HADIAH

PERPUSTAKAAN
UNIVERSITI SAINS MALAYSIA

Tesis/latihan ilmiah ini adalah untuk kajian dan penyelidikan sahaja. Bahagian-bahagian, data-data atau idea-idea yang asal dari karya ini tidak boleh dipetik atau disebutkan tanpa akuan

This thesis/academic exercise must be used for study or research purposes. No portion of this work, original data or ideas may be taken/ quoted without due acknowledgement

STUDIES ON KALLIKREIN-KININ SYSTEM IN NORMOTENSIVE AND HYPERTENSIVE RATS WITH AND WITHOUT DIABETES

by

UMA KESAVARAO

Thesis submitted in fulfilment of the
requirements for the degree
of Master of Science
(Pharmacology)

December 1995

Oom Ganeṣaaya Namaha

This thesis is dedicated
to
my most reverent elders
Mr. R. Periyaswamy,
Mr. S. Kesavarao and Mrs. Vasanthi Kesavarao
and to my most beloved husband
Dr. S.P. Ram and
children
Ranjith and R. Dinesh.

ACKNOWLEDGEMENTS

It is my duty to acknowledge my heartfelt gratitude to Prof. J.N. Sharma, my supervisor, who has been a pillar of support in stimulating and guiding me at all stages of this work. I am indebted to thank him for imparting his wide experience and knowledge in this field.

I am thankful to Dr. Abdul Rahman Noor, Head of Department of Pharmacology and co-supervisor of my research, for his kind co-operation and providing an oppourtunity to work in his department.

I am most grateful to Dato' Prof. Mustaffa Embong, Dean of Medical School for his kind support and providing an opportunity to work for higher degree.This work was supported by a research and development grant from the Ministry of Science and Technology of Malaysia, under the Sixth Malaysian Plan (IRPA) to Professor .J.N.Sharma.

My sincere thanks to Puan Che Nin, Encik Lokman Ali, Encik Jammalludin Ramli, Encik Abdul Razak, Encik Mohd. Aminorddin, En. Mohd Lukmi, Puan Shaheela and En.Shahrul, Department of Pharmacology for their support during the course of my study.

I am also indebted to acknowlege Dr.Afifi, Vetenerian and En. Maarof, School of Medical Sciences, Kubang Kerian, for providing and taking care of the animals,and to the Staff of the Department of Chemical pahology and histopathology for rendering their help and allowing me to utilize their laboratory services.

I sincerely thank Cik Noor Syamsinaz, Prof. Madya Dr.and Mrs Sirisinghe and Puan Ajindar Kaur who have given their support and encouragement in completing this task.

I also thank En.Saaban Mohd.Shukor, En.Izaham Musa and En.Arifin Harun from the CAI Laboratory and other staff of the School of Medical Sciences ,USM ,Kubang Kerian,Kelantan for their help in completing this thesis.

CONTENTS

LIST OF FIGURES x
LIST OF TABLES xvi
ABSTRAK xvii
ABSTRACT xix
INTRODUCTION 1
1.The kallikrein kinin system 2
1.1 A brief history 4

1.2.Chemistry and characterization 6
1.2.1 Bradykinin and its analogues 6
1.2.2 T-kinin 6
1.2.3 Other mammalian kinin 7
1.2.4 Non-mammalian kinins 7

1.3.Kinin-forming enzymes 10
1.3.1 Plasma kallikrein 11
1.3.2 Plasma kallikrein inhibitor 12
1.3.3 Tissue kallikrein 12
1.3.4 Tissue kallikrein inhibitors 14
1.3.5 T-kininogenases 15
1.3.6 T-kinin inhibitors 16

1.4. Plasma Kininogens 16
1.4.1. Types of kininogens 16
1.4.1a High molecular-weight kininogen 16
1.4.1b Low molecular-weight kininogen 16
1.4.1c T-kininogen 18

