol ester enhance isoproterenol- and vasoactive intestinal peptide-induced cyclic AMP accumulation in vascular smooth muscle cells. Biochem Biophys Res Commun 131:30-36, 1985.

Naeije R, Golstein J, Clumeck N, Meinhold H, Wenzel KW and Vanhaelst L. A low T3 syndrome in diabetic ketoacidosis. Clin Endo. 8(6):467-72, 1978.

Nechcheret AP. Changes in the adrenergic mechanisms regulating cardiac activity and coronary circulation in experimental diabetes mellitus. Cardiologiia 25(4):94-98, 1985.

Nishio Y, Kashiwagi A, Kida Y et al. Deficiency od cardiac β-adrengergic receptor in streptozocin-induced diabetic rats. Diabetes 37:1181, 1988.

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

Notarbartoto A, Rini G, Licata G, Scaglione R, Di Fede G, Averna MR, Montalto G and Butturini U. Correlation between different degree and duration of metabolic control and thyroid hormone levels in type 1 and 2 diabetes. Acta Diabetotogica Latina 20(4):341-6, 1983.

Nukatsuka M, Sakurai H and Kawada J. Generation of alloxan free radicals in chemical and biological systems: implication in the diabetogenic action of alloxan. Biochemical & Biophysical Research Communications. 165(1):278-83, 1989.

Nukatsuka M, Sakurai H, Yoshimura Y, Nishida M and Kawada J. Enhancement by streptozotocin of O2- radical generation by the xanthine oxidase system of pancreatic Beta-cells. FEBS Lett 239(2):295-8, 1988.

Nukatsuka M, Yoshimura Y, Nishida M and Kawada J. Importance of the concentration of ATP in rat pancreatic beta cells in the mechanism of streptozotocin-induced cytotoxicity. Journal of Endocrinology. 127(1):161-5, 1990a.

Nukatsuka M, Yoshimura Y, Nishida M, Kawada J. Allopurinol protects pancreatic beta cells from the cytotoxic effect of streptozotocin: in vitro study. Journal of Pharmacobio-Dynamics. 13(4):259-62, 1990b.

Okamoto H and Yamamoto H. DNA strand breaks and poly (ADP-ribose) synthetase in pancreatic islets - a new aspect to development of inulin-dependent diabetes and pancreatic tumors. Int Symp Princess Takamatsu Cancer Res Fund 13L297-308, 1983.

Okamoto H, Yamamoto H and Uchigata Y. Protection by superoxide dismutase, catalase and poly(ADP-ribose) synthetase inhibitors against alloxan- and streptozotocin-induced islet DNA strand breaks and against proinsulin synthesis. In: Algerti JGG, Ogata T, Alouch JAA et al. eds. Proc 11th Cong International Diabetes Federation. Excerpta Medica:1982:13.

Okamoto H. Regulation of proinsulin synthesis in pancreatic islets and new aspect to insulin-dependent diabetes. Mol Cell Biochem 37:43-61, 1981.

OKamura K, Kawai T, Hashomoto H, Ito T, Ogawa K and Satake T. Sustained diacylglycerol formation in norepinephrine-stimulated rat hearts is associated with α $_1$-adrenergic receptor. J Cardiovasc Pharmacol 11:651, 1988.

Ortiz-Caro J, Gonzalez C and Jolin T. Diurnal variations of plasma growth hormone, thyrotropin, thyroxine, and triiodothyronine in streptozotocin-diabetic and food-restricted rats. Endocrinology 115(6):2227-32, 1984.

Ortiz-Caro J, Obregon MJ, Pascual A and Jolin T. Decreased T4 to T3 conversion in tissues of streptozotocin-diabetic rats. Acta Endocrinologica 106(1):86-91, 1984.

Otani H and Das DK. Alpha$_1$-adrenoceptor-mediated phosphoinositides breakdown and inotropic response in rat left ventricular papillary muscles. Circ Res 62:8-17, 1988.

Ozuari A, Ozturk Y, Yildizoglu-Ari N, Ozcelkay AT and Altan VM. The effects of glyburide and insulin on the cardiac performance in rats with non-insulin-dependent diabetes mellitus. General Pharmacology 24(1):165-9, 1993.

