ALTERATIONS IN CARDIAC RESPONSES TO ADRENERGIC AGONISTS AND IN CIRCULATING THYROID HORMONES IN ALLOXAN INDUCED DIABETIC RATS

by

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We accept this thesis as conforming to the required standard

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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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<tr>
<td>$\alpha$</td>
<td>alpha</td>
</tr>
<tr>
<td>AC</td>
<td>adenylyle cyclase</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine 5'-diphosphate</td>
</tr>
<tr>
<td>ADR</td>
<td>adrenergic receptor</td>
</tr>
<tr>
<td>ALX</td>
<td>alloxan</td>
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<tr>
<td>ATP</td>
<td>adenosine trisphosphate</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
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<td>$\beta$</td>
<td>beta</td>
</tr>
<tr>
<td>CA</td>
<td>catecholamine</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>calcium</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine 3',5' monophosphate</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
</tr>
<tr>
<td>CEC</td>
<td>chlorethylclonidine</td>
</tr>
<tr>
<td>CI</td>
<td>cardiac index</td>
</tr>
<tr>
<td>CPM</td>
<td>counts per minute</td>
</tr>
<tr>
<td>DAG</td>
<td>diacylglycerol</td>
</tr>
<tr>
<td>Db-cAMP</td>
<td>Dibutyryl cyclic adenosine monophosphate</td>
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<tr>
<td>dl</td>
<td>decilitre</td>
</tr>
<tr>
<td>E</td>
<td>epinephrine</td>
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<tr>
<td>EC$_{50}$</td>
<td>concentration at which a drug produces half of the maximal effect</td>
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<tr>
<td>E$_{MAX}$</td>
<td>maximal effect</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>Fig.</td>
<td>figure</td>
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<td>FOR</td>
<td>free oxygen radicals</td>
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<tr>
<td>FSK</td>
<td>forskolin</td>
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<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
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</tr>
<tr>
<td>G</td>
<td>GTP activated protein(s)</td>
</tr>
<tr>
<td>IP3</td>
<td>inositol-1,4,5-trisphosphate</td>
</tr>
<tr>
<td>ISO</td>
<td>isoproterenol</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
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<tr>
<td>I.V.</td>
<td>intravenous</td>
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<tr>
<td>Kg</td>
<td>kilogram</td>
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<tr>
<td>LA</td>
<td>left atria</td>
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<tr>
<td>LPL</td>
<td>lipoprotein lipase</td>
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<tr>
<td>LVP/LVSP</td>
<td>left ventricular (systolic) pressure</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
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<tr>
<td>mg</td>
<td>milligram</td>
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<td>ml</td>
<td>millilitre</td>
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<td>mm</td>
<td>millimetre</td>
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<td>mmHg</td>
<td>millimetre of mercury</td>
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<td>mM</td>
<td>millimolar</td>
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<tr>
<td>M</td>
<td>molar concentration, mole/L</td>
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<tr>
<td>ms</td>
<td>millisecond</td>
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<tr>
<td>N</td>
<td>number of the observations</td>
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<tr>
<td>NE</td>
<td>norepinephrine</td>
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<tr>
<td>nm</td>
<td>nanometre</td>
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<tr>
<td>P</td>
<td>probability</td>
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<tr>
<td>PE</td>
<td>phenylephrine</td>
</tr>
<tr>
<td>pD2</td>
<td>agonist affinity constant</td>
</tr>
<tr>
<td>PK</td>
<td>protein kinase</td>
</tr>
<tr>
<td>PKA</td>
<td>cAMP dependent protein kinase</td>
</tr>
<tr>
<td>PM</td>
<td>papillary muscle</td>
</tr>
<tr>
<td>pmol</td>
<td>picomole</td>
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<td>%</td>
<td>percentage</td>
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PX peroxidase
RA right atria
RIA radioimmunoassay
rT3 3,3,5'-triiodothyronine, reverse T3
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
T3 3,5,3'-triiodothyronine
T4 thyroxine
TRH thyrotropin-releasing hormone
TSH Thyroid-stimulating hormone, thyrotropin
μCi microcurie
μl microliter
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, α1 and α2 (Berthelson and Pettinger 1977, Hoffman et al 1979) and β1 and β2 (Lands et al 1967). α2 and β2 also known as autoreceptors, mainly exist presynaptically, modulating the release of catecholamine (CA) from the nerve terminals negatively (α2) or positively (β2). The cardiac α1- and β1- ADRs are positively linked to the contractility and chronotropic response when the receptors are occupied. α1-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). β1-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 - Gs. The activation of Gs 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, α2-ADRs are negatively linked to AC by coupling of the receptor to a Gi 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 α1-ADRs in various tissues including the cardiomyocytes, can be further subdivided into at least two distinct subtypes, α1A and α1B. 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 α1A-subtype has a higher affinity than the α1B-subtype for the antagonists 5-methyl-urapidil, WB-4101 and (+)-niguldipine or the novel prazosin derivative, SZL-49. The α1B-subtype is characterized
by its irreversible alkylation by CEC. Based on their respective sensitivity toward selective 
$\alpha_{1A}$-antagonists, it was found that 20% of cardiac $\alpha_1$-ADR belong to the $\alpha_{1A}$-subtype and 
80% of the binding sites correspond to the $\alpha_{1B}$-subtype in rat (Groß and Hanft 1988; Groß et 
al 1988a). Evidence indicates at least two subtypes of pharmacologically distinct cardiac $\alpha_1$-
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 $\alpha_1$-adrenoceptors encoded by two different genes (Lomasney et al 1991).

