Sympathetic Activation and Heart Failure

Danelle Badenhorst

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ABSTRACT

Chronic activation of the sympathetic nervous system, via β-adrenoreceptor (AR) stimulation, contributes toward progressive heart failure. However, in this regard there are some outstanding issues which require clarity. First, in addition to contributing toward progressive heart failure, it is not clear whether chronic β-AR activation can also initiate cardiac decompensation. If so, the mechanisms of this effect also need to be determined. Second, the role of functional variants of β-AR genes as determinants of either the development or progression of heart failure requires elucidation. Moreover, whether there is any practical value in genotyping of patients for these variants has yet to be determined. These questions were addressed in the present thesis.

With respect to the question of whether chronic β-AR activation initiates cardiac decompensation, the mechanisms responsible for the transition from compensated cardiac hypertrophy to heart failure in pressure overload states, such as hypertension, are uncertain. In this thesis I explored whether chronic sympathetic nervous system activation, produced by daily administration of a β-AR agonist, could promote the transition to cardiac pump failure in spontaneously hypertensive rats (SHR) with compensated cardiac hypertrophy. After 5 months of daily administration of a β-AR agonist, SHR developed marked left ventricular pump dysfunction, whereas normotensive control rats maintained pump function. The pump dysfunction noted in SHR was attributed to marked chamber dilatation with wall thinning, whilst myocardial contractile function appeared to be intact. The changes in cardiac structure and function noted after chronic β-AR activation in SHR were similar to those noted in SHR with advanced heart failure. These data provided the first evidence to indicate that chronic β-AR activation can promote the transition to decompensated cardiac hypertrophy in pressure overload states, and that this effect is principally mediated by adverse structural remodeling of the cardiac chamber.

The mechanisms responsible for the effect of chronic β-AR activation on cardiac chamber dilatation were subsequently studied. The identified mechanisms included activation of an enzyme that degrades myocardial collagen (matrix metalloproteinase 2) and an increase of myocardial collagen of the type that is susceptible to collagen degradation (non-cross-linked collagen). I also excluded alternative potential mechanisms such as necrosis, apoptosis and an accumulation of type III collagen. However, previous studies have indicated that increases in myocardial collagen concentrations determine myocardial stiffness and not cardiac chamber dilatation. Hence, I performed a study to examine whether the impact of increases in myocardial collagen concentrations on cardiac structure and function depends on the qualitative changes in myocardial collagen. Indeed, using a variety of models of pressure overload hypertrophy associated with increases in myocardial collagen concentrations, I was able to provide evidence to support the theory that increases in myocardial collagen of the cross-linked phenotype will promote myocardial stiffness, whereas increase in myocardial collagen of the non-cross-linked phenotype promotes cardiac dilatation.

With respect to the question of whether functional variants of β-AR genes contribute toward either the development or progression of heart failure, I studied the role of both functional β_1-AR and β_2-AR (together with a α_{2C}-AR) gene variants in black South Africans with idiopathic dilated cardiomyopathy (IDC). In a prospective study I obtained data to indicate that the relationship between functional β_2-AR genotypes and the progression to hospitalization, death or transplantation; a reduced exercise capacity, and left ventricular functional responses to β-blocker therapy, as described by other groups, is unlikely to be attributed to an independent effect of genotype on cardiac chamber dimensions and pump function. Moreover, I was able to show that contrary to what had previously been suggested, genotyping black subjects for functional α_{2C}-AR

and β_1-AR gene variants is of little use when predicting the development or severity of IDC in this population group.

DECLARATION

I declare that this thesis is my own unaided work. It is being submitted for the degree of Doctor of Philosophy in the Faculty of Science, University of Witwatersrand, Johannesburg. The work contained in this thesis has not been submitted for any degree or examination in this University, or any other University.

Danelle Badenhorst ……..……..

