RENIN ANGIOTENSIN SYSTEM & SYMPATHETIC NERVOUS SYSTEM IN THE PROGRESSION OF PRESSURE OVERLOAD CARDIAC HYPERTROPHY AND THE ROLE OF α_1 ADRENOCEPTOR SUBTYPES IN THE CONTROL OF RENAL HAEMODYNAMICS IN LEFT VENTRICULAR HYPERTROPHY

HASSAAN ANWER RATHORE

UNIVERSITI SAINS MALAYSIA

2008

RENIN ANGIOTENSIN SYSTEM & SYMPATHETIC NERVOUS SYSTEM IN THE PROGRESSION OF PRESSURE OVERLOAD CARDIAC HYPERTROPHY AND THE ROLE OF α_1 ADRENOCEPTOR SUBTYPES IN THE CONTROL OF RENAL HAEMODYNAMICS IN LEFT VENTRICULAR HYPERTROPHY

by

HASSAAN ANWER RATHORE

Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

UNIVERSITI SAINS MALAYSIA

December, 2008

To

My beloved father Mr. Mohammad Anwer Rathore
My beloved mother Mrs. Salma Bano Rathore
&
Zarina Hanim Ismail

ACKNOWLEDGEMENTS

Bismillah-ir-rehman-ir-rahim. All praises and gratitude to Allah who has guided me to this day to successfully complete the study that I started a few years ago.

I am extremely thankful to my father and mother without whose support, training, advice, prayers and finances I would not have been as blessed as I am today. Affaf, my younger sister, her husband, Mansoor and my nephew Mohammad Ibrahim, I have missed you a lot throughout my stay in Malaysia. Without your prayers and support it would have been difficult for me to complete my research here. Sohaib, my younger brother, his wife Afshan and my niece Azka, I have always wished that we could spend more time together. Thank you for taking care of our parents in my absence.

No doubt there were difficult times, times when I missed my home and family and friends, times when I was stuck in my research, getting frustrated and times when I just needed to talk to some one. At those times there was just one person, Dr. Munavavr Zubaid, my PhD supervisor, who was always available (without any exceptions) to support me, to guide me, to give me a direction in my research and be my family at the same time. He has been kind enough to be my mentor, my elder brother, my friend as well as my teacher, all at the same time. I will be in debt to him throughout my life for sharing his knowledge with me as well as for training me not just to be a good researcher but also to be a good human being. It will be not fair if I don't mention the kindness of his wife who invited me to join her family for dinner at several occasions and who treated me like a younger brother. Thanks to the coffees she made that kept me agile. Shazwi Nabila, Dr. Munavvar's daughter, with her cheerful smiles is soon going to be a medical doctor.

My warmest regards to my field supervisor, Prof. Dr. Nor Azizan, University of Malaya and Prof. Dr. Edward James Johns, University College cork, Ireland, for their inputs, support, mentoring and help in my research. Without Prof. Edward's generosity of letting me use some of his lab equipments I would not have been able to complete my research.

My heartiest gratitude to my lab mates, Dr. Aidi, Dr. Tito, Fathihah (the little noisy sister), Jannah, Raisa, Anand, Ibrahim and Mohamamd. The valuable discussions that I had with you guys made me learn a lot about our field of research as well as complexities and different colors of life.

Thanks to all the staff of school of pharmaceutical sciences. Mr. Yousuf and Mr. Adnan from the animal house, Mr. Wan our lab assistant (supposedly), Puan Yong, Puan. Zahida, the former deputy dean research Dr. Pazilah, the present deputy dean research, Dr. Izham, the present dean of school Dr. Syed Azhar and the former dean Dr. Abbas Hussain. Without your help and support it would have been difficult for me to complete my study smoothly.

Many thanks to all my Pakistani, Malaysian, Taiwanese, Japanese, Arab, Persian, Thai and Chinese friends in USM whose presence, support and sharing made life more colorful.

Thanks to IPS for appointing me a graduate assistant teaching for one and a half years and many thanks to the Ministry of Higher Education Malaysia who granted me a scholarship under a technical co-operation program with the government. of Pakistan (MTCP). Here I would like to mention my gratitude for Mrs. Rukhsana Afzaal, the first secretary of Pakistan High commission in Kuala Lumpur for going out of the way to help me in making my stay more comfortable in Malaysia.

My warmest thanks to Dr. Syed Atif Abbas who introduced me to USM and Dr. Munavvar Zubaid. He has just been blessed with a baby girl, Abeera.

Last but not the least; I am thankful to a person who has been my corner and the force behind my strengths. You know who you are. I am indebted to your support forever.

Hassaan A. Rathore

Dated: 14th August 2008

TABLE OF CONTENTS

	Page
Acknowledgements	ii
Table of Contents	iv
List of Tables	ix
List of Figures	xi
List of Abbreviations	xviii
Abstrak	xxi
Abstract	xxiii
CHAPTER 1 – INTRODUCTION	1
1.1 Anatomy of the Neurons & the neural bed	2
1.1.1 Central Nervous System	3
1.1.2 Peripheral Nervous System	6
1.1.2.a Autonomic Nervous System	7
1.1.2.b Somatic Nervous System	8
1.2. Sympathetic nervous system	9
1.2.1 Adrenergic receptors	11
1.2.1.1 Classification, characterization & distribution	12
1.2.2 α adrenoceptors	15
1.2.1.2.a α_1 adrenoceptors	15
1.2.1.2.a.i α_{1A}	16
1.2.1.2.a.ii α_{1B}	17
1.2.1.2.a.iii α_{1D}	18
1.2.1.2.a.iv α_{1L} & α_{1H}	20
1.2.1.2.b α_2 adrenoceptors	21
1.2.3 Beta adrenoceptors	22
1.3. Renin angiotensin system	25
1.3.1 Angiotensin II	27
1.3.2 Angiotensin converting enzyme & Inhibitors	32
1.3.3 Angiotensin receptors	33
1.4. Cardiovascular system	38
1.4.1 Anatomy of heart	38
1.4.2 Extrinsic control of cardiovascular function	41
1.4.3 Electrical conductivity and electro cardiogram	45
1.5. Pressure overload cardiac hypertrophy & heart failure	50
1.5.1 Anatomical and molecular changes	51
1.5.2 Changes in cardiac function	57
1.5.3 Neurohormonal changes	60
1.5.4 Etiology and Diagnosis of LVH and heart failure	66
1.6. Kidney	68
1.6.1 Anatomy of Nephron & basic renal processes	69
1.6.2 Neurohumoral control of kidney function	73
1.6.2.1 Role of sympathetic nervous system	73
1.6.2.2 Role of renin-angiotensin system	77
1.7 Objectives of the Study	79

CHAPTER 2 – MATERIALS AND METHODS 80
2.1 Animals 80
2.1.1 General description & animals' ethics committee clearance 80
2.1.2 Induction of left ventricular hypertrophy (LVH) 80
2.2 Experimental Groups 82
2.2.1 Vasopressor experiment 83
2.2.1.a Surgical preparation 83
2.2.1.b Determination of mean arterial pressure and acute peripheral vasopressor responses 85
2.2.1.c Chemical sympathectomy with 6OHDA 86
2.2.1.d Treatment with losartan 87
2.2.1.e Combined treatment with losartan and 6OHDA 88
2.2.1.f Statistical analysis 88
2.2.2 Progression of LVH experiment 90
2.2.2.a Surgical preparation 90
2.2.2.b Determination of MAP, acute vasopressor responses and ventricular function 92
2.2.2.c Haemodynamic and ventricular function parameters 93
2.2.3 Intra renal adrenoceptor function experiment 96
2.2.3.a Surgical preparation 97
2.2.3.b Measurement of MAP and intra renal vasoconstrictor responses 100
2.2.3.c Statistical analysis 102
2.3 Measurement of ECG 104
2.4 Measurement of heart, left ventricle and kidney index 104
2.5 Physiological data collection 105
2.6 Preparation of drugs 105
2.7 List of Chemicals 106
2.8 List of Equipment 107

CHAPTER 3 – RESULTS 109
3.1 Results of vasopressor experiment 109
3.1.1 General observations 109
3.1.1.a Body weight, daily water intake and urine output in Normal, Sham, AB, ABSYMP, ABLOS and ABSYMPLOS groups 110
3.1.1.b ECG changes in Normal, Sham and Aortic Banded groups 110
3.1.1.c Heart index, left ventricle index, kidney index of Normal, Sham and Aortic Banded groups 111
3.1.1.d ECG changes in all groups 111
3.1.2 Baseline MAP of all groups 114
3.1.3 Vasopressor responses to adrenergic and vasoactive agents 115