1.5.Fate of kinins 19
1.5.1 Kininases 19

1.6. Role of kinins 20
1.6.1 Physiological conditions 20
1.6.2 Pathological conditions 22

1.7. The Antihypertensive agent-
Angiotensin converting enzyme inhibitors 23

2. AIM OF THE PRESENT STUDY 25

3. MATERIALS 27

4. METHODS 29

4.1. Experimental methods 29

4.1.1 Animal model **29**

4.1.2 Recording blood pressure and removal of heart **29**

4.1.3 Induction of Diabetes **30**

4.1.4 Administration of captopril **30**

4.2.ESTIMATION OF KALLIKREIN-KININ COMPONENTS 31

4.2.1.Determination of tissue kallikrein activity 31

4.2.1a Preparation of standard curve **32**

4.2.1b Preparation of cardiac tissue homogenate **33**

4.2.1c Determination of Active Tissue Kallikrein **34**

4.2.1d Determination of Total Tissue Kallikrein **34**

4.2.1e Estimation of Inactive Tissue Kallikrein **34**

4.2.2.Estimation of cardiac tissue kininogen activity 35

4.2.3. Determination of plasma prekallikrein activity and kallikrein-like activity 36

4.2.3a Preparation of standard Curve of plasma kallikrein activity **37**

4.2.3b Determination of Plasma Prekallikrein Activity **38**

4.2.3c Determination of Plasma Kallikrein-Like Activity **38**

4.2.4.Determination of plasma kallikrein inhibition activity . 39

4.2.4a Preparation of standard Curve 39

4.2.4b Determina[illegible]llikrein Inhibition Activity 41

4.2.5. Estimation o[illegible] and kininogens 41

4.2.5a Preparation of standard curve 42

4.2.5b Estimation of total Plasma kininogen 43

4.2.5c Estimation of high molecular weight plasma Kininogen 44

4.2.5d Estimation of low molecular weight plasma Kininogen 44

4.2.6.Determination of urine kallikrein 44

4.2.6a Preparation of standard Curve 45

4.2.6b Determination of Active Urinary Kallikrein 46

4.2.6c Determination of Total Urinry Kallikrein 47

4.2.6d Estimation of Urinary prokallikrein 47

4.2.7.MORPHOLOGICAL EVALUATION OF THE HEART 47

4.2.7a Preparation of slides 47

4.2.7b Staining with haematoxylin and eosin 47

4.2.7c Measurement of left ventricular wall thickness 48

4.2.8. Statistical analysis 50

5.RESULTS 51

5.1.STANDARD CURVES 51

5.1.1 Urinary kallikrein activity 51

5.1.2 Tissue kallikrein 51

5.1.3 Plasma kallikrein 55

5.1.4 Plasma kallikrein inhibitory activity 55

5.1.5 Bradykinin 55

5.2.ALTERATIONS IN KALLIKREIN-KININ SYSTEM IN NORMOTENSIVE WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSVE RATS WITH AND WITHOUT DIABETES 59

5.2.1. Subjects characteristics 59

5.2.1a Urine glucose 59

5.2.1b Urine total protein 60

5.2.1c Blood glucose 60

5.2.2.Blood pressure 60

5.2.2 a Systolic blood pressure 60

5.2.2 b Diastolic blood pressure 60

5.2.2 c Mean arterial pressure 65

5.2.3. Urinary kallikrein activity 65

5.2.3a Active 65

5.2.3b Total 69

5.2.3c Prokallikrein 69

5.2.4.Plasma kallikrein activity 73

5.2.4a Prekallikrein activity 73

5.2.4b Kallikrein-like activity 73

5.2.5.Plasma kallikrein inhibitory activity 73

5.2.6.Cardiac tissue kallikrein 73

5.2.6a Active 73

5.2.6b Total 76

5.2.6c Inactive 76

5.2.7.Cardiac tissue kininogen 76

5.2.8.Plasma kininogens 76

5.2.8a Total plasma kininogen 81

5.2.8b High molecular weight kininogen 81

5.2.8c Low molecular weight kininogen 81
5.2.8d Plasma Bradykinin 81
5.2.9.Measurement of left ventricular wall thickness including interventricular septum 83