Perez de Gracia B, Rhoads AR and West WL. The repsonses of cyclic 3',5'-AMP and cyclic 3',5'-GMP phosphodiesterases to experimental diabetes. Experientia 36(7):824-5, 1980.

Pericas I and Jolin T. The effect of streptozotocin-induced diabetes on the pituitary-thyroid axis in goitrogen-treated rats. Acta Endocrinologica. 86(1):128-39, 1977.

Pershadsingh Ha, McDaniel ML et al. Ca^{2+} activated ATPase and ATP-dependent calmodulin-stimulated Ca^{2+} transport in islet cell plasma membrane. Nature 288:492-5, 1980.

Poggioli J et al. Inositol phosphate production following α_1-adrenergic, muscarinic or electrical stimulation of phosphoinositide hydrolysis is adult rat cardiomyocytes. FEBS Lett 206:9185-9190, 1985.

Pucéate M, Clément O, Scamps F et al. Neurohormonal control of calcium sensitivity of myofilaments in rat single heart cells. Circ Res 67:517-524, 1990.

Pucéate M, Clément O, Terzic A and Vassort G. α_1-Adrenoceptor and purinoceptor agonists modulate the Na/H antiport in single cardiac cells. Am J Physiol 264:H310-319, 1993.

Pucéate M, Terzic A, Clément O et al. Cardiac α_1-Adrenoceptors mediate a positive inotropic effect via myofibrillar Ca^{2+}-sensitization. Trends Pharmacol Sci 13:263-265, 1992.

Ramanadham S and Tenner TE. Alterations in the myocardial β-adrenoceptor system on streptozotocin-induced diabetic rats. Eur J Pharmacol 136:377-379, 1987.

Rapiejko PJ, Watkins DC, Ros M and Malbon CC. Thyroid hormones regulate G-protein beta-subunit mRNA expression in vivo. J Biol Chem 264(27):16183-9, 1989.

Robbins MJ, Sharp RA, Slonim AE et al. Protection against streptozotocin-induced diabetes by superoxide dismutase. Diabetologia 18:55-58, 1980.

Rokosh DG and Sulakhe PV. Characteristics of alpha$_1$-adrenoceptors coupled to inotropic response and phosphoinositide metabolism in rat myocardium. Circulation 84:II-389, 1991.

Rondeel JM, de Greef WJ, Heide R and Visser TJ. Hypothalamo-hypophysial-thyroid axis in streptozotocin-induced diabetes. Endocrinology. 130(1):216-20, 1992.

Sakurai H, Tsuchiya K, Nukatsuka M, Sofue M, Kawada J. Insulin-like effect of vanadyl ion on streptozotocin-induced diabetic rats. Journal of Endocrinology 126(3):451-9, 1990.

Sandler S and Anderson A. Partial protective effect of the hydroxyl radical scavenger dimethylurea on streptozotocin-induced diabetes in the mouse. Diabetologia 23:374-8, 1982.

Sandler S. Protection by demethylurea against hyperglycemia, but not insulitis, in low-dose streptozotocin-induced diabetes in vivo and in vitro. Diabetologia 26:386-8, 1984.

Sato M, Hashimoto H, Takiguchi Y and Nakashiwa M. Altered responsiveness to sympathetic nerve stimulation and agonists of islolated left atria of diabetic rats: No evidence for involvement of hypothyroidism. J Pharmcol and Experimental Therapeutics 248(1):367-371, 1989.

Saunders J, Hall SE and Sonksen PH. Thyroid hormones in insulin requiring diabetes before and after treatment. Diabetologia. 15(1):29-32, 1978.

Sauviat MP and Feuvray D. Electrophysiological analysis of the sensitibity to calcium in ventricular muscle from alloxan diabetic rats. Basic Research in Cardiology 81(5):489-96, 1986.

Savarese JJ and Berlowits BA. β-adrenergic receptor decrease in diabetic rat hearts. Life Sci 25:2075-8, 1979.

Schein PS, Cooney DA and Vernon ML. The use of nicotinamide to modify the toxicity of streptozotocin diabetes without loss of antitumor activity. Cancer Research. 27(12):2324-32, 1967.