3.1.3.1 Noradrenaline 115
3.1.3.2 Phenylephrine 116
3.1.3.3 Methoxamine 118
3.1.3.4 Angiotensin II 119

3.2 Progression of LVH and cardiac function experiment results 120
3.2.1 Baseline values of ventricular function 120
3.2.1.a Maximum pressure 120
3.2.1.b Minimum pressure 121
3.2.1.c End diastolic pressure 121
3.2.1.d Mean pressure 122
3.2.1.e Max-Min pressure 122
3.2.1.f Systolic duration 123
3.2.1.g Diastolic duration 123
3.2.1.h Cycle duration 124
3.2.1.i Heart rate 124
3.2.1.j Maximum dp/dt 125
3.2.1.k Minimum dp/dt 125
3.2.1.l Contractility index 126
3.2.1.m Pressure-time index 126
3.2.2 Baseline values of arterial function 127
3.2.2.a Systolic pressure 127
3.2.2.b Diastolic pressure 128
3.2.2.c Dicrotic notch pressure 128
3.2.2.d Mean pressure 129
3.2.2.e Pulse pressure 129
3.2.2.f Ejection duration 130
3.2.2.g Non ejection duration 130
3.2.2.h Cycle duration 131
3.2.2.i Heart rate 131
3.2.2.j Time to peak 132
3.2.3 Changes in left ventricular parameters caused by adrenergic and vasoactive agents 132
3.2.3.a Isoprenaline 132
3.2.3.a.i Maximum pressure 133
3.2.3.a.ii Minimum pressure 133
3.2.3.a.iii End distolic pressure 133
3.2.3.a.iv Mean pressure 134
3.2.3.a.v Max-Min pressure 134
3.2.3.a.vi Systolic duration 134
3.2.3.a.vii Diastolic duration 135
3.2.3.a.viii Cycle duration 135
3.2.3.a.ix Heart rate 135
3.2.3.a.x Maximum dp/dt 136
3.2.3.a.xi Minimum dp/dt 136
3.2.3.a.xii Contractility index 136

3.2.3.a.xiii Pressure-time index 137
3.2.3.b Noradrenaline 137
3.2.3.b.i Maximum pressure 137
3.2.3.b.ii Minimum pressure 137
3.2.3.b.iii End distolic pressure 138
3.2.3.b.iv Mean pressure 138
3.2.3.b.v Max-Min pressure 138
3.2.3.b.vi Systolic duration 139
3.2.3.b.vii Diastolic duration 139
3.2.3.b.viii Cycle duration 139
3.2.3.b.ix Heart rate 140
3.2.3.b.x Maximum dp/dt 140
3.2.3.b.xi Minimum dp/dt 140
3.2.3.b.xii Contractility index 141
3.2.3.b.xiii Pressure-Time index 141
3.2.3.c Phenylephrine 141
3.2.3.c.i Maximum pressure 141
3.2.3.c.ii Minimum pressure 142
3.2.3.c.iii End distolic pressure 142
3.2.3.c.iv Mean pressure 142
3.2.3.c.v Max-Min pressure 143
3.2.3.c.vi Systolic duration 143
3.2.3.c.vii Diastolic duration 143
3.2.3.c.viii Cycle duration 144
3.2.3.c.ix Heart rate 144
3.2.3.c.x Maximum dp/dt 144
3.2.3.c.xi Minimum dp/dt 145
3.2.3.c.xii Contractility index 145
3.2.3.c.xiii Pressure-time index 145
3.2.3.d Angiotensin II 146
3.2.3.d.i Maximum pressure 146
3.2.3.d.ii Minimum pressure 146
3.2.3.d.iii End distolic pressure 146
3.2.3.d.iv Mean pressure 147
3.2.3.d.v Max-Min pressure 147
3.2.3.d.vi Systolic duration 147
3.2.3.d.vii Diastolic duration 148
3.2.3.d.viii Cycle duration 148
3.2.3.d.ix Heart rate 148
3.2.3.d.x Maximum dp/dt 149
3.2.3.d.xi Minimum dp/dt 149
3.2.3.d.xii Contractility index 149
3.2.3.d.xiii Pressure-time index 150

3.3 Intra renal adrenoceptors function experiment 151
3.3.1 Baseline and changes in mean arterial pressure (MAP) in control and aortic banded groups 151
3.3.2 Baseline cortical perfusion of left kidney in control and aortic banded groups 151
3.3.3 Adrenergic and vasoactive agents induced vasoconstriction in the renal vasculature of rats in control and aortic banded groups 152
3.3.3.a Renal vasoconstrictor responses to noradrenaline 153
3.3.3.b Renal vasoconstrictor responses to phenylephrine 155
3.3.3.c Renal vasoconstrictor responses to methoxamine 158
3.3.3.d Renal vasoconstrictor responses to ANGII 161

CHAPTER 4 – DISCUSSION 266

CHAPTER 5 – CONCLUSION 313

BIBLIOGRAPHY

APPENDICES

LIST OF TABLES

		Page
Table 1.1	Summary of the factors involved in extrinsic control of MAP	46
Table 2.1	Summary of the experimental groups	88
Table 2.2	Summary of the physiological data collection and 6OHDA Treatment	92
Table 2.3	Summary of the physiological data collection and losartan treatment	93
Table 2.4	Summary of physiological data and treatment with 6OHDA and losartan	94
Table 2.5	Summary of the haemodynamic and ventricle function parameters studied during the progression of LVH	100
Table 2.6	Summary of the intra renal adrenoceptor characterization experiment groups	102
Table 3.1	Body weight, daily water intake and daily urine output of Normal, Sham and Aortic Banded groups	165
Table 3.2	Body weight, daily water intake and daily urine output of ABLOS, ABSYMP and ABSYMPLOS groups	166
Table 3.3	Electrocardiography (ECG) changes	167
Table 3.4	Baseline MAP of Normal, Control, AB, ABSYMP, ABLOS, ABSYMPLOS	168
Table 3.5	Average percentage change in vasopressor responses caused by adrenergic and vasoactive agents in Normal, Sham, AB, ABSYMP, ABLOS and ABSYMPLOS	169
Table 3.6	Baseline values of ventricular function	170
Table 3.7	Baseline values of arterial function	171
Table 3.8	Average percentage change in ventricular function caused by isoprenaline	172
Table 3.9	Average percentage change in ventricular function caused by noradrenaline	173

Table 3.10	Average percentage change in ventricular function caused by phenylephrine	174
Table 3.11	Average percentage change in ventricular function caused by ANGII	175
Table 3.12	Baseline MAP of 5MeU, CEC and BMY treated control group and AB group	176
Table 3.13	Baseline cortical blood perfusion (CBP) of 5MeU, CEC and BMY treated control group and AB group	177
Table 3.14	Average/Mean percentage change in cortical perfusion caused by noradrenaline in 5MeU, CEC and BMY treated control group and AB group	178
Table 3.15	Average/Mean percentage change in cortical perfusion caused by phenylephrine in 5MeU, CEC and BMY treated control group and AB group	179
Table 3.16	Average/Mean percentage change in cortical perfusion caused by methoxamine in 5MeU, CEC and BMY treated control group and AB group	180
Table 3.17	Average/Mean percentage change in cortical perfusion caused by angiotensin II in 5MeU, CEC and BMY treated control group and AB group	181
Table 3.18	Heart, left ventricle and kidney indices at day 45 of surgical induction of pressure overload compared to the control group	182

LIST OF FIGURES

		Page
Figure 1.1.	Structure of a typical neuron	5
Figure 1.2	Organization of the nervous system	6
Figure 1.3	Bioenzymatic cascade of the RAS	27
Figure 1.4	Signal transduction mechanism of ANGII.	31
Figure1.5	Chemical structure of losartan	33
Figure 1.6	Gross anatomy of human heart	40
Figure 1.7	Longitudinal section of the heart exposing chambers	40
Figure 1.8	Evolution of the electrocardiogram from the electrometer.	47
Figure 1.9	ECG leads and their positions.	47
Figure 1.10	Typical lead II ECG recording	49
Figure 1.11	Signaling pathways of the major cardiac adrenoceptor subtypes.	55
Figure 1.12	Gross anatomy of the kidney	70
Figure 1.13	Cortical & juxtamedullary nephron anatomy	70
Figure 2.1	Summary of the acute vasopressor experiment	89
Figure 2.2	Summary of the acute progression of LVH experiment	95
Figure 2.3	Summary of the intra renal acute experiment protocol	103
Figure 3.1	R-R interval changes of all groups	183
Figure 3.2	Changes in heart rate of all groups	184
Figure 3.3	QRS changes of all groups	185
Figure 3.4	QT changes of all groups	186
Figure 3.5	R amplitude changes of all groups	187

Figure 3.6	Baseline mean arterial pressure (MAP) of experimental groups in vasopressor experiment.	188
Figure 3.7	Vasopressor responses of noradrenaline	189
Figure 3.8	Vasopressor responses of phenylephrine	190
Figure 3.9	Vasopressor responses of methoxamine	191
Figure 3.10	Vasopressor responses of angiotensin II.	192
Figure 3.11	Baseline values of ventricular function, max pressure.	193
Figure 3.12	Baseline values of ventricular function, min pressure	194
Figure 3.13	Baseline values of ventricular function, EDP	195
Figure 3.14	Baseline values of ventricular function, mean pressure	196
Figure 3.15	Baseline values of ventricular function, Max-Min pressure	197
Figure 3.16	Baseline values of ventricular function, systolic duration	198
Figure 3.17	Baseline values of ventricular function, diastolic duration	199
Figure 3.18	Baseline values of ventricular function, cycle duration	200
Figure 3.19	Baseline values of ventricular function, heart rate	201
Figure 3.20	Baseline values of ventricular function, Max dp/dt	202
Figure 3.21	Baseline values of ventricular function, contractility index	203
Figure 3.22	Baseline values of ventricular function, Min dp/dt	204
Figure 3.23	Baseline values of ventricular function, pressure-time index	205
Figure 3.24	Baseline values of arterial function, systolic pressure	206
Figure 3.25	Baseline values of arterial function, diastolic pressure	207
Figure 3.26	Baseline values of arterial function, dicrotic notch pressure	208
Figure 3.27	Baseline values of arterial function, mean pressure	209
Figure 3.28	Baseline values of arterial function, pulse pressure	210