5.3.EFFECTS OF CAPTOPRIL ON NORMOTENSIVE WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS WITH AND WITHOUT DIABETES 83
5.3.1.Subjects characteristics 83
5.3.1a Blood glucose 84
5.3.1b Urine protein and glucose 84
5.3.2.Blood pressure
5.3.2a Systolic blood pressure 88
5.3.2b Diastolic blood pressure 88
5.3.2c Mean arterial pressure 92
5.3.3. Urinary kallikrein activity 92
5.3.3a Active urinary kallikrein 97
5.3.3b Total urinary kallikrein 97
5.3.4.Plasma kallikrein activity 102
5.3.5.Plasma kallikrein inhibitory activity 102

5.3.6.Plasma kininogens and bradykinin 107
5.3.6a Total kininogen 107
5.3.6b High molecular weight kininogen 107
5.3.6c Low molecular weight kininogen 114

5.3.7.Measurement of left ventricular wall thickness including interventricular septum **114**

6.DISCUSSION **115**

7.CONCLUSION **121**

8.REFERENCES **1**

9.APPENDICES

10.PUBLISHED PAPERS AND COMMUNICATIONS

List of figures

Figure1.1. Formation and destruction of kinins........................3

Figure 4.1. Measurement of left ventricular wall thickness including interventricular septum by projecting the image of its slide on to a calibrated screen...49

Figure 5.1. Standard curve for urinary kallikrein activity using standard pancreas kallikrein . Absorbance (y axis) was plotted against different concentration of standard pancreas kallikrein (x axis).....52

Figure 5.2. Optimation of time for rate assay of tissue kallikrein activity. Time in minutes (x axis) was plotted against increase in absorbance (y axis)...53

Figure 5.3. Standard curve for tissue kallikrein using standard pancreas kallikrein..Different concentrations of standard pancreas kallikrein (x axis) were plotted against increase in absorbance at 405nm(y axis)...54

Figure 5.4. Standard curve for plasma prekallikrein and kallikrein-like activity . Different concentrations of standard plasma pool (x axis) were plotted against absorbance at 405nm (y axis)...........................56

Figure 5.5. Standard curve for plasma kallikrein inhibition activity. Different concentrations of standard plasma pool (x axis) were plotted against absorbance at 405nm (y axis).......................................57

Figure 5.6. Standard curve for measurement of bradykinin, total, high molecular weight kininogen and low molecular weight kininogen using Markit M bradykinin kit...58

Figure 5.7. Changes in systolic blood pressure in diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to control Wistar kyoto and control spontaneously hypertensive rats..........................62

Figure 5.8. Changes in diastolic blood pressure in diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared with control Wistar kyoto and control spontaneously hypertensive rats..............63

Figure 5.9. Changes in mean arterial pressure in diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to control Wistar kyoto and control spontaneously hypertensive rats..........................64

Figure 5.10. Level of active urinary kallikrein activity in diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to control Wistar kyoto and control spontaneously hypertensive rats....66

Figure 5.11. Level of total urinary kallikrein activity in control Wistar kyoto, diabetic Wistar kyoto, control spontaneously hypertensive rats and diabetic spontaneously hypertensive rats..............................67

Figure 5.12.Urinary level of total, active and prokallikrein activity in control Wistar kyoto, diabetic Wistar kyoto, control and diabetic spontaneously hypertensive rats...68

Figure 15.13. Changes in plasma prekallikrein activity associated with control and diabetic Wistar kyoto and control and diabetic spontaneously hypertensive rats...70

Figure5.14.Changes in plasma kallikrein inhibition acitvity in diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to their respective nondiabetic controls.......................................71

Figure 5.15. Changes in active cardiac tissue kallikrein activity associated with diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to their respective nondiabetic controls..72

Figure 5.16. Changes in total cardiac tissue kallikrein activity associated with diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to control Wistar kyoto and control spontaneously hypertensive rats... 74

Figure 5.17. Level of cardiac tissue kininogen in control and diabetic Wistar kyoto and control and diabetic spontaneously hypertensive rats..75