The nature of the $\alpha_1$-ADR subtypes responsible for the positive inotropic effect is a 
matter of current investigation. In rabbit papillary muscle, the involvement of $\alpha_{1B}$-subtype 
was suggested based on the concentration-dependent inhibition by CEC of the $\alpha_1$-ADR-
mediated positive inotropic effects (Takanashi et al 1991). The $\alpha_{1A}$ subtype may also 
participate in this effect, although to a smaller extent than the $\alpha_{1B}$-subtype, since WB-4101, 
an $\alpha_{1A}$-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 $\alpha_{1A}$ appears to 
be responsible for the $\alpha_1$-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 $\alpha_1$-ADRs are coupled to at least two cellular mechanisms: the $\alpha_{1A}$-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, $\alpha_{1B}$-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 (IP$_3$) and a hydrophobic 
product 1,2-diacylglycerol (DAG) from the cleavage of phosphotidylinositol 4,5 
bisphosphate (PIP$_2$) (Berridge 1982). The occupancy of the cardiac $\alpha_1$-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 $\mu$M PE or 100 $\mu$M NE
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 \(\alpha_1\)-ADR-mediated positive inotropic effect, probably mainly by
phosphorylation of myosin light chain kinase (Puceat et al 1990, Khalil et al 1992) or
increasing the sensitivity of contractile proteins to Ca\(^{2+}\) rather than increasing [Ca\(^{2+}\)]\(_i\)

1.3 Adrenoceptor-Coupled Second Messengers in the Heart

1.3.A \(\alpha_1\)-ADR-linked IP\(_3\)/DAG

The role of IP\(_3\) as a second messenger in initiation of \(\alpha_1\)-mediated contraction in
smooth muscles has been generally recognized, and it may play the same role in the
cardiomyocyte. Enhanced IP\(_3\) production following \(\alpha_1\)-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 \(\alpha_1\)-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 \(\alpha_1\)-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 \(\alpha_1\)-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 ($\alpha_1$, $\alpha_2$ blocker), prazosin ($\alpha_1$ blocker), yohimbine ($\alpha_2$ blocker) and propranolol ($\beta$ 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 $\alpha_1$-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 \(\alpha_1\)-ADR agonists or other positive inotropic interventions, implied that the \(\alpha_1\)-ADR agonists may increase the myofibrillar Ca\(^{2+}\) sensitivity. The increase in myofibrillar sensitivity to Ca\(^{2+}\) following the administration of \(\alpha_1\)-ADR was later confirmed in single cardiomyocyte preparation (Capogrossi et al 1988). The definite evidence that \(\alpha_1\)-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 \(\alpha_1\)-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, \(\alpha_1\)-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 \(\alpha_1\)-ADR agonists, several lines of evidence suggest that the activation of Na\(^+\)/H\(^+\) antiporter participates in the positive inotropic effects of \(\alpha_1\)-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 α1-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 α1-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 α1-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 dibutryryl 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 \((-\)^3H-dihydroalprenolol showed a significant 28% reduction in the number of ventricular \(\beta\)-receptor binding sites in STZ-induced diabetic rats. The reduced diabetic ventricular \(\beta\)-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 \(\beta\)-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 epipherine-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\textsubscript{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\textsubscript{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\textsubscript{1}-receptor agonist has been observed (Jackson et al 1986, Yu and McNeill, 1990). The increase in the α\textsubscript{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\textsubscript{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 IP3 formation was observed concomitantly with the increase in contractile response to α1-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 α1-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 $\beta$-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 $\beta$-ADR to AC in the heart of hypothyroid rats; although, a similar significant decrease in the number of $\beta$-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 $\beta$-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 $\beta$-ADR and hypothyroidism seem to be less conclusive. The underlying mechanism of the concomitant reduced $\beta$-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 (Saander et al 1978). It was found that in diabetic patients, the circulating thyroxine (T4) and triiodothyronine (T3) were low, but reversed triiodothyronine (T3) was elevated and all three thyroid hormones returned to within normal range following insulin treatment. The T3 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 T4, as well as a decrease in rT3 clearance rate, may contribute to a decreased T3 and increased rT3 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 T4 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 (Gonzalez 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, T4 and T3 were significantly decreased while rT3 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 T4 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 T3 administration. The results indicated that the treatment with T3 alone did not improve cardiac function in diabetic rats although the T3 level was restored to normal or above normal (Tahiliani and McNeill 1984, Xiang et al 1988). In addition to the fact that T3 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 \( E_{\text{max}} \) of norepinephrine (NE) was decreased without any significant alteration in \( EC_{50} \) and a decrease in \( E_{\text{max}} \) 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 T4 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 (\( \text{H}_2\text{O}_2 \)), superoxide anion (\( \text{O}_2^- \)) and hydroxyl free radicals (OH·) (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 produce 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·.