Figure 3.29	Baseline values of arterial function, ejection duration	211
Figure 3.30	Baseline values of arterial function, Non-Ejection duration	212
Figure 3.31	Baseline values of arterial function, cycle duration	213
Figure 3.32	Baseline values of arterial function, heart rate	214
Figure 3.33	Baseline values of arterial function, time to peak	215
Figure 3.34	Effect of isoprenaline on left ventricular function (contractility index)	216
Figure 3.35	Effect of isoprenaline on left ventricular function (cycle duration)	217
Figure 3.36	Effect of isoprenaline on left ventricular function (diastolic duration)	218
Figure 3.37	Effect of isoprenaline on left ventricular function (EDP)	219
Figure 3.38	Effect of isoprenaline on left ventricular function (heart rate)	220
Figure 3.39	Effect of isoprenaline on left ventricular function (Max dp/dt)	221
Figure 3.40	Effect of isoprenaline on left ventricular function (maximum pressure)	222
Figure 3.41	Effect of isoprenaline on left ventricular function (Max-Min pressure)	223
Figure 3.42	Effect of isoprenaline on left ventricular function (min dp/dt)	224
Figure 3.43	Effect of isoprenaline on left ventricular function (min pressure)	225
Figure 3.44	Effect of isoprenaline on left ventricular function (pressure-time index)	226
Figure 3.45	Effect of isoprenaline on left ventricular function (systolic duration)	227
Figure 3.46	Average percentage change in ventricular function (max pressure) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	228

Figure 3.47	Average percentage change in ventricular function (min pressure) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	229
Figure 3.48	Average percentage change in ventricular function (EDP) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	230
Figure 3.49	Average percentage change in ventricular function (mean pressure) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	231
Figure 3.50	Average percentage change in ventricular function (max-min Pressure) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	232
Figure 3.51	Average percentage change in ventricular function (systolic duration) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	233
Figure 3.52	Average percentage change in ventricular function (diastolic duration) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	234
Figure 3.53	Average percentage change in ventricular function (cycle duration) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	235
Figure 3.54	Average percentage change in ventricular function (Heart rate) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	236
Figure 3.55	Average percentage change in ventricular function (max dp/dt) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	237
Figure 3.56	Average percentage change in ventricular function (contractility index) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO)	238

Figure 3.57 Average percentage change in ventricular function (min dp/dt) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO) 239

Figure 3.58 Average percentage change in ventricular function (pressure-time Index) caused by noradrenaline (NA), phenylephrine (PE), angiotensin II (ANGII) and isoprenaline (ISO) 240

Figure 3.59 Effect of noradrenaline on renal haemodynamics in Normal SD rats in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 241

Figure 3.60 Effect of phenylephrine on renal haemodynamics in Normal SD rats in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 242

Figure 3.61 Effect of methoxamine on renal haemodynamics in Normal SD rats in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 243

Figure 3.62 Effect of ANGII on renal haemodynamics in Normal SD rats in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 244

Figure 3.63 Effect of NA on renal haemodynamics in Aortic Banded rats (AB) in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 245

Figure 3.64 Effect of PE on renal haemodynamics in Aortic Banded rats (AB) in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 246

Figure 3.65 Effect of ME on renal haemodynamics in Aortic Banded rats (AB) in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 247

Figure 3.66 Effect of ANGII on renal haemodynamics in Aortic Banded rats (AB) in the presence of o 5MeU High Dose, ■ 5MeU Low Dose and Δ Saline 248

Figure 3.67 Effect of NA on renal haemodynamics in Normal SD rats in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 249

Figure 3.68 Effect of PE on renal haemodynamics in Normal SD rats in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 250

Figure 3.69 Effect of ME on renal haemodynamics in Normal SD rats in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 251

Figure 3.70 Effect of ANGII on renal haemodynamics in Normal SD rats in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 252

Figure 3.71 Effect of NA on renal haemodynamics in Aortic Banded rats (AB) in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 253

Figure 3.72 Effect of PE on renal haemodynamics in Aortic Banded rats (AB) in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 254

Figure 3.73 Effect of ME on renal haemodynamics in Aortic Banded rats (AB) in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 255

Figure 3.74 Effect of ANGII on renal haemodynamics in Aortic Banded rats (AB) in the presence of o CEC High Dose, ■ CEC Low Dose and Δ Saline 256

Figure 3.75 Effect of NA on renal haemodynamics in Normal SD rats in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 257

Figure 3.76 Effect of PE on renal haemodynamics in Normal SD rats in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 258

Figure 3.77 Effect of ME on renal haemodynamics in Normal SD rats in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 259

Figure 3.78 Effect of ANGII on renal haemodynamics in Normal SD rats in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 260

Figure 3.79 Effect of NA on renal haemodynamics in Aortic Banded rats (AB) in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 261

Figure 3.80 Effect of PE on renal haemodynamics in Aortic Banded rats (AB) in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 262

Figure 3.81 Effect of ME on renal haemodynamics in Aortic Banded rats (AB) in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 263

Figure 3.82 Effect of ANGII on renal haemodynamics in Aortic Banded rats (AB) in the presence of o BMY7378 High Dose, ■ BMY7378 Low Dose and Δ Saline 264

Figure 3.83 Effect of Renal Nerve Stimulation (RNS) on renal haemodynamics in AB4weeks group, AB8weeks group, ABSYMP group and the Control group 265

LIST OF ABBREVIATIONS

-ve	negative
%	percent
%age	Percentage
+ve	positive
±	Plus minus
1/s	one per second
5MeU	5 Methyl Urapidil
6OHDA	6 Hydroxy dopamine
α	Alpha
α_1	Alpha one (subtype of alpha adrenoceptors)
α_{1A}	Alpha one A (subtype of α_1 adrenoceptors)
α_{1B}	Alpha one B (subtype of α_1 adrenoceptors)
α_{1C}	Alpha one C (subtype of α_1 adrenoceptors)
α_{1D}	Alpha one D (subtype of α_1 adrenoceptors)
α_{1H}	Alpha one H (subtype of α_1 adrenoceptors)
α_{1L}	Alpha one L (subtype of α_1 adrenoceptors)
α_2	Alpha two adrenoceptor
α_{2A}	Alpha two A adrenoceptor
α_{2B}	Alpha two B adrenoceptor
α_{2C}	Alpha two C adrenoceptor
α_{2D}	Alpha two D adrenoceptor
β	Beta
β_1	Beta one adrenoceptors
β_2	Beta two adrenoceptors
β_3	Beta three adrenoceptors
β_4	Beta four adrenoceptors
µg	Micro gram
AB	Aortic banded
AB4weeks	Aortic banded for 4 weeks
AB4weeksSYMP	Aortic banded for 4 weeks and sympethectomized
AB8weeks	Aortic banded for 8 weeks
ABLOS	Aortic banded treated with losartan
ABSYMP	Aortic banded and sympathectomised
ABSYMPLOS	Aortic banded sympethectomized & treated with losartan
ACE	Angiotensin converting enzyme
ACEi	Angiotensin converting enzyme inhibitor
AMPK	Adenosine monophosphate activated protein kinase
ANGII	Angiotensin type II
ANP	Atrial naturetic peptide
ANS	Autonomic nervous system
ARBs	Angiotensin receptor blockers
AT_1	Angiotensin type 1 receptor
AT_{1A}	Angiotensin type one A receptor

AT_{1B}	Angiotensin type one B receptor
AT_2	Angiotensin type 2 receptor
ATG	Angiotensin gene
AV	Atrio-ventricular
BMY7378	8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione dihydrochloride
BNP	Brain natriuretic peptide
BP	Blood pressure
BPM	Beats per minute
BPU	Blood perfusion unit
Ca^{+2}	Calcium
CBP	Cortical blood perfusion
CEC	Chlorethylclonidine
cGMP	Cyclic guanine mono phosphate
CHF	Congestive heart failure
Cl^-	Chloride
CNS	Central nervous system
CT	Computed tomography
CVS	Cardiovascular system
DVR	Desecending vasa recta
ECG	Electro cardiogram
ECM	Extra cellular matrix
EDP	End diastolic Pressure
EF	Ejection fraction
ET-1	Endothelin type 1
g	Gram
GFR	Glomerular filtration rate
GI	gastrointestinal
GPCRs	G protein coupled receptors
HR	Heart rate
i.m	Intramuscular
i.p	Intraperitoneal
i.v	Intravenous
ISO	Isoprenaline
K^+	Potassium
LCa	L-type calcium channel
LVEF	Left Ventricular Ejection Fraction
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
Max	Maximum
Max-Min	Maximum minus Minimum
ME	Methoxamine
mg	Milli gram
Min	Minimum
ml	Milli liter
mmHg	Millimeter mercury

mmHg/s	Millimeter mercury per second
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MUGA	Multiple gated acquisition
n	number of animals
NA	noradrenaline
Na^+	Sodium
NaCl	Sodium Chloride
ng	Nano gram
NO	Nitric oxide
p	Probability
PE	Phenylephrine
PNS	Peripheral nervous system
QRS	Part of ECG wave
RAS	Renin angiotensin system
RBF	Renal blood flow
ROS	Reactive oxygen species
s.e.m	Standard error of mean
SA	Sino atrial
SD	Sprague Dawley
Sec	Seconds
SNS	Sympathetic nervous system
TGF β	Tumor growth factor beta
U.O	Urine output
W.I	Water intake
wt.	Weight