……day of ……..2007

I certify that the studies contained in this thesis have the approval of the Committee for Research in Human Subjects and the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The ethics approval numbers are 97/44/5, 98/28/4, 99/01/2b, 2000/40/5, 2002/37/5, 2002/39/5, 2006/37/04 and M951122.

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Angela J. Woodiwiss (supervisor) Gavin R. Norton (supervisor)

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Date Date

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STATEMENT OF MY CONTRIBUTION TO DATA COLLECTION AND ANALYSIS

The studies described in this thesis were designed by myself in consultation with my supervisors. I collected all animal haemodynamic and cellular/molecular data under the supervision of and with the assistance of my supervisors. I established the genotyping techniques and genotyped all subjects. Clinical data were collected by clinical personnel registered to practice in South Africa. I performed all of the data analysis for this thesis and interpreted these data.

LIST OF ABBREVIATIONS

AC	adenylate cyclase
ACE	angiotensin-converting enzyme
ACEI	ACE inhibitor
AESC	Animal Ethics Screening Committee
α_{2c}AR Del322-325	deletion of four amino acids at amino acid positions 322-325 of the α_{2c}AR gene
ANCOVA	analysis of covariance
ANOVA	analysis of variance
Arg16Gly	substitution of arginine for glycine at amino acid position 16 of the β_2-AR gene
β-AR	β-adrenoreceptor
β-ARK	β-adrenoreceptor kinase
BMI	body mass index
$b.min^{-1}$	beats per minute
bp	base pair
BP	blood pressure
BW	body weight
cAMP	cyclic adenosine monophosphate
cDNA	copy deoxyribonucleic acid
CI	confidence interval
CNBr	cyanogen bromide
DAG	diacylglycerol
DBP	diastolic blood pressure
DNA	deoxyribonucleic acid

E	slope of the linear portion of the LV peak systolic P-V relation
E/A	early-to-atrial transmiatral velocity ratio
E_n	slope of the systolic stress (σ)-strain relation
G protein	guanosine trisphospate protein
GDP	guanosine diphosphate
Gi	inhibitory G protein
Gln27Glu	substitution of glutamine for glutamic acid at amino acid postion 27 of the β_2-AR gene
Gly389Arg	substitution of glycine for arginine at amino acid position 389 of the β_1-AR gene
Gs	stimulatory G protein
GTP	guanosine trisphosphate
h	wall thickness
HPRO	hydroxyproline
h/r	wall thickness-to-radius ratio
IDC	idiopathic dilated cardiomyopathy
IP_3	inositol trisphosphate
ISO	isoproterenol
$kg.m^{-2}$	kilogram per metres squared
LV FS_{end}	LV endocardial fractional shortening
LV FS_{mid}	LV midwall fractional shortening
LV V_0	left ventricular volume intercepts of the LV diastolic P-V relation at 0 mm Hg
LVEDD	left ventricular end diastolic diameter
$LVED_{h/r}$	left ventricular end diastolic relative wall thickness

$LVED_{h/ro}$	intercept of LVEDP-$LVED_{h/r}$ relation
LVEDP	left ventricular end diastolic pressure
$LVED_r$	left ventricular end diastolic radius
$LVED_{r0}$	$LVED_r$ intercept of the LVEDP-$LVED_r$ relation
LVEF	left ventricular ejection fraction
LVESD	left ventricular end systolic diameter
LVH	left ventricular hypertrophy
MANCOVA	multivariate analysis of covariance
$\mu g.mg^{-1}$ dry LV	microgram per milligram dry left ventricle
$mg.kg^{-1}$	milligram per kilogram
$ml.m^{-2}$	millilitres per meter squared
$ml.min^{-1}$.g wet heart weight	millilitres per minute per gram wet heart weight
mm Hg	millimeters of mercury
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
myocardial k	myocardial stiffness
NE	norepinephrine
NYHA	New York Heart Association
OR	odds ratio
P	pressure
p value	probability value
PCR	polymerase chain reaction
PDE	phosphodiesterase
PIP_2	phospho-inositol-bisphopshate
PLC	phopholipase C
POH	pressure overload hypertrophy