In vitro studies in isolated islet cells measuring rubidium accumulation and Trypan blue exclusion support a role of FOR in ALX-induced diabetes. Diethylenetriaminepentaacetic acid (DETA-PAC) protected islet cells from the cytotoxicity of ALX (Grankvist et al 1979a). Copper(II) which catalyses production of OH· 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 diabetogenicity 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 produced 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-nitrosoureaed o 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$_2$O$_2$ 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$_2$O$_2$ mimicked the effects of ALX and STZ on DNA breakdown in the same preparation. These effects of ALX or STZ on the H$_2$O$_2$ and DNA fragmentation were concentration and time-dependent, with a Emax > 1mM and taking 10-15 min to generate a significant increase in H$_2$O$_2$ (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 diabetogenicity 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 responses 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\textsuperscript{2+} metabolism, ultrastructural changes, low circulating thyroid hormones and alterations of activity of cardiac Ca\textsuperscript{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 \(\alpha\)- and \(\beta\)-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 \(\beta\)-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 administration 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, CaCl2 2.2, MgCl2 2.1, NaHCO3 25 and glucose 10) which was continuously oxygenated with 95% O2 and 5% of CO2. 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 α₁-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, pD₂ 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 pD₂ 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 O₂-saturated (with 95% O₂ and 5% CO₂) 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 peristaltic 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 [125I]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}\text{I}]\text{cAMP}\ that bound to the primary rabbit antibody is immobilized on the fluorosphere, which produces light. Measurement in a β-scintillation counter enables the amount of fluorosphere 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}\text{I}]\text{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}\text{I}]\text{cAMP}\ bound to the fluorospheres 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:

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

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 \(E_{\text{max}}\) and estimated pD2 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 T3 and T4 were reduced in diabetic animals and the reductions were significant. The magnitude of reduction in T4 (93%) was greater than that of T3 (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 $E_{\text{MAX}}$ in right atria. There was an decrease in $E_{\text{MAX}}$ of ISO in diabetic RA. On the other hand, $E_{\text{MAX}}$ of PE was increased significantly in diabetic tissue.