SISTEM RENIN ANGIOTENSIN DAN SISTEM SARAF SIMPATETIK DALAM PERKEMBANGAN TEKANAN BERLEBIHAN HIPERTROFI KARDIAK DAN PERANAN ADRENOSEPTOR SUBJENIS α_1 DALAM HIPERTROFI VENTRIKEL KIRI

ABSTRAK

Hipertrofi ventrikel kiri (LVH) telah dikenal pasti sebagai faktor penyebab tunggal bagi penyakit arteri koronari, kematian mengejut, lumpuh dan kegagalan jantung. Kadar pertambahan penyakit hipertrofi ventrikel kiri bergabungan dengan hipertensi secara puratanya adalah sebanyak 40%. Oleh itu, pencegahan atau kemerosotan bagi LVH menjadi sasaran rawatan sama ada secara farmakologi, mekanikal, pembedahan atau manipulasi genetik. Tesis ini membincangkan tentang perkembangan LVH yang mengarah kepada kegagalan jantung dengan penekanan terhadap penglibatan sistem saraf simpatetik (SNS) dan sistem renin angiotensin (RAS). Faktor-faktor yang menyebabkan LVH yang boleh membawa kepada kegagalan jantung termasuklah lebihan mekanikal, pertambahan tekanan oksidatif, pertambahan penghasilan spesis oksigen yang reaktif (ROS), perubahan neurohormonal dan kecacatan aktiviti nitrogen oksida synthase (NOS). Perubahan neurohormonal yang berlaku semasa pengubahsuaian kardiak adalah disebabkan oleh rangsangan adrenergik α dan β, angiotensin, endotelin, dan faktor nekrosis tumor α. Peningkatan tahap angiotensinogen, ACE, ANGII dan mRNA reseptor ANGI yang telah ditunjukkan, mencadangkan aktiviti RAS yang berlebihan di dalam model kajan LVH. Selain daripada itu, penyekatan aktiviti RAS telah memberi faedah ke atas pertunjukan kardiak malah ke atas kadar penyakit dan kematian. Noradrenalin adalah faktor pertumbuhan yang sangat penting untuk kardiomiosit dan ia telah dicadangkan mengandungi kesan langsung ke atas

kardiomiosit tanpa mempengaruhi kesan selepas pengepaman darah masuk ke jantung (afterload). Dengan latar belakang ini, kajian ini telah diaturkan untuk menjelaskan sumbangan SNS dan RAS di peringkat vascular periferi dan saluran darah berintangan ginjal. Kajian ini telah dibahagikan kepada 3 bahagian dengan objektif penyelidikan yang berbeza dalam mencapai tujuan yang satu. Eksperimen vasopressor telah dijalankan untuk mengenali peranan adrenoseptor α dan reseptor AT_1 serta mengenalpasti interaksi antara RAS dan SNS dalam vaskulatur periferi. Untuk mengetahui perkembangan LVH pada sela masa tertentu, beberapa eksperimen telah dijalankan dan adrenoseptor intrarenal dan AT_1 telah dikaji melalui eksperimen yang telah dirancang secara spesifik untuk menyelidik reseptor α_1 dan subjenisnya dalam vaskulatur ginjal dan buah pinggang. Dalam vaskulatur periferi, reseptor AT_1 dan adrenoseptor α_1 telah terlihat mengandungi mekanisma tindak balas yang kompleks di mana secara keseluruhannya RAS bertindak balas positif terhadap SNS dengan beberapa pengecualian. Selepas stenosis aorta, fungsi ventrikel kiri telah dimodulasi oleh SNS dan RAS. Fasa pertama terdiri daripada disfungsi sistolik dan kemudian diikuti oleh disfungsi diastolik. Peranan SNS telah dipertingkatkan dalam pengubahsuaian fungsi jantung semasa LVH dan antagonism SNS telah mengarah kepada fungsi ventrikel kiri yang lebih baik. Pada peringkat saluran darah berintangan ginjal, pelbagai perubahan subjenis α_1 adrenoseptor telah dilihat yang mana telah menjana tindak balas yang kompleks terhadap reseptor AT_1 di dalam ginjal. Peranan adrenoseptor subjenis α_{1D} seolah-olah telah mendapat kepentingan asas dengan sedikit perubahan dalam peranan adrenoseptor α_{1A} dan α_{1D}.

RENIN ANGIOTENSIN SYSTEM & SYMPATHETIC NERVOUS SYSTEM IN THE PROGRESSION OF PRESSURE OVERLOAD CARDIAC HYPERTROPHY AND THE ROLE OF α_1 ADRENOCEPTOR SUBTYPES IN THE CONTROL OF RENAL HAEMODYNAMICS IN LEFT VENTRICULAR HYPERTROPHY

ABSTRACT

Left ventricular hypertrophy (LVH) has been identified as an independent risk factor in coronary artery disease, sudden death, stroke and heart failure. The prevalence of LVH in patients with essential hypertension has been found to be 40% on average. Therefore, prevention and/or regression of LVH is a major therapeutic goal whether achieved by pharmacologic, mechanical, surgical or genetic means. This thesis discusses the progression of LVH that leads to heart failure with an emphasis on the involvement of sympathetic nervous (SNS) and the renin angiotensin systems (RAS) The pathogenesis of LVH leading to a failing heart might include mechanical overload, increased oxidative stress, increased production of reactive oxygen species (ROS), neurohormonal changes and impaired nitric oxide synthase activity. The neurohormonal changes that occur during this cardiac remodeling are caused by α and β adrenergic stimulation, angiotensin, endothelin and tumor necrosis factor α. Increased levels of angiotensinogen, ACE, ANGII and ANGI receptor mRNA have been shown which suggests an overactive RAS in experimental models of LVH. Moreover, inhibition of RAS has been shown to be of benefit for cardiac performance as well as morbidity and mortality. Noradrenaline is a potent growth factor for cardiomyocytes and it has been postulated to have a direct effect on cardiomyocytes without affecting afterload. With this background, this study sets out to elucidate the contribution of SNS and RAS at

peripheral vasculature and renal resistance vessel level. The complete study was divided into three parts with different experiments aimed to achieve individual objectives. The vasopressor experiments were carried out to identify the role of α adrenoceptors and AT_1 receptors and identify the interaction of RAS and SNS in the peripheral vasculature. To study the progression of LVH over a period of time some experiments were done. Thirdly, intra renal adrenoceptors and AT_1 were studied in an experiment which was designed specifically to study the role of α_1 adrenoceptors and their subtypes in the renal vasculature and kidney. It was found that in peripheral vasculature AT_1 receptors and α_1 adrenoceptors have a complex feedback mechanism whereby in general RAS had a positive feedback on the SNS with a few exceptions. After aortic stenosis the left ventricular function is greatly modulated by both SNS and RAS. The initial phase constitutes of systolic dysfunction which is followed by diastolic dysfunction in later stage. The role of SNS is greatly enhanced in the modulation of cardiac function during LVH and antagonism of SNS leads to improved left ventricular function. At the renal resistance vessels, a multiple subtype shift of α_1 adrenoceptor subtypes was observed which generated a complex feedback on the renal AT_1 receptors. The role of α_{1D} adrenoceptor subtype seemed to have gained functional importance in LVH with a minimal altered role of α_{1A} and α_{1B} adrenoceptors.

CHAPTER 1

INTRODUCTION

Heart failure is one of the leading causes of cardiovascular morbidity and mortality in the modern age. About 60-70% of the patients presented with congestive heart failure (CHF) have left ventricular systolic dysfunction. Rest of the patients who preserve left ventricular ejection fraction (LVEF) exhibit left ventricular diastolic dysfunction (Vasan *et al*., 1995).

Heart failure can be defined as a clinical syndrome caused by an abnormality of the heart and recognized characteristic patterns of haemodynamic, renal, neural and hormonal responses accompanied by structural alterations of the ventricles of heart. Reports from the Framingham study suggested a 6-fold increased risk in overall mortality in individuals with definite ECG-LVH and 45% deaths due to cardiovascular events in these patients (Alan *et al*., 2006). Moreover, left ventricular hypertrophy (LVH) has been identified as an independent risk factor for coronary artery disease, sudden death, stroke and also heart failure (Elieser, 1994; Alan *et al*., 2006).