Figure 5.18.Changes in total plasma kininogen associated with diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to their respective nondiabetic controls.............................. . ..77

Figure 5.19. Changes in high molecular weight plasma kininogen associated with diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to their respective nondiabetic controls...78

Figure 5.20. Changes in low molecular weight plasma kininogen associated with diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to their respective nondiabetic controls...79

Figure 5.21. Level of plasma bradykinin in control and diabetic Wistar kyoto and control and diabetic spontaneously hypertensive rats..80

Figure 5.22. Changes in left ventricular wall thickness associated with diabetic Wistar kyoto and diabetic spontaneously hypertensive rats compared to their respective nondiabetic controls....................82

Figure 5.23. Effect of different doses of captopril on systolic blood pressure of nondiabetic Wistar kyoto and diabetic Wistar kyoto rats compared with their respective nontreated controls 85

Figure 5.24.Effect of different doses of captopril on systolic blood pressure of nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats compared with their respective nontreated
controls...86

Figure 5.25. Changes in diastolic blood pressure of nondiabetic Wistar kyoto and diabetic Wistar kyoto rats with different doses of captopril compared with their respective nontreated controls................87

Figure 5.26. Effect of different doses of captopril on diastolic blood pressure of nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats compared with their respective nontreated controls 89

Figure 5.27. Changes in mean arterial pressure of nondiabetic Wistar kyoto and diabetic Wistar kyoto rats with different doses of captopril compared with their respective nontreated controls......................90

Figure 5.28. Effect of different doses of captopril on mean arterial pressure of nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats compared with their respective nontreated controls.. .. 91

Figure 5.29. Urinary levels of active kallikrein activity in nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls.....93

Figure 5.30. Urinary levels of active kallikrein activity in nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated controls..94

Figure 5.31. Effect of different doses of captopril on total urinary kallikrein activity in nondiabetic Wistar kyoto and diabetic Wistar kyoto rats compared with their respective nontreated controls 95

Figure 5.32. Effect of different doses of captopril on total urinary kallikrein activity of nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats compared with their respective nontreated controls...96

Figure 5.33. Changes in plasma prekallikrein activity associated with nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls..98

Figure 5.34. Changes in plasma prekallikrein activity associated with nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated controls......................... 99

Figure 5.35. Changes in plasma kallikrein inhibition activity associated with nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls.. 100

Figure 5.36. Level of plasma kallikrein inhibition activity in the nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated controls............................... 101

Figure 5.37. Plasma level of total kininogen in nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls... 103

Figure 5.38. Plasma level of total kininogen in nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated controls.. 104

Figure 5.39. Changes in plasma levels of high molecular weight kininogen associated with nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls.. ...105

Figure 5.40. Changes in plasma level of high molecular weight kininogen associated with nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated controls.. 106

Figure 5.41. Plasma level of low molecular weight kininogen in nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls... 108

Figure 5.42. Plasma level of low molecular weight kininogen in nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated controls............................ 109

Figure 5.43. Level of plasma bradykinin in nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls...............110

Figure 5.44. Level of plasma bradykinin in the nondiabetic spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated controls..111

Figure 5.45. Changes in left ventricular wall thickness in nondiabetic Wistar kyoto and diabetic Wistar kyoto rats treated with different doses of captopril compared with their respective nontreated controls...112

Figure 5.46. Changes in left ventricular wall thickness in nondiabetic spontaneously hypertensive and diabetic spontaneously hypertensive rats treated with different doses of captopril compared with their respective nontreated control..113

LIST OF TABLES

Table 4.1. Steps in dilution of standard pancreas kallikrein in preparation of standard curve for tissue kallikrein activity....31.