The positive inotropic $E_{\text{MAX}}$ of adrenoceptor agonists in left atria and papillary muscles are illustrated in Figure 12. In left atria, the difference in $E_{\text{MAX}}$ of ISO or PE between diabetic and control was not significant although the $E_{\text{MAX}}$ 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 $E_{\text{MAX}}$ of ISO, and a significantly increase in $E_{\text{MAX}}$ 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N=40)</th>
<th>ALX-diabetic (N=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain in Body Weight (g)</td>
<td>293 ± 3.7</td>
<td>57.6 ± 9*</td>
</tr>
<tr>
<td>[Insulin]_p (μIU/ml)</td>
<td>42.6 ± 2.06</td>
<td>8.65 ± 0.74*</td>
</tr>
<tr>
<td>[Glucose]_p (mM)</td>
<td>7.6 ± 0.2</td>
<td>19.7 ± 0.8*</td>
</tr>
<tr>
<td>[Triglyceride]_p (mM)</td>
<td>1.42 ± 0.11</td>
<td>6.37 ± 0.57*</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Control (N=40)</th>
<th>ALX-diabetic (N=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight</td>
<td>1.481 ± 0.026</td>
<td>1.047 ± 0.027*</td>
</tr>
<tr>
<td>Cardiac Index</td>
<td>2.93 ± 0.054</td>
<td>4.00 ± 0.118*</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Control (N=40)</th>
<th>ALX-diabetic (N=54)</th>
<th>% Reduction in diabetic rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>[T3]p (ng/dl)</td>
<td>42.5 ± 1.2</td>
<td>25.2 ± 1.24*</td>
<td>40.7%</td>
</tr>
<tr>
<td>[T4]p (µg/dl)</td>
<td>4.1 ± 0.1</td>
<td>0.28 ± 0.06*</td>
<td>93.2%</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>RA (beat/min)</th>
<th>LA (g)</th>
<th>PM (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Control</td>
<td>232 ± 5 (N=24)</td>
<td>0.55 ± 0.05 (N=24)</td>
</tr>
<tr>
<td></td>
<td>ALX</td>
<td>197 ± 6* (N=22)</td>
<td>0.58 ± 0.06 (N=25)</td>
</tr>
<tr>
<td>$E_{MAX}$</td>
<td>Control</td>
<td>206 ± 6</td>
<td>1.2 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>ALX</td>
<td>171 ± 6*</td>
<td>0.98 ± 0.10</td>
</tr>
</tbody>
</table>
Table 5. Basal rate of RA, basal contractile force of LA and PM, maximal effects ($E_{\text{MAX}}$) 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.

<table>
<thead>
<tr>
<th></th>
<th>RA (beat/min)</th>
<th>LA (g)</th>
<th>PM (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>Control</td>
<td>232 ± 5 (N=22)</td>
<td>0.55 ± 0.05 (N=26)</td>
</tr>
<tr>
<td></td>
<td>ALX</td>
<td>197 ± 6* (N=23)</td>
<td>0.58 ± 0.06 (N=25)</td>
</tr>
<tr>
<td>$E_{\text{MAX}}$</td>
<td>Control</td>
<td>26 ± 3</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>ALX</td>
<td>73 ± 11*</td>
<td>0.78 ± 0.07</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>LA</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>pD$_2$ of ISO</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.22 ± 0.11</td>
<td>7.76 ± 0.12</td>
<td>7.28 ± 0.12</td>
</tr>
<tr>
<td>ALX</td>
<td>8.34 ± 0.09</td>
<td>7.72 ± 0.15</td>
<td>7.06 ± 0.2</td>
</tr>
<tr>
<td>pD$_2$ of PE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.08 ± 0.18</td>
<td>5.32 ± 0.14</td>
<td>5.18 ± 0.18</td>
</tr>
<tr>
<td>ALX</td>
<td>5.66 ± 0.15</td>
<td>5.45 ± 0.11</td>
<td>5.47 ± 0.12</td>
</tr>
</tbody>
</table>
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.

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

\(^1\) 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.