In the US, it has been estimated that there are 550,000 new cases of CHF every year and approximately 4,800,000 Americans are currently diagnosed to have congestive heart failure. After the age of 65 incidence of CHF has approached ten per 1,000 population (1%) in the US (Wayne *et al*., 2007). CHF patients are believed to have a 5 year mortality of approximately 50% (National Heart, Lung & Blood Institute CHF fact sheet, 1996) with roughly 20% dying in the first year (Wayne *et al*., 2007).

Myocardial infarction disables 22% males and 46% females within 6 years of diagnosis of CHF. 80% of men, while 70% of women under the age of 65 with CHF die within 8 years. After CHF is diagnosed, survival is poorer in men than in women, but fewer than 15% of women survive more than 8-12 years. Their one-year mortality rate is higher with one in five dying. In USA, CHF deaths have increased by 145% between 1979 and 1999.

The prevalence of LVH in patients with essential hypertension has been found to be 40% on average (Alexander *et al*., 2003). Therefore, prevention and/or regression of LVH are major therapeutic goals whether achieved by pharmacologic, mechanical, surgical or genetic means.

Here we will discuss the progression of LVH that leads to heart failure with an emphasis on the involvement of sympathetic nervous and the renin angiotensin systems.

1.1 Anatomy of the neurons & the neural bed

The system of cells, tissues and organs that regulate the body's response to internal and external stimuli, through electrical and/or chemical transmission, is known as the nervous system (William *et al*., 2001). It constitutes of brain, spinal cord, nerves, ganglia and receptors which harmonize the transmission, reception and response to these signals in order to capacitate the body to function.

Neurons are the building blocks of the nervous system and constitute of a cell body, the dendrites and axon. The cell body contains the nucleus and performs most of the functions as a somatic cell except that after maturation most of the neuronal cells loose the ability to undergo cell division. Dendrites are small thread like structures originating from the cell body and function to receive inputs from the surroundings. They may also form junctions with the surrounding neurons to form synapse(s). A pre-synaptic neuron may release a chemical transmitter called neurotransmitter to communicate with the dendrite of a post-synaptic neuron cell body. Axon or a nerve fiber is usually elongated and unlike dendrite, it functions to transmit electrical signals in the form of action potential, from the cell body to either another synapse or to an effector organ. Axons may also branch and thus form collaterals. The beginning end of an axon is known as hillock and the terminal end of the axon is called terminal. Axon hillock, in most of the neurons, is specialized in initiating the action potential while terminal is specialized to release a neurotransmitter (William *et al*., 2001).

In general the nervous system can be divided into two main subcategories; central nervous system (CNS) and the peripheral nervous system (PNS).

1.1.1 Central Nervous System

The central nervous system (CNS) consists of brain and spinal cord. These two organs probably consist of the most delicate tissues in our body. Pertaining to their importance they are well protected in our body by three different substances; bone, connective tissue and fluid. The outer most layer that protects this delicate tissue is

cranium (skull). It surrounds the brain and also constitutes the bony vertebral column which surrounds the spinal cord. Between the bone and the nervous tissue are three layers of membranes called meninges (dura mater, arachnoids mater and pia mater). In between the archnoid mater and the pia mater, the space called subarachnoid space, is filled with a fluid called cerebrospinal fluid. The cerebrospinal fluid is a clear watery fluid that bathes the CNS and provides a cushion against sudden jerks. It is similar but not identical to the plasma in constitution (William *et al*., 2001). The structure of a typical neuron is shown in Fig. 1.1.

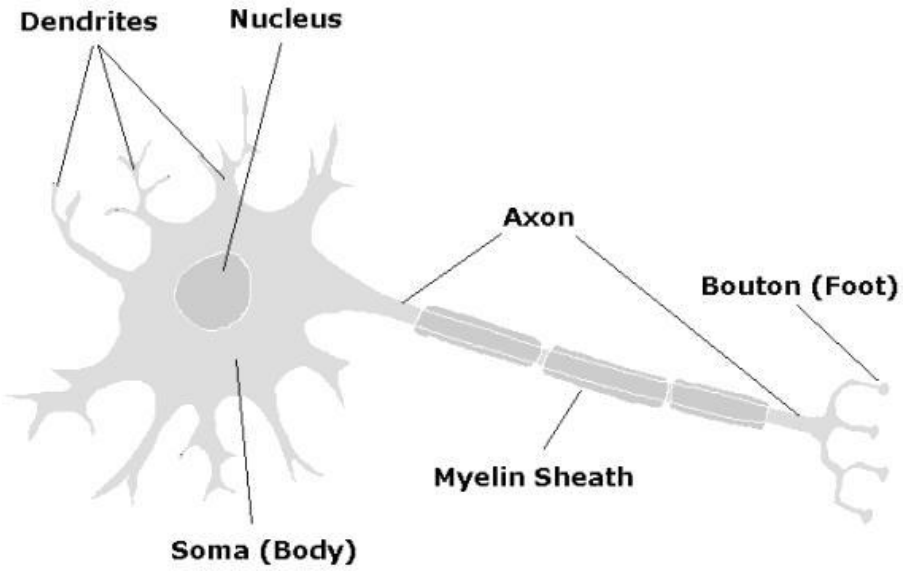

Figure 1.1. Structure of a typical neuron

1.1.2 Peripheral Nervous System

Peripheral nervous system (PNS) consists of nerve cells that enable communication between the CNS and the effector organs throughout the body. The peripheral nervous system can be subdivided into two divisions; Afferent PNS and Efferent PNS.

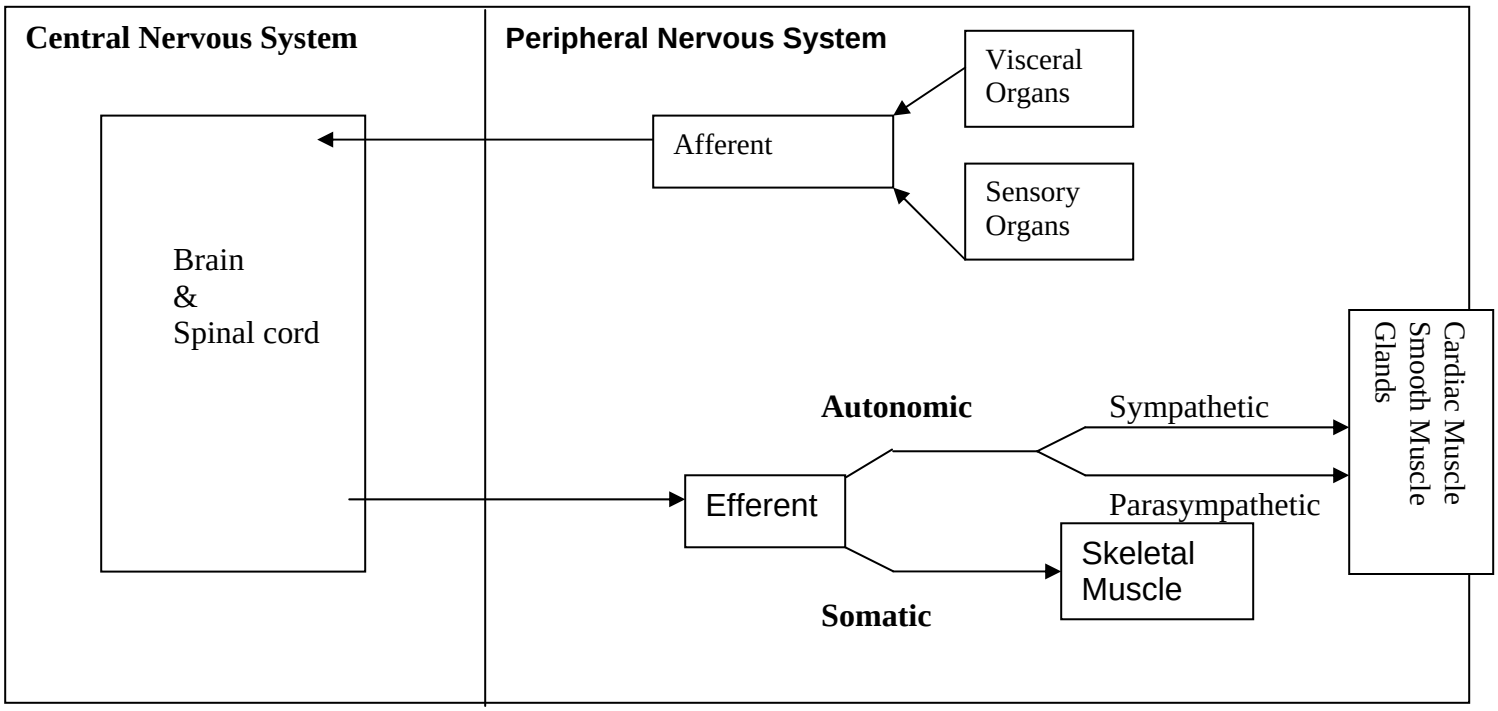

Figure 1.2 Organization of the nervous system
[Adapted from William *et al*., 2001]

Afferent division of the PNS consists of neurons that transmit electrical signals from organs to the CNS. Information from the visceral organs is transmitted to the CNS via these neurons. This information is in regard to blood pressure, hunger, thirst etc while the sensory neurons transmit the information related to the external stimuli such as vision, touch, taste etc.