Schein PS, Rakieten N, Cooney DA, Davis R and Vernon ML. Streptozotocin diabetes in monkeys and dogs, and its prevention by nicotinamide. Proc of Soc for Exp Biol & Med. 143(2):514-8, 1973.

Schmitz W, Scholz H, Scholz J and Steinfath. Increase in IP$_3$ precedes alpha-adrenoceptor-induced increase in force of contraction in cardiac muscle. Eur J Pharmacol 140:109-111, 1987.

Scholz H, Hackbarth I, Schmitz W and Wetzel E. Effect of vanadate on myocardial force of contraction. Basic Res in Cardiol. 75(3):418-22, 1980.

Scholz J, Scholz H and Steinfath M. Effect of alpha$_1$-adrenoceptor stimulation on phosphoinositide metabolism and force of contraction in rat heart. (abstract) Naunyn-Schmiedeberg's Arch. Pharmacol 335:R58, 1987.

Scholz J, Troll U, Sandig P, Schemitz W et al. Existence and α_1-adrenergic stimulation of inositol polyphosphates in mammalian heart. Mol Pharmacol 42:134-140, 1992.

Sibley DR, Nambi P, Peters JR and Lefkowitz RJ. Phorbol diesters promote β-adrenergic receptor phosphorylation and adenylate cyclase desensitization in duck erythrocytes. Biochem Biophys Res Commun 121: 973-979, 1984.

Simpson PC. Stimulation of hypertrophy of cultured neonatal heart cells through an α_1-adrenergic receptor and induction of beating through β_1-adrenergic receptor interaction. Evidence for independent regulation of growth and beating. Circ Res 56:884-894, 1985.

Sims JL, Berger SJ, Berger NA. Effects of nicotinamide on NAD and poly(ADP-ribose) metabolism in DNA-damaged human lymphocytes. Journal of Supramolecular Structure & Cellular Biochemistry. 16(3):281-8, 1981.

Sims JL, Juarez-Salinas H, Jacobson MK. A new highly sensitive and selective chemical assay for poly(ADP-ribose). Analytical Biochemistry. 106(2):296-306, 1980.

Slonim AE, Fletcher T, Burke V et al. Effect of streptozotocin on red-blood-cell-induced glutathione: modification by glucose, nitotinamide and epinephrine. Diabetes 25:216-22, 1976.

Slonim AE, Reinhardt J, Surber MH et al. Evidence for the oxidant action of streptozotocin. In: Waldhausl WW, Luft R and Alberti KGGM eds. Proc 10th Cong Int Diabetes Federation. Amsterdam: Excerpta Medica 1979:219.

Slonim AE, Surber ME, Page DE et al. Modification of chemically induced diabetes in rats by vitamin E: supplementation minimizes and depletion enhances development of diabetes. J Clin Invest 71:1282-8, 1983.

Smith CI, Pierce GN and Dhalla NS. Alterations in adenylate cyclase activity due to streptozotocin-induced diabetic cardiomyopathy. Life Sciences 34(3):1223-30, 1984.

Sochor M, Gonzalez AM and McLean P. Regulation of alternative pathways of glucose metabolism in rat heart in alloxan diabetes: changes in the pentose phosphate pathway. Biochem & Biophys Res Commun 118(1):110-6, 1984.

Sofue M, Yoshimura Y, Nishida M, Kawada J. ADP modifies the function of the glucose transporter: studies with reconstituted liposomes. Biochemical Journal. 292(Pt3):877-81, 1993.

Sofue M, Yoshimura Y, Nishida M, Kawada J. Possible multifunction of glucose transporter. Transport of nicotinamide by reconstituted liposomes. Biochemical Journal. 288(Pt 2):669-74, 1992.

Sofue M, Yoshimura Y, Nishida M, Kawada J. Uptake of nicotinamide by rat pancreatic beta cells with regard to streptozotocin action. Journal of Endocrinology. 131(1):135-8, 1991.

Solomon SS, Deaton J, Harris G and Smoake JA. Studies of insulin resistance in the streptozotocin diabetic and BB rat: Activation of low Km cAMP phosphodiesterase by insulin. Am J Med Sci 297(6):372-376, 1989.

Sperelakis N. Regulation of calcium slow channels of cardiac muscle by cyclic nuleotides and phosphorylation. J bol Cell Cardiol 20(Suppl II):75-105, 1988.