Table 4.2. Steps in procedure for measurement of active and total tissue kallikrein activity..32

Table 4.3. Experimental steps in preparation of cardiac tissue for measurement of tissue kallikrein and tissue kininogen............33

Table 4.4. Preparation of different percentages of the standard plasma pool for establishing the standard curve for plasma prekallikrein and kallikrein-like activity..37

Table 4.5. Procedure for measurement of plasma prekallikrein activity...38

Table 4.6. Preparation of different percentages of standard plasma pool for establishing standard curve for plasma kallikrein inhibitory activity... 40

Table 4.7. Procedure for measurement of plasma kallikrein inhibition activity...41

Table 4.8. Steps in dilution of standard pancreas kallikrein in preparation of standard curve for urinary kallikrein activity...45

Table 4.9. Procedure for measurment of total and active urinary kallikrein activity...46

ABSTRAK

KAJIAN SISTEM KALLIKREIN-KININ DALAM TIKUS-TIKUS NORMOTENSI DAN HIPERTENSI DENGAN DAN TANPA DIABETES

Penyelidikan ini bertujuan untuk mengkaji fungsi sistem kallikrein-kinin dalam tikus-tikus normotensi dan hipertensi dengan dan tanpa diabetes. Lanjutan dengan itu pengaruh sistem kallikrein-kinin ke atas kesan perencat enzim penukar angiotensin (ACE inhibitor), captopril ke atas diabetes dan hipertensi juga ditentukan.

Tikus-tikus Wistar kyoto dan hipertensi spontan telah di-induskan diabetes untuk selama lima minggu dan dibandingkan dengan haiwan kawalan tanpa diabetik masing-masing. Tekanan darah kedua-dua kumpulan tikus Wistar kyoto dan hipertensi spontan telah meningkat dengan signifikennya ($p < 0.001$). Kallikrein urin aktif telah menurun dengan signifikan ($p < 0.001$) pada tikus-tikus diabetes Wistar kyoto dan hipertensi spontan (SHR). Buat pertama kalinya penyelidikan kami mendapati ada penurunan kedua-due jenis kallikrein total dan aktif pada tisu kardiak dalam tikus-tikus Wistar kyoto diabetes, hipertensi spontan diabetes bila dibandingkan dengan tikus Wistar kyoto kawalan. Walaubagaimana pun prekallikrein plasma dan kininogen berat molekul tinggi (LMWK) telah meningkat secara signifikan dalam kumpulan diabetik bila dibandingkan masing-masing kumpulan kawalan tanpa diabetik. Penganggaran morfologi jantung mendapati adanya peningkatan pada ketebalan dinding ventrikel kiri pada tikus-tikus Wistar kyoto diabetes, hipertensi spontan dan hipertensi spontan yang berdiabetes berbanding dengan tikus Wistar kyoto kawalan.

Pengaruh sistem kallikrein-kinin ke atas perencat enzim penukar angiotensin, captopril yang diberi dalam dua dos berlainan, 40mg/kg dan 80mg/kg berat badan untuk selama lima minggu bagi kedua -dua kumpulan tikus diabetik , Wistar kyoto dan hipertensi spontan telah dibandingkan dengan kumpulan tikus tanpa diabetik Wistar kyoto dan hipertensi spontan yang juga diberi captopril mendapati; Penurunan

tekanan darah dan gula darah yang signifikan (**p <0.001) telah dapati pada tikus-tikus diabetik, Wistar kyoto dan hipertensi spontan yang telah diberi captopril. Kedua-dua aktif dan total kallikrein pada urin telah meningkat kumpulan-kumpulan diabetik yang dirawat dengan captopril. Prekallikrein plasma dan kininogen berberat molekul tinggi telah didepresikan dalam kedua-dua tikus diabetik, Wistar kyoto dan hipertensi spontan berbanding dengan kawalan masing-masing. Pengukuran ketebalan dinding ventrikel kiri menunjukan tiada perubahan hipertrofi. Suka dinyatakan yang captopril pada dos 40mg/kg dapat menghalang hipertrofi ventrikel kiri tetapi gagal untuk menghasilkan normalisasi tekanan darah pada tikus-tikus hipertensi spontan yang diabetik.