1.1.2.a Autonomic nervous system

Autonomic nervous system (ANS) regulates organs of the body that are not under voluntary control. It regulates the function of internal organs, glands, vessels, cardiac rhythm, kidney function etc. The ANS receives input from parts of the CNS that process stimuli from the body and the external environment. These parts of CNS include the hypothalamus, nucleus of the solitary tract, reticular formation, amygdala, hippocampus, and olfactory cortex.

ANS can be divided into two groups; sympathetic nervous system and parasympathetic nervous system. Both sympathetic and parasympathetic systems consist of two sets of nerve bodies. The pre-ganglionic body is in CNS while the post-ganglionic body is usually outside the CNS and these post-ganglionic efferent fibers lead to effector organs.

The pre-ganglionic fibers of the sympathetic nervous system are located in the inter-mediolateral horn of the spinal cord. The ganglia are usually within the vertebral column and long post-ganglionic fibers innervate the effector organs, usually vascular smooth muscles, viscera, lungs, scalp, lachrymal and salivary glands, smooth muscles of viscera and glands, and ocular muscles. The sympathetic nervous system is catabolic in nature and exhibits flight and fright responses. Thus, sympathetic output increases heart rate and contractility, bronchodilation, hepatic glycogenolysis and glucose release and muscular strength. Less immediately life-preserving functions (e.g. digestion, renal filtration) are decreased. Most post-ganglionic sympathetic fibers release noradrenaline

and therefore sometimes termed as noradrenergic fibers; i.e., they act by releasing noradrenaline (NA).

The pre-ganglionic fibers of the parasympathetic system are located in the brain stem and sacral portion of the spinal cord. Pre-ganglionic fibers exit the brain stem in the form of cranial (vagus) nerves. The vagus nerve contains most of the parasympathetic fibers. Parasympathetic ganglia are found within the effector organ and post-ganglionic fibers are usually very small in length. The parasympathetic nervous system is anabolic; it conserves and restores, therefore, exhibits rest and digest responses. GI secretions and motility (including evacuation) are stimulated, heart rate is slowed, and BP decreases.

Erection is a parasympathetic function while ejaculation is a sympathetic function.

1.1.2.b Somatic nervous system

Somatic nervous system consists of nerve cells called motor neurons which control the function of skeletal muscles. These nerves constitute the voluntary control of organs.

Here we will focus more on the contribution of sympathetic nervous system and renin angiotensin system to LVH progression and thus will discuss them in detail.

1.2 Sympathetic nervous system

The autonomic nervous system (ANS) plays an important role in both healthy and diseased states. It regulates the function of all tissues and organs innervated by sympathetic nerves, except the skeletal muscles. ANS is independent in its activities and is not under voluntary control. It consists of three components; noradrenergic nerves, cholinergic nerves and the enteric system. Adrenergic nervous system (sympathetic nervous system) is physiologically the most important component of ANS and innervates all the vital organs of the body. The cholinergic nerves constitute the parasympathetic nervous system. The enteric system regulates the intestinal functions and projects from sympathetic and parasympathetic nerves (Furness *et al*., 1980, Vizi *et al*., 1991)

Here we will emphasize more on the sympathetic nervous system and the role of its components in the modulation of haemodynamics.

Sympathetic nervous system consists of a complex relay system consisting of nerves, neurotransmitters and receptors. Following are the components that are vital to the sympathetic nervous system:

- Pre-ganglionic sympathetic nerves
- Post-ganglionic sympathetic nerves
- Catecholamines
- Adrenal Medulla
- Adrenergic receptors

Sympathetic nerves originate in the brain stem and give rise to pre-ganglionic efferent nerve fibers that leave the CNS in the thoraco-lumbar region of spinal cord. Most of these pre-ganglionic sympathetic nerves form synapses with post-ganglionic sympathetic nerves in the para-vetebral chains located on either side of the spinal column. Remaining sympathetic ganglia are located in the pre-vertebral ganglia which lie in front of the vertebrae. Post-ganglionic sympathetic nerves transmit the signals to the innervated tissues by the release of NA and thereby activating the adrenergic receptors in the effector tissue/organ and thus producing its actions (Ilia *et al*., 2000).

The major paracrine factor released by adrenal medulla is adrenaline and to a lesser extent noradrenaline (NA). The chromaffin cells of the adrenal medulla are innervated by pre-ganglionic sympathetic nerve terminals which uses acetylcholine as the neurotransmitter (Ilia *et al*., 2000). Upon activation of the chromaffin cells, adrenaline and noradrenaline are produced. Therefore, we can say that adrenaline and noradrenaline are the principle end products of the sympathetic nervous system.

Adrenaline and noradrenaline are catecholamines. They are synthesized from tyrosine which is transported into the noradrenergic varicosities by a sodium dependent carrier, where tyrosine is converted to di hydroxy phenylalanine (DOPA) by an enzyme tyrosine hydroxylase and is finally hydroxylysed to dopamine and a carrier which can transport dopamine into the vesicles. Dopamine is converted to NA in the vesicles by dopamine bihydroxylase (DBH). In the adrenal medulla, NA is further converted to adrenaline.

The nerve impulse carried by the sympathetic nerves exerts its actions on the effectors through activation of certain receptors in the effector organs. The post-ganglionic sympathetic nerve terminals release NA to activate these receptors (DiBona, 2000). Pertaining to the fact that primarily NA and adrenaline activate these receptors, they are referred to as adrenergic receptors.

1.2.1 Adrenergic receptors

All adrenergic receptors are G-protein coupled receptors. Each major type has an affinity for a specific G protein. α_1 adrenoceptors show preference for Gq, α_2 adrenoceptors link with Gi while β_2 adrenoceptors couple with Gs (Shelli *et al*., 2004). These receptors exhibit a relatively rapid response to agonists. Upon exposure to agonists, responses depend on the desensitized state of the receptor (Shelli *et al*., 2004). There are multiple mechanisms involved in the desensitization process. A relatively rapid route to desensitization is through phosphorylation of the receptor by both G protein receptor kinase and by signaling kinases such as protein kinase A or protein kinase C (Luttrell *et al*., 2002). A slower process to desensitized state of the adrenoceptors might exhibit in the form of receptor endocytosis / internalization and degradation, which ultimately leads to down regulation of the receptors (Taso *et al*., 2001).

Adrenergic receptors are membrane bound receptors in nature and are present in cells of both peripheral tissues and CNS. Adrenoceptors mediate a variety of actions and therefore have been in the limelight of research for several decades. Ahlquist first

classified them as alpha and beta adrenoreceptors in 1948 (Ahlquist., 1948). Adrenoceptors excite on exposure to both adrenaline and noradrenaline. Both adrenaline and noradrenaline play an important role in the control of blood pressure (BP), myocardial contractile rate and force as well as metabolism. Adrenoceptors have affinity for many synthetic drugs and with the newer pharmacological tools, scientists have been able to identify and subdivide the adrenoceptors. Drugs interacting with subtypes have proven useful in a variety of diseases, involving most of the vital organs in the body. Some of the pathophysiological conditions in which adrenoceptors have been targeted and found to be of use include hypertension, angina pectoris, CHF, cardiac arrhythmia and asthma (David *et al*., 1994).

1.2.1.1 Classification, characterization & distribution

In early 20th century, Dale reported that certain responses to adrenoceptor stimulation, such as the vasopressor action of adrenaline, are insensitive to ergot alkaloids that are classical adrenoceptor antagonists. This opened up an exciting new avenue for researchers to explore adrenoceptors. Up till then, the focus of adrenoceptor classification was based either on “excitatory” or “inhibitory” actions. These qualitative approaches of classification were not so helpful and even lead to proposal of different neurotransmitters. However, in 1948 Ahlquist’s quantitative approach was based on different order of potency of the two different adrenoceptor subtypes. He evaluated effects of structurally related natural and synthetic agonists in different tissues. He proposed alpha and beta adrenoceptors based on the findings that beta receptors are insensitive to blockade by ergot alkaloids (Powell *et al*., 1957; Moran *et al*, 1958). In

1967, Lands and his colleagues described that there were two subtypes of β receptors. They explained that β_1 adrenoceptor subtype was equally sensitive to adrenaline and NA and that it is the dominant subtype in heart and adipose tissue. Whereas β_2 adrenoceptors were much less sensitive to noradrenaline as compared to adrenaline and that they are responsible for relaxation of vascular, uterine and airway smooth muscles (Lands *et al*., 1967 a&b).