Spiro MJ and Crowley TJ. Increased rat myocardial type VI collagen in diabetes mellitus and hypertension. Diabetologia 36(2):93-8, 1993.

Stiles GL and Lefkowitz RJ. Thyroid hormone modulation of agonist-beta-adrenergic receptor interactions in the rat heart. Life Sciences. 28(22):2529-36, 1981.

Stiles GL, Caron MG and Lefkowitz RJ. Beta-adrenergic receptors: biochemical mechanisms of physiological regulation. Physiol Rev. 64(2):661-743, 1984.

Stiles GL, Strasser RH, Kilpatrick BF, Taylor SR and Lefkowitz RJ. Endogenous proteinases modulate the function of the beta-adrenergic receptor-adenylate cyclase system. Biochimica et Biophysica Acta. 802(3):390-8, 1984.

Sugden D, Vanacek J, Klein DC, Thomas TP and Anderson WB. Activation of protein kinase C potentiates isoprenalin-induced cyclic AMP accumulation in rat pinealocytes. Nature 314:359-361, 1985.

Sundaresan PR, Sharma VK, Gingold SI and Banerjee SP. Decreased beta-adrenergic receptors in rat heart in streptozotocin-induced diabetes: role of thyroid hormones. Endocrinology. 114(4):1358-63, 1984.

Sutherland E. Studies on the mechanism of hormone action. Science 177:401-408, 1972.

Tahiliani AG and McNeill JH. Lack of effect of thyroid hormone on diabetic rat heart function and biochemistry. Can J Physiol Pharmacol 62(6):617-621, 1984.

Tahirovic HF. Thyroid hormones changes in infants and children with metabolic acidosis. J Endocrin Invest 14(9):723-6, 1991.

Takanashi M, Norota I and Endoh M. Potent inhibitory action of chlorethylclinidine on the positive inotropic effect and phosphoinositide hydrolysis mediated via myocardial α $_1$-adrenoceptors in the rabbit ventricular myocardium. Naunyn Schmiedebergs Arch. Pharmacol 343: 669-673, 1991.

Takasu N, Komiya I, Asawa T et al. Streptozocin- and alloxan-induced H_2O_2 generation and DNA fragmentation in pancreatic islets. Diabetes 40:1141-1145, 1991.

Tani M and Neely JR. Hearts from diabetic rats are more resistant to in vitro ischemia: possible role of altered Ca^{2+} metabolism. Circ Res 62(5):931-40, 1988.

Terzic A, Anagnostopoulos T and Vogel SM. Opposite modulation of ouabain cardiotoxicity by hexamethylamilaride and phenylephrine. Naunyn Schmiedebergs Arch. Pharmacol 343:511-518, 1992.

Terzic A, Pucéate M, Clément O et al. α_1-adrenergic effects on intracellular pH and calcium, and on myofilaments in single rat cardiac cells. J Physiol 447:275-292, 1992a.

Thaete LG, Crouch RK, Buse MG et al. The protective role of copper-zinc superoxide dismutase against alloxan-induced diabetes: morphological aspects. Diabetologia 28:677-82, 1985.

Tibaldi J, Benjamin J, Cabbat FS et al. Protection against alloxan-induced diabetes by various urea derivatives: relationship between protective effects and reactive with the hydroxyl radical. J Pharmacol Exp Ther 211:415-8, 1979.

Tomlinson DR and Yusof AP. Autonomic neuropathy in the alloxan-diabetic rat. J of Auton Pharmacol 3(4):257-63, 1983.

Tsien RW. Cyclic AMP and contractile activity in heart. Adv Cylic Nucleotide Res 8:363-420, 1977.

Tsujimoto G, Tsujimoto A, Suzuki E and Hashimoto K. Glycogen phosphorylase activation by two different alpha 1-adrenergic receptor subtypes: methoxamine selectively stimulates a putative alpha1-adrenergic receptor subtype (alpha 1a) that couples with Ca2+ influx. Molecular Pharmacol. 36(1):166-76, 1989.

Uchigata Y, Yamamoto H, Kawamura A and Okamoto H. Protection by superoxide dismutase, catalase and poly(ADP-ribose) synthetase inhibitors against alloxan- and streptozotocin-induced islet DNA strand breaks and against inhibition of proinsulin synthesis. J Biol Chem 6084-8, 1982.