Kajian ini mengesahkan terdapatnya pengawalan yang tidak normal sistem kallikrein kinin dalam diabetes dan hypetensi, juga terdapat pengaruh yang jelas dari sistem ini terhadap antihipertensi, hypoglisemia dan kesan kardioprotektif , enzim perencat pengubah angiotension iaitu captopril. Mekanisma yang mungkin berlaku terhadap komponen kinin dalam keadaan patologi ini dan perawatannya telah dibincangkan.

ABSTRACT

This research investigated the role of kallikrein-kinin system in normotensive and hypertensive rats with and without diabetes. Furthermore, the influence of kallikrein-kinin system on the effects of angiotensin converting enzyme inhibitor, captopril on diabetes and hypertension were evaluated.

Diabetes was induced in Wistar kyoto and spontaneously hypertensive rats by a single intraperitoneal injection of streptozocin 50mg/kg, followed up for five weeks and compared with respective nondiabetic controls. Blood pressure was significantly elevated (**p < 0.001) in the diabetic groups of both Wistar kyoto and spontaneously hypertensive rats compared wit their respective nondiabetic controls.. Active urinary kallikrein was significantly depressed in the diabetic Wistar kyoto and diabetic spontaneously hypertensive rats (**p < 0.001). For the first time, to our knowledge, this research has revealed a lowering of cardiac tissue kallikrein, both total and active, in diabetic Wistar kyoto, spontaneously hypertensive rats and diabetic spontaneously hypertensive rats when compared to control Wistar kyoto rats. However, the plasma prekallikrein and high molecular weight kininogen were raised significantly in the diabetic groups when compared to respective nondiabetic controls of both Wistar kyoto and spontaneously hypertensive rats. Morphological estimation of heart revealed an increase in left ventricular wall thickness including the interventricular septum thus indicating hypertrophic changes in the diabetic Wistar kyoto, spontaneously hypertensive rats and diabetic spontaneously hypertensive rats when compared to control Wistar kyoto rats.

Influence of kallikrein-kinin system on angiotensin converting enzyme inhibitor, captopril, administered in two different doses, 40mg/kg and 80mg/kg body weight for five weeks in diabetic rats both Wistar kyoto and spontaneously hypertensive groups was compared with nondiabetic control Wistar kyoto and control spontaneously hypertensive rats treated with captopril. A significant decrease (**p < 0.001) in blood pressure and blood sugar was observed in the diabetic Wistar kyoto and diabetic spontaneously hypertensive rats treated with captopril. The urinary kallikrein, both active and total were increased in the diabetic Wistar kyoto and spontaneously hypertensive rats treated with captopril. Total and active cardiac tissue kallikrein were significantly decreased(p<0.001)in the diabetic groups .when compared to their

respective nondiabetic controls. Plasma prekallikrein and high molecular weight kininogen were depressed in both diabetic Wistar kyoto and diabetic spontaneously hypertensive rats when compared to their respective control.Measurement of left ventricular wall thickness showed no hypertrophic changes in diabetic Wistar kyoto, control spontaneously hypertensive rats and diabetic spontaneously hypertensive rats treated with captopril. It is of interest to note that captopril at a dose of 40mg/kg which prevented left ventricular hypertrophy failed to produce normalization of blood pressure in the diabetic spontaneously hypertensive rats.

Our study confirms the abnormal regulation of kallikrein-kinin system in diabetes and hypertension and a definite influence of this system on the antihypertensive, hypoglycemic and cardioprotective effects of angiotensin converting enzyme inhibitor, captopril. The possible mechanisms of action of kinin components in these pathological conditions and their treatment have been discussed.

INTRODUCTION

The diversity and significance of recent researches on the kallikrein kinin system has provided the impetus for understanding of the pathophysiological processes of human diseases. The kallikrein-kinin system can no longer be regarded as an isolated system. The recent introduction of specific bradykinin receptor subtype antagonists has greatly advanced our understanding of the kallikrein-kinin system in various physiological and pathological conditions. Early studies focussed on the role of kinins in shock, inflammation and the function of exocrine glands. Interest has broadened recently as the numerous studies over the last few years have demonstrated the participation of kallikrein-kinin system in essential hypertension, aldosteronism, diabetes mellitus, asthma and rheumatoid arthritis. Our study is focussed on the role of kinins and the effects of angiotensin converting enzyme inhibitors on hypertension and diabetes mellitus in experimental animals.