In 1974, Langer presented the classification of α adrenoceptors based on the anatomical distribution. Reports started to come in regarding the pharmacological differences amongst pre and post junctional α adrenoceptors (Starke *et al*., 1974). Langer suggested that pre junctional α adrenoceptors be named as α_2 while post-junctional as α_1. Not much time had passed when a functional subdivision of α adrenoceptors was viewed in literature in late 1970's. These studies used agonists-antagonists interaction as the basis to oppose anatomical subdivision discussed by Langer and other researchers and suggested a classification of adrenoceptors based on their function (Berthelsen *et al*., 1977). These researchers suggested that α_1 adrenoceptors mainly induce excitation while α_2 adrenoceptors play a role in inhibition. A few years later, Drew and Whiting argued the presence of two distinct types of post synaptic α adrenoceptors based on their findings that NA induced vasoconstriction is inhibited not only by α_1 adrenoceptor antagonists such as prazosin but also with yohimbine, a selective α_2 antagonist (Drew *et al*., 1979). These findings lead scientists to re-evaluate the way to classify adrenoceptors. In late 1980's and early 1990's the pharmacological subdivision of α_1 and α_2 adrenoceptors and their subtypes stirred

interest in most scientists. This new classification was based on highly specific agonist and antagonist interactions (Ruffolo *et al*., 1988; Ruffolo *et al*., 1991). Over the passage of time, pharmacological basis of sub classifying adrenoceptors strengthened. Scientists agreed that α adrenoceptors that are activated by methoxamine, cirazoline or phenylephrine and blocked competitively by low concentration of prazosin, WB-1401 or corynanthin should be classified as α_1 adrenoceptors. Whereas, adrenoceptors activated by α-methylnorepinephrine, UK-14, 304, B-HT 920 and/or B-HT 933 and blocked by low concentrations of yohimbine, rauwolscine and/or idazoxin be classified as α_2 adrenoceptors (Ruffolo *et al*., 1991).

In late 1980's the advancement in the radio-ligand binding assays, gene recombinant technology and development of molecular biology techniques had a major impact on adrenoceptor classification. Since then, the presence of several subtypes of α and β adrenoceptors have been shown to exist. Sometimes multiple subtypes are expressed in a single tissue.

Adrenoceptors have been historically classified into α and β adrenoceptors, however, in 1994, David and his co-workers suggested that it is more appropriate to classify adrenoceptors into α_1, α_2 and β adrenoceptors based on differences in affinity to pharmacological tools, differences in the second messenger responses and the amino acid sequence of these receptors (David *et al*., 1994). However, in the later studies it was shown that there is more than one subtype of β adrenoceptors. These subclasses of adrenoceptors are discussed in detail below.

1.1.1.2 α adrenoceptors

Information regarding modulation of vascular tone through α adrenoceptors gained interest by researchers as well as clinicians pertaining to their possible manipulation in hypertension. However, what we know about these adrenoceptors today is based on their discovery in 1948 by Ahlquist and pioneering work by Langer and his co-workers. With the development of selectively binding drugs, these receptors have enabled insight into pathological states and their possible treatment. They can be largely subdivided into α_1 and α_2 adrenoceptors.

1.2.1.2.a α_1 adrenoceptors

α_1 adrenoceptors are involved in smooth muscle growth, regulation of blood pressure, maintenance of the vascular tone and myocardial contractility (Brodde *et al*., 2001; Guimaraes *et al*., 2001; Koshimizu *et al*., 2002). A cascade of reactions occurs upon stimulation of these receptors, stimulating the activity of Phospholipase C, which promotes the hydrolysis of phosphatidylinositol bisphosphate producing inositol trisphosphate and diacylglycerol. These molecules act as second messengers mediating intracellular Ca^{2+} release from non-mitochondrial pools and activate protein kinase C (Guimaraes *et al*., 2001). These membrane-bound receptors excite upon coupling with both sympathetic neurotransmitters; adrenaline and noradrenaline. Also, each of the α_1 adrenoceptor subtype shows linkage to Gq protein and activates phospholipase C (Shelli *et al*., 2004).

With the development in receptor cloning techniques, advancements in molecular biology approach and radio-ligand binding assays, α_1 adrenoceptors were first divided into α_{1A} and α_{1B} subtypes based on relative affinity of these receptors for 5-MeU, WB-4101 and CEC (Morrow *et al*., 1986; Han *et al*., 1987; Gross *et al*., 1988; Hanft *et al*., 1989 and Boer *et al*., 1989). These researchers showed that α_{1A} adrenoceptors were sensitive to 5-MeU and WB-4101 and insensitive to CEC. They also reasoned that α_{1B} adrenoceptors were sensitive to CEC and had a very low affinity to 5-MeU and WB-4101. At present researchers discuss at least three sub types of α_1 adrenergic receptors based on a pharmacological classification, namely; α_{1A}, α_{1B} and α_{1D}.

1.2.1.2.a.i α_{1A}

In vitro studies have shown that α_{1A} mRNA is highly expressed in peripheral arteries and that up to 90% of total α_1 adrenoceptor mRNA is of α_{1A} subtype in peripheral vasculature (Guimaraes *et al*., 2001). Discrepancies exist between the knowledge of the expression of these receptors *in vitro* and their functional implications *in vivo* (Hrometz *et al*., 1999; Ohmi *et al*., 1999). It has also been suggested that α_{1A} adrenoceptor predominates in 8 different types of rat arteries which had a dense innervation of sympathetic nerves (Frank *et al*., 1998). By inducing chemical sympathectomy they also argued that reshuffling of α_{1A} adrenoceptor density might be of importance in pathophysiological states with high sympathetic activity. Using molecular biology techniques, the presence of another α_1 adrenoceptor subtype, termed as α_{1C} has been shown to exist (Schwin *et al*., 1990). In the following years a consensus

was reached that $\alpha_{1A/C}$ are the same sub type of alpha adrenoceptor and α_{1A} is to be referred to this receptor (Ruffolo *et al*., 1991). Using binding competition experiments, co-expression of α_{1A} and α_{1B} adrenoceptors in rat tail arteries has been shown in intact tissue segments and membranes (Tanaka *et al*., 2004). Recent studies have more clearly addressed the functional attributes of these receptors and it has been demonstrated that noradrenaline induced contractions in rat tail artery are mediated by both α_{1A} and α_{1B} adrenoceptors (Sven *et al*., 2004). α_{1A} and α_{1B} adrenoceptors have also been shown to play a role in the inotropy of mice heart. Using a gene knock out approach, it was shown that both α_{1A} and α_{1B} adrenoceptor subtypes play similar roles in exhibiting negative inotropy in mouse myocardium. It was also discussed by the same group of researchers that the inotropic effects induced by α_1 adrenoceptors differ dramatically in mouse and rat myocardium (Diana *et al*., 2002). In humans, α_{1A} and α_{1B} subtypes of adrenoceptors have also been shown to be the major expressed and functionally important sub types in the sphenous vein (Ming *et al*., 2001).

1.2.1.2.a.ii α_{1B}

α_{1B} was probably the first α_1 adrenoceptor subtype to be cloned using molecular biology techniques. Cotecchia and his fellow workers isolated a clone from a hamster vas deferens cell line (DD_1MF2), which expressed a protein with a sequence similar to that of a G protein coupled receptor (Cotecchia *et al*., 1988). Upon further analysis and expression of this cDNA resulted in a protein with properties consistent with α_{1B} adrenoceptor. It had a high affinity for prazosin and a low affinity for phentolamine, 5 methyl urapidil (5-MeU) and yohimbine. It was also highly sensitive to irreversible

inactivation by CEC. Voigt and fellow colleagues identified rat α_{1B} adrenoceptors in liver, spleen, heart and cerebral cortex and showed that its pharmacological profile was similar to that of hamster (Voigt *et al*., 1990). In one of the recent studies, it was shown that noradrenaline mediates sympathetic neurotransmission in murine mesenteric arteries by acting on α_{1B} adrenoceptors and to a lesser extent on α_{1A} adrenoceptors. The same experiment also revealed that in DOCA salt hypertensive mice, neurogenic constrictions are mediated only by α_{1B} adrenoceptors (Alex *et al*., 2005). Using α_{1B} knock out mice, a definite but minor role of α_{1B} adrenoceptors in the rat tail arteries has also been discussed recently (Daley *et al*., 2002).

1.2.1.2.a.iii α_{1D}

In 1991, Lomasney and his fellow researchers reasoned for the presence of another α_1 adrenoceptor subtype based on their findings from molecular cloning and cDNA expression from the rat cerebral cortex. Their theory was fortified by Perez and co-workers who called this as α_{1D} adrenergic receptor (Lomasney *et al*., 1991). They found low affinity for the more selective α_{1A} adrenoceptor antagonist 5-MeU and (+) niguldipine (Lomasney *et al*., 1991). It has been established by gene knock out studies in mice that α_{1D} play an important role in homeostasis. *In vivo* studies showed that α_{1D} adrenoceptors play an important role in the vascular contractions caused by α_1 adrenoceptor agonists (Piascik *et al*., 1995; Hussain *et al*., 2000). Removal of the genes regulating α_{1D} reduced the resting blood pressure in mice but α_{1B} knock out mice did not show any baseline change in the pressure, suggesting an important role of α_{1D} adrenoceptor subtype in maintaining homeostasis. These scientists also suggested that

α_{1D} is an important adrenoceptor subtype in the development of secondary hypertension in acute renal dysfunction (Chihiro *et al*., 2005). One of the studies has shown that α_{1D} adrenoceptors do not play a role in neurogenic constrictions in both the arteries of sham and DOCA hypertensive mice (Alex *et al*., 2005). Moreover, a recent study suggests that in hyper homocysteinemia, the contractile responses to phenylephrine are due to impaired modulation performed by α_{1D} adrenoceptor mediated endothelial vasorelaxation in rat carotid arteries (Claudia *et al*., 2006).