P-V	pressure-volume
$PWT_{diastole}$	LV end diastolic posterior wall thickness
$PWT_{systole}$	LV end systolic posterior wall thickness
r	correlation coefficient
RFLP	restriction fragment length polymorphism
RT-PCR	reverse transcription polymerase chain reaction
SBP	systolic blood pressure
SEM	standard error of the mean
SHAM	sham-operated controls
SHR	spontaneously hypertensive rats
TdT	terminal deoxynucleotidyl transferase
TIMP	tissue inhibitor of matrix metalloproteinase
V	volume
V_0	unstressed left ventricular volume
V_m	left ventricular muscle volume
WKY	Wistar Kyoto

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PREFACE

Chronic sympathetic activation is now well recognized as mediating progressive cardiac dysfunction. As such the use of β-adrenoreceptor (AR) blockers has become standard care in the management of heart failure. However, the role of sympathetic activation as a stimulus for the development of heart failure has never been given due consideration. Moreover, factors that may influence the impact of sympathetic activation on the progression of chronic heart failure have not been clarified. In the present thesis I have assessed whether chronic sympathetic activation contributes toward the development of heart failure in pressure overload states (hypertension). The structural, functional, cellular and molecular mechanisms by which this effect may be mediated were explored. Identifying these mechanisms is of importance as there is substantial controversy as to whether β-AR blockers should be used in hypertension and hence drugs targeting downstream mechanisms may be required. In the present thesis, I also studied the role of gene variants that modify sympathetic actions via β_1- and β_2-ARs as potential determinants of progressive heart failure or the development of heart failure.

Data from the present thesis lend support for the notion that chronic β-AR activation promotes the transition to heart failure in pressure overload states. Moreover, I provide evidence to indicate that in pressure overload states adverse cardiac chamber remodeling (cardiac dilatation) is more important as a cause of the transition to cardiac failure associated with pump dysfunction than are intrinsic myocardial contractile abnormalities. The potential mechanisms responsible for β-AR-induced left ventricular dilatation appeared to include interstitial modifications. The interstitial changes of importance were noted to be an accumulation of myocardial collagen susceptible to matrix metalloproteinase (MMP) digestion (non-cross-linked collagen) and activation of MMPs.

In the present thesis, I also provide evidence to indicate that functional variants of the β_2-AR gene have no independent effect on adverse structural remodeling and pump function in idiopathic dilated cardiomyopathy. These data therefore suggest that relationships between these genetic variants and heart failure as previously shown are unlikely to be through effects on chamber remodeling or pump function *per se*. Moreover, data from the present thesis do not support a role for α_{2C}-AR Del322-325 and β_1-AR Gly389Arg genotyping in predicting the development or severity of heart failure in black South Africans as has previously been suggested.

As a consequence of the work presented in this thesis I have therefore a) provided the first evidence to suggest that chronic β-AR activation promotes the transition to heart failure in pressure overload states b) provided cellular and molecular evidence to suggest a therapeutic target other than β-ARs themselves (which if blocked therapeutically could produce a multitude of side effects) and c) further clarified the potential role of β-AR gene variants in human heart failure.

In support of this thesis, the work presented in this thesis has been published in the journals *Hypertension* (Badenhorst et al 2003; 41: 499-504) and *Cardiovascular Research* (Badenhorst et al 2003; 57: 632-641) and is also presently *in press* in the journal *Pharmacogenomics* (Badenhorst et al *in press*). Two further manuscripts are under review or in-preparation.

Importantly, the work outlined in this thesis is presented in a series of semi-independent chapters to ensure that each chapter deals with a separate hypothesis. Chapter 1 places the work in the context of the present scientific literature. Each subsequent chapter, with the exception of the conclusions chapter, consists of an abstract, introduction, methods, results, and discussion section. Finally, a conclusions chapter is provided to integrate the main findings of the thesis.