1.The KALLIKREIN KININ SYSTEM

Kinins are paracrine peptides derived from circulating precursors by the action of serine proteases called kininogenases or kallikreins (Sharma, 1988a,b). They participate in a wide range of functions including the regulation of local organ blood flow, systemic blood pressure, transepithelial water and electrolyte transport, cellular growth, capillary permeability and inflammatory responses. Once released into the circulation, the kallikreins are rapidly inactivated by a group of plasma protease inhibitors, while kinins in circulation are destroyed by a group of enzymes known as kininases (fig.1.1).

The two most potent kallikreins are plasma and tissue kallikreins.Tissue kallikrein is present in organs like the kidney, brain and heart. When released into the circulation, it releases kinin before being inactivated by plasma inhibitors (Scicli et al.,1983). Plasma kallikrein is found in plasma in zymogen form and differs from tissue kallikrein in its biochemical and functional properties. In addition to kinin releasing function, it is associated with coagulation, fibrinolysis and inactivation of the complement system (Sundsmo and Fair.,1983).

The two main forms of kininogens or kallikrein substrates are low molecular-weight kininogen and high molecular-weight kininogen, both of which are found in plasma. A rat kininogen (T-kininogen) has been identified as a major acute phase reactant of inflammation (Muller-Esterl et al.,1986).

Kinins are produced from the kininogen substrates by the plasma and tissue kallikreins and other kininogenases. The important kinins described in mammals are:- bradykinin, lys-bradykinin, met-lys-bradykinin, hyp-lys-bradykinin, and ile-ser-bradykinin (T-kinin).

The kinins are rapidly destroyed by enzymes known as kininases found in blood, endothelial cells and other tissues.The main kininases are kininase I, an arginine carboxypeptidase and kininase II, also known as angiotensin I converting enzyme.