<table>
<thead>
<tr>
<th>(mmHg)</th>
<th>Basal</th>
<th>7-8 sec</th>
<th>15 sec</th>
<th>60 sec</th>
<th>4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control¹</td>
<td>69 ± 2.3</td>
<td>78.2 ± 3.4</td>
<td>74.5 ± 2.7</td>
<td>78.2 ± 3.4</td>
<td>81.7 ± 2.75</td>
</tr>
<tr>
<td></td>
<td>(N=15)</td>
<td>(N=11)</td>
<td>(N=15)</td>
<td>(N=7)</td>
<td>(N=3)</td>
</tr>
<tr>
<td>Diabetic¹</td>
<td>75.1 ± 1.7*</td>
<td>90.1 ± 2+*</td>
<td>86.9 ± 1.9+*</td>
<td>93.5 ± 2.3+*</td>
<td>96.8 ± 2.38+*</td>
</tr>
<tr>
<td></td>
<td>(N=21)</td>
<td>(N=16)</td>
<td>(N=17)</td>
<td>(N=12)</td>
<td>(N=4)</td>
</tr>
<tr>
<td>Control²</td>
<td>10.0 ± 3.0</td>
<td>6.4 ± 3.5</td>
<td>11.0 ± 4.7</td>
<td>15.0 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>Diabetic²</td>
<td>16.5 ± 2.5</td>
<td>12.7 ± 2.81</td>
<td>21.6 ± 3.6</td>
<td>31.0 ± 5.4</td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>15 sec</th>
<th>30 sec</th>
<th>60 sec</th>
<th>4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control¹</td>
<td>9.5 ± 1.23</td>
<td>23.6 ± 1.6+</td>
<td>13 ± 1.6</td>
<td>9.49 ± 1.5</td>
<td>7.8 ± 1.2</td>
</tr>
<tr>
<td>(N=7)</td>
<td>(N=4)</td>
<td>(N=4)</td>
<td>(N=5)</td>
<td>(N=7)</td>
<td></td>
</tr>
<tr>
<td>Diabetic¹</td>
<td>9.3 ± 1.7</td>
<td>28.1 ± 2.2+</td>
<td>24.4 ± 2+*</td>
<td>19.7 ± 1.8*</td>
<td>15.4 ± 2+*</td>
</tr>
<tr>
<td>(N=7)</td>
<td>(N=4)</td>
<td>(N=5)</td>
<td>(N=6)</td>
<td>(N=5)</td>
<td></td>
</tr>
<tr>
<td>Control²</td>
<td>1.6 ± 0.19</td>
<td>3.5 ± 0.35+</td>
<td>2.3 ± 0.29</td>
<td>1.9 ± 0.29</td>
<td>2.4 ± 0.23</td>
</tr>
<tr>
<td>Diabetic²</td>
<td>1.8 ± 0.21</td>
<td>2.2 ± 0.34</td>
<td>2.9 ± 0.26</td>
<td>3.1 ± 0.27+*</td>
<td>2.8 ± 0.27</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>15 sec</th>
<th>60 sec</th>
<th>4 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control¹</td>
<td>9.4 ± 0.87</td>
<td>7.0 ± 0.87</td>
<td>11.3 ± 0.92</td>
<td>10.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>(N=4)</td>
<td>(N=4)</td>
<td>(N=3)</td>
<td>(N=3)</td>
</tr>
<tr>
<td>Diabetic¹</td>
<td>9.1 ± 0.28</td>
<td>11.5 ± 0.92*</td>
<td>6.5 ± 0.78*</td>
<td>6.6 ± 0.29*</td>
</tr>
<tr>
<td></td>
<td>(N=4)</td>
<td>(N=5)</td>
<td>(N=7)</td>
<td>(N=4)</td>
</tr>
<tr>
<td>Control²</td>
<td>2.0 ± 0.14</td>
<td>4.1 ± 0.47+</td>
<td>4.2 ± 0.17+</td>
<td>3.7 ± 0.11+</td>
</tr>
<tr>
<td>Diabetic²</td>
<td>4.0 ± 0.14*</td>
<td>3.1 ± 0.42+</td>
<td>1.42 ± 0.09+/*</td>
<td>1.3 ± 0.09+/*</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Protein content</th>
<th>Control hearts</th>
<th>Diabetic hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.99 ± 0.06</td>
<td>0.88 ± 0.06 *</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>LVP</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ISO</td>
<td>Control</td>
<td>15 sec</td>
<td>30 sec</td>
<td>60 sec</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>77.4 %</td>
<td>58.7 %</td>
<td>44.2 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic</td>
<td>65.5 %</td>
<td>60.8 %</td>
<td>61.9 %</td>
</tr>
<tr>
<td></td>
<td>PE</td>
<td>Control</td>
<td>10.2 %</td>
<td>-</td>
<td>18.7 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic</td>
<td>21.4 %</td>
<td>-</td>
<td>31.7 %</td>
</tr>
<tr>
<td></td>
<td>cAMP</td>
<td>ISO</td>
<td>148.4 %</td>
<td>36.8 %</td>
<td>0 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic</td>
<td>202.2 %</td>
<td>162.4 %</td>
<td>111.8 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PE</td>
<td>-2.4 %</td>
<td>-</td>
<td>1.9 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diabetic</td>
<td>26.4 %</td>
<td>-</td>
<td>-28.6 %</td>
</tr>
</tbody>
</table>
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 $E_{\text{MAX}}$ 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 $E_{\text{MAX}}$ 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 ($\mathrm{pD}_2$) of ISO calculated from the results obtained from concentration-response curves.
Figure 14. Estimated affinity constant ($\text{pD}_2$) 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 T3 and T4 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 T3 level with an increase in rT3 (Naeije et al 1978), and in diabetic animals the deiodonization of T4 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 T4 into T3 in the adipose and hepatic tissues (Gavin et al 1981). However, there had been a lack of explanation of the concomitant decrease in T4, 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 T4 to T3 is a causative factor of the "low thyroid state" in diabetes, the circulating T4 would not decrease to a greater extent than T3 as found in the present study and in other reports (Garber and Neely 1983). A normal rT3 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 T3 and T4 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 T3 will in turn increase TRH and TSH release from the higher centres, thus leading to increases in circulating T4 and correction of the decreased circulating T3. But increases in TRH, TSH and T4 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 T4 as well as T3 (Garber and Neely 1983, Karasu et al 1990), suggesting mechanisms other than a reduced peripheral deiodonization of T4 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 T4, T3, and reciprocal increase in rT3 in Type I and II diabetes. The reduced T3 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
Among the altered thyroid hormones levels, only T₃ 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 T₃ 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 T₃ in insulin resistance as well as the lipid metabolism. The decreased T₃ 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 rT₃ and to rT₃/T₃ ratio were significantly increased in both types of diabetic patients. There is a positive correlation of rT₃ to only HbA1c (Type I: r = 0.63, Type II: 0.53) and to rT₃/T₃ 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 T₃ levels in diabetes may reflect the glucose levels thus serving as an indicator for glucose control in Type I diabetic patients, and the reduced T₃ 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 T₃ 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, T₃, T₄, TSH in all the patients and TRH in 10 randomly selected 10 patients from each groups were measured. A lower T₃ level was found in all of the pathological groups versus the control
group, but T₄ in diabetic patients was not significantly lower than the control. However, rT₃ was significantly higher in diabetic and anorexia nervosa groups, suggesting the reciprocal low T₃ and high rT₃ 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 T₃, T₄ and rT₃ 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 T₄, T₃ and rT₃ were concerned, there were no differences between the low T₃, T₄ and high rT₃ 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 T₃ and T₄, but not an increase in rT₃ (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 T₄ starting from 2 days after injection.
The fact that the T₄ 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 T₄, T₃ 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 T₄.