Uchigata Y, Yamamoto H, Nagai H et al. Effect of poly-(ADP-ribose) synthetase inhibitor administration to rats before and after injection of alloxan and streptrozotocin on islet proinsulin synthesis. Diabetes 32:316-318, 1983.

Vadlamudi RSVS and Tenner TE. Effects of experimental diabetes on rat cardiac cAMP, phosphorylase and inotropy. Am J of Physiol 244:H844, 1983.

Vadlamudi RV and McNeill JH. Effect of experimental diabetes on isolated rat heart responsiveness to isoproterenol. Can J of Physiol & Pharmacol 62(1):124-31, 1984.

Vadlamudi RV, Rodgers RL and McNeill JH. The effect of chronic alloxan- and stroptoxotocin-induced diabetes on isolated rat heart performance. Can J Physiol & Pharmacol 60(7):902-11, 1982.

Vanacek J, Sugden D, Weller J and Klein DC. Atypical synergistic α_1- and β-adrenergic regulation of adenosine 3', 5'-monophosphate and guanosine 3',5'-monophosphate in rat pinealocytes. Endocrinology 116:2167-2173,1985.

Vaughan-Jones RD, Eisner Da and Legerer J. Effects of changes of intracellular pH on contraction in sheep cardiac Purkinje fibers. J Gen Physiol 89:1015-1032, 1987.

Wald M, Borda ES and Sterin-Borda L. Alpha-adrenergic supersensitivity and decreased number of alpha-adrenoceptors in heart from acute diabetic rats. Can J of Physiol Pharmacol 66(9):1154-60, 1988.

Wallert MA and Frohlich O. Adrenergic stimulation of Na-H exchange in cardiac myocytes Am J Physiol 263:C1096-C1102, 1992.

Weisse RB. Streptozotocin: a review of its pharmacology, efficacy and toxicity. Cancer Treat Rep 66:427-38, 1982.

Williames IH, Chua BH, Sahms RH, Siehl D and Morgan HE. Effects of diabetes on protein turnover in cardiac muscle. Am J Physiol 239(3):E178-85, 1980.

Williams RS, Schaible TF, Scheuer J and Kennedy R. Effects of experimental diabetes on adrenergic and cholinergic receptors of rat myocardium. Diabetes 32:881, 1983.

Wise BC, Glas DB, Chou CHJ, Rayer RL, Katoh N, Schatzman TC, Turner RS, Kibler RF and Kou JF. Phospholipid-sensitive Ca^{2+}-dependent protein kinase from heart. II. substrate specificity and inhibition by various agents. J Biol Chem 257:8489, 1982.

Wohaieb SA and Godin DV. Alterations in tissue antioxidant systems in the spontaneously diabetic (BB Wistar) rat. Can J of Physiol & Pharmacol. 65(11):2191-5, 1987.

Xiang H and McNeill JH. α_1-adrenoceptor-mediated phosphoinositide breakdown and inotropic responses in diabetic hearts. Am J Physiol 260:H557-H562, 1991.

Xiang H, Heyliger CE and McNeill JH. Effect of myo-inositol and T_3 on myocardial lipids and cardiac function in streptozocin-induced diabetic rats. Diabetes 37(11):1542-1548, 1988.

Yamamoto H, Uchigata Y and Okamoto H. Streptozotocin and alloxan induced DNA strand breaks and poly (ADP-ribose) synthetase in pancreatic islets. Nature 294: 284-6, 1981.

Yoon JW, Kim CJ and McArthur RG. Enviromental factors and IDDM. Clin Invest Med 10:457-469, 1987.

Yu Z and McNeill JH. Altered inotropic responses in diabetic cardiomyopathy and hypertensive-diabetic cardiomyopathy. J Pharmacol and Expt Ther 257(1):64-71, 1991.

Zarich SW and Nesto RW. Diabetic Cardiomyopathy. Am Heart Journal 118(5pt1):1000-1012, 1989.

Zola BE, Miller B, Stiles GL et al. Heart rate control in diabetic rabbits: blunted response to isoproterenol. Am J of Physio 255(5pt1):E636-41, 1988.