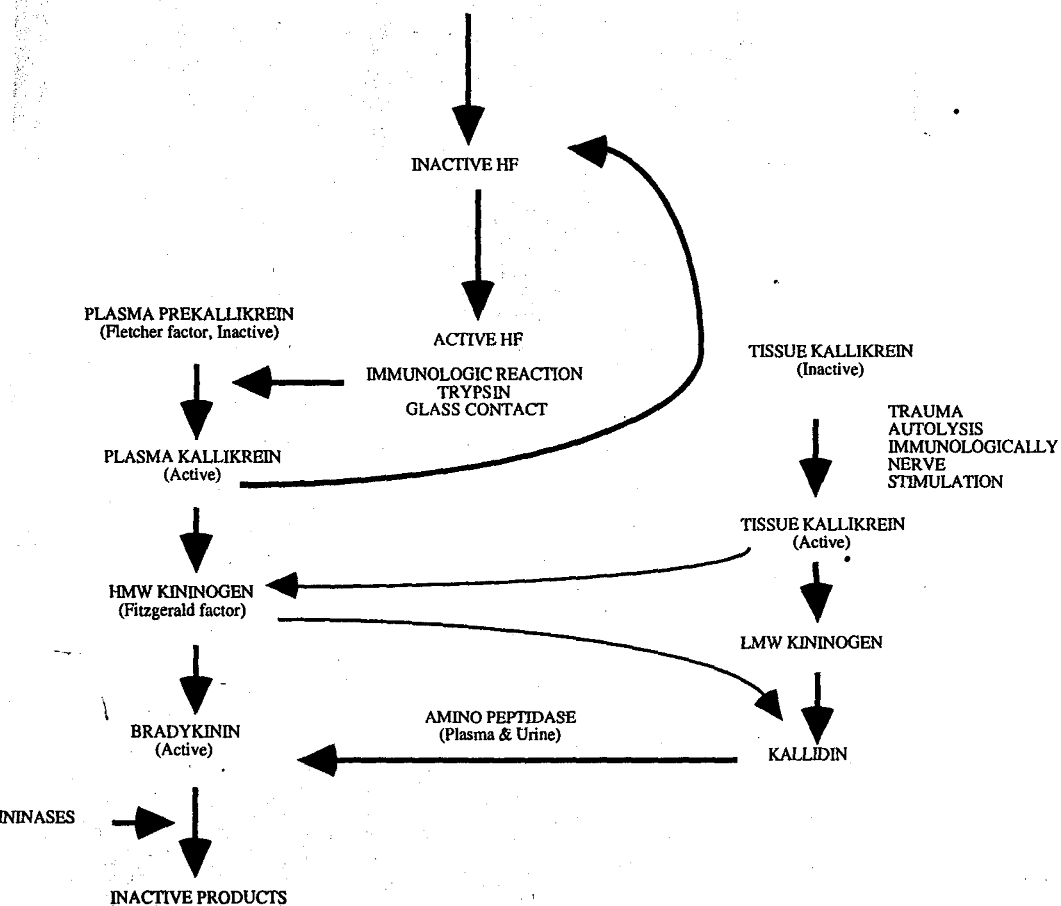

Figure 1. 1 Formation and destruction of kinins.
(HF: Hageman factor)

1.1. A brief history

The history of kallikrein kinin system dates back to the early 20th century when Abelous and Bardier (1908, 1909 a,b,c,d.) detected the presence of a hypotensive substance in normal human urine which they called urohypotensin. In 1926, Frey observed a considerable reduction in blood pressure when the urine of human or any other mammals was injected into dogs. Together with Kraut (Frey and Kraut, 1928) and Schultz (Frey et al., 1930) he isolated the causative substance from human urine and other organs. This hormone type substance was called `kallikrein' after the greek synonym for pancreas as it occurred in high concentrations in this organ. A short time after the discovery of kallikrein, Frey and Kraut (Kraut et al., 1928) found an inactivator of this substance in the blood, which blocked the efficacy of kallikrein by being bound to it, the formed complex releasing the substance again on acidification. This led to the discovery of a potent kallikrein inhibitor in bovine organs (Bauer et al., 1929; Kraut et al., 1930) which was later known as Aprotinin or Trasylol.

A further mile-stone in the history of kallikrein-kinin system was the publication by Werle et al., (1937) who found that kallikrein did not act directly but released an effector substance from an inactive precursor in plasma to produce contraction of isolated smooth muscle preparation of guinea pig ilieum.This substance, thought to be a polypeptide, was initially called Dermkontrahieirende substanz (gut contracting substance, substanz D.K). Werle and Berek (1948) later renamed it kallidin, and its inactive precursor kallidinogen. Much later, it was shown by Werle et al., (1961) and by Pierce and Webster (1961) that kallidin, a decapeptide, was the lysyl derivative of bradykinin. Bradykinin was first described by Rocha e Silva et al., (1949), in Brazil. They found that incubating dog plasma with venom of certain snakes and trypsin produced a polypeptide with hypotensive and smooth muscle contracting properties. Kallidin and bradykinin were found to be derived from the same substrate (Werle et al.,1953). The purification and synthesis of these substances, a decade later, confirmed that the trypsin or snake venom - bradykininogen - bradykinin system and kallikrein - kallidinogen - kallidin system were closely related (Elliot et al., 1960a; Boissonass et al.,1960a). The adoption of the generic name "kinins" for the whole group (Schachter and Thain, 1954) led to

the use of the term "kininogenases" for such enzymes as kallikrein, trypsin, snake venom enzyme and bacterial proteases which released kinin from an inactive protein substrate known as "kininogen".

In 1965, a third kinin, met-lys-bradykinin, was found by Elliot and Lewis in acidified ox plasma. It bore one additional methionyl group at the amino end of the kallidin molecule (Erdos 1970). Many different free kinins of non-mammalian origin were found first by Erspamer in amphibians (Anastasi et al., 1966) and then by Schachter in some insect venoms (Jaques and Schachter, 1954). Much later another kind of kinin was found by Greenbaum, liberated from the lysosomes of polymorphonuclear leucocytes (