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 T3 and T4 in diabetes were significantly lower than that of the control group and the percentage reduction of T4 in diabetes was greater than that of T3. 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 rT3 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 T3, T4 and TSH was 40-60% lower in diabetic animals while free T4 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\textsubscript{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 $\text{Ca}^{2+}$ 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 $\text{Ca}^{2+}$-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 $E_{\text{MAX}}$ 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 Gs, 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 Ca\(^{2+}\) transporter and attenuated PKA-induced enhancement of SR Ca\(^{2+}\) 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 subsequent 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. T₃ administration to diabetic animals at a dose sufficient to restore the circulating levels of T₃ 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 T₄ 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 T₄ 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\textsubscript{MAX} of PE with unchanged pD\textsubscript{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 \(\alpha\)-ADR was found to be decreased without a binding affinity change (Heyliger et al 1982, Williams et al 1983). This enhanced responsiveness to the \(\alpha\)-ADR has been suggested to be related to the low thyroid state in diabetes since T\textsubscript{3} administration in STZ-induced diabetic rats restored the cardiac responsiveness to PE to normal (Goyal et al 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\textsubscript{2} producing IP\textsubscript{3} or other PI metabolites and the DAG/PKC cascade; in addition to increased intracellular Ca\textsuperscript{2+} and/or increases in [Ca\textsuperscript{2+}] close to myofibrils and other contractile components may lead to an increased response. Among these potential mechanisms, only increase in the IP\textsubscript{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\textsubscript{3} may underlie the increased cardiac \(\alpha\)-adrenergic response; however, little is known about the post \(\alpha\)-ADR alterations in diabetes since the intracellular events between activation of the cardiac \(\alpha\)-ADR and increase in intracellular Ca\textsuperscript{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\textsuperscript{2+}-activated myosin ATPase in 2 week ALX-induced diabetic rats which was also observed in fasting rats (Garber and Neely 1983). However, T\textsubscript{3} replacement did restore cardiac function at doses which successfully restored circulating T\textsubscript{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\textsubscript{2}O 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\textsubscript{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\textsuperscript{2+} transport system (Dhalla et al. 1980) and a higher Ca\textsuperscript{2+} binding, uptake and higher activity of Ca\textsuperscript{2+}-ATPase, thus a lower Ca\textsuperscript{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\textsubscript{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 of the 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.
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