At present a consensus has been reached as to the presence of three α_1 adrenoceptors namely; α_{1A}, α_{1B} and α_{1D}. Identifying the subtype of α_1 adrenoceptors involved in vasoconstrictive responses to sympathomimetic agonists is by no way straightforward. *In vitro* studies suggested that vascular smooth muscles express mixed subtypes of α_1 adrenoceptor subtypes (Miller *et al*., 1996). Evidence also suggests that responses produced are probably due to the activation of more than one subtype (Zhong *et al*., 1999). Although mRNA of all three subtypes have been found in several blood vessels, the contraction in response to phenylephrine is primarily mediated by α_{1D} and secondarily by α_{1B} adrenoceptors (Xu *et al*., 1997; Hussain *et al*., 2000). In another study Piascik and co-workers made similar observation. They found that expression of mRNA for α_1 adrenoceptor subtypes, in several arteries of rat, were in order $\alpha_{1A}>\alpha_{1B}>\alpha_{1D}$ but only α_{1B} played a functionally important role in mesenteric artery (Piascik *et al*., 1997) whereas α_{1D} adrenoceptor subtype dominated the functional importance in aorta, femoral and iliac arteries (Piascik *et al*., 1997). Some functional studies show that in the rat, α_{1A} and α_{1D} adrenoceptor subtypes regulate the larger

vessels, while α_{1B} dominate the functional importance in the small resistance vessels (Piascik *et al*., 1997; Gisbert *et al*., 2000). Some studies have also discussed the possibility of junctional versus extra junctional localization of α_1 adrenoceptor subtypes. However, their physiological significance and differential localization based on the functional studies remain to be clinically exploited (Alex *et al*., 2005).

1.2.1.2.a.iv α_{1L} & α_{1H}

Another classification of α_1 adrenoceptors based on the relative affinity for prazosin exists. α_{1H} showed high affinity to prazosin while α_{1L} showed low affinity (Holck *et al*., 1983; Langer *et al*., 1984; Flavahan *et al*., 1986). This point of view emerged because some scientists observed that two types of α_1 adrenoceptors did not show any response to noradrenaline induced contractile responses of propranolol in prostate and bladder of rat and guinea pig (Cohen *et al*., 1989). More recently, scientists have shown that α_{1L} adrenoceptors mediate the noradrenaline induced contraction in mouse prostate (Katherine *et al*., 2006). These results are in accordance with the findings of Ford and co-workers, that the same receptor induces contractile responses in human prostate (Ford *et al*., 1996).

1.2.1.2.b α_2 adrenoceptors

α_2 adrenoceptors were first discussed in 1980's. Pertaining to the non-availability of selective agonists, as in the case of α_1 adrenoceptors, α_2 adrenoceptors and their subtypes classification was by no means easy. Binding studies initially revealed two subtypes of α_2 adrenoceptors namely α_{2A} from human platelets and α_{2B} from rat kidney, lungs and cerebral cortex (Van Zwieten *et al*., 1982; McGrath *et al*., 1989). To date, three subtypes of α_2 adrenoceptors have been shown to exist, namely; $\alpha_{2A/D}$, α_{2B} and α_{2C} (Guimaraes *et al*., 2001). These findings were initially based on radioligand binding assays but later functional studies and molecular approaches confirmed their expression as well. The amino acid composition of α_{2B} and α_{2C} are similar in mammalian species except for α_{2A} adrenoceptor, which differs slightly. In humans the gene coding for α_{2A}, α_{2B} and α_{2C} adrenoceptors are located in chromosomes 10, 2 and 4 respectively (Regan *et al*., 1988; Lomasney *et al*., 1990; Weinshank *et al*., 1990). Pharmacologically α adrenoceptor antagonists possess different affinity for α_2 adrenoceptor subtypes (Guimaraes *et al*., 2001). Prazosin has a relatively higher affinity for α_{2B} and α_{2C} adrenoceptors and a very low affinity for $\alpha_{2A/D}$ adrenoceptors. Yohimbine and rauwolscine are more selective than phentolamine and idazoxanon for α_{2A} in comparison to α_{2D} adrenoceptors.

A vast majority of tissues express more than one subtype of α_2 adrenoceptors with a few exceptions; α_{2A} in human platelets, α_{2B} in the rat neonatal lung and α_{2C} in opossum cells (Bylund *et al*., 1988). It has been discussed that α_2 adrenoceptors are an essential part of the neural system regulating cardiovascular function (Ruffalo *et al*.,

1991). However, at the post junctional level, α_2 adrenoceptors were not found *in vitro* in most of the arterial vessels. Also, in general, the vasoconstrictive effect of α_2 adrenoceptors is restricted in general to the small arteries and arterioles but not in large arteries (Guimaraes *et al*., 2001).

The involvement of α_2 adrenoceptors and/or imidazoline receptors in the anti-hypertensive effects of drugs like clonidine and moxonidine is still controversial. The results of one study suggests that the effects of moxonidine acting in the forebrain (renal vasodilatation) depends on α_1 adrenoceptor activation, while the cardiovascular effects, including renal vasodilatation, produced by moxonidine acting in the brainstem depends at least partially on the activation of α_2 adrenoceptors (Thiego *et al*., 2007).

1.2.3 β adrenoceptors

These are G protein coupled receptors and mediate cardiovascular responses to noradrenaline released from sympathetic nerve terminals and to circulating adrenaline. Also, β adrenoceptor mediated vasodilatation plays an important physiological role in the regulation of vascular tone. Stimulation of peripheral β adrenoceptors lead to relaxation of the vascular smooth muscles, thereby controlling the peripheral vascular resistance. β adrenoceptors can be stimulated or blocked by many compounds. Such compounds are therefore used to treat important and common diseases, such as hypertension, asthma, cardiac arrhythmias and ischemic heart disease (Guimaraes *et al*., 2001).

All β adrenoceptor subtypes signal by coupling to the stimulatory G-protein; Ga. This leads to the activation of adenylyl cyclase and release of the second messenger cAMP (Dixon *et al*., 1986; Frielle *et al*., 1987; Emorine *et al*., 1989). However, some recent studies indicate that, under certain circumstances, β adrenoceptors, particularly β_3 adrenoceptor, can couple to Gi as well as to Gs (Chaudry *et al*., 1994; Xiao *et al*., 1995; Gauthier *et al*., 1996, Gauthier *et al*., 2000). Intracellular events following β-adrenoceptor activation are also linked to ion transport. It is well known that protein kinase A activated by cAMP, phosphorylates L-type Ca^{2+} channels to facilitate Ca^{2+} entry and thus producing the positive inotropic effect in atria and ventricles, increased heart rate in the sino-auricular node, and accelerated conduction in the atrio-ventricular node (Guimaraes *et al*., 2001). Multiple mechanisms control the signaling and density of G-protein-coupled receptors (GPCRs). The termination of GPCR signals involves binding of proteins to the receptor as a first step. This process is initiated by serine-threonine phosphorylation of the receptors. This phosphorylation takes place both by members of the GPCR kinase family and by second-messenger-activated protein kinases A and C. Receptor phosphorylation by GPCR kinase is followed by binding of proteins named arrestins. Arrestins bind to the phosphorylated receptor and inhibit further G-protein activation (Luttrell *et al*., 1999).

In late 1960s Lands and coworkers classified β receptors into β_1 and β_2 (Lands *et al*., 1967 a&b). So far three distinct β adrenoceptor subtypes have been cloned; β_1, β_2, and β_3. β_1 adrenoceptors mediate +ve chronotropy and ionotropy as well as relaxation of the coronary arteries and stimulates the renin release (Bylund *et al*., 1994).

The indigenous catecholamines adrenaline and noradrenalin have an excitatory action on both β_1 and β_2 adrenoceptors whereas propranolol antagonizes them. Three different genes located on human chromosomes 10, 5 and 8 encode β_1, β_2, and β_3 respectively. The human β_3 adrenoceptor has 49% and 51% overall homology at the amino acid level with β_2 and β_1 adrenoceptors, respectively (Emorine *et al*., 1989; Granneman *et al*., 1994). β_3 adrenoceptors are not blocked by propranolol and other conventional β adrenoceptor antagonists. However, this subtype of β adrenoceptors can be selectively blocked by SR-59230. Moreover, they are activated by selective β_3 adrenoceptor agonists like BRL 37344 and CL 316243 (Manara *et al*., 1995; Summers *et al*., 1997; Fischer *et al*., 1998).

On the basis of many pharmacological and molecular studies, the existence of a fourth β adrenoceptor subtype was postulated (Brodde *et al*., 1999). Studies using more selective agonists and antagonists showed that relaxation of vascular smooth muscle cells resulted from activation of either β_1 or β_2 adrenoceptor subtypes and that the involvement of each subtype depended on the vascular bed and the species under investigation (O'Donnell *et al*., 1984; Guimaraes *et al*., 1993; Shen *et al*., 1994; Shen *et al* 1996; Begonha *et al*., 1995).

In the heart, maximum +ve ionotropy is obtained by stimulation of only β_1 adrenoceptors (Kaumann *et al*., 1989; Motomura *et al*., 1990) while the –ve ionotropy induced by β_2 adrenoceptors is larger than that induced by the β_1 adrenoceptor stimulation in the vessels (Guimaraes *et al*., 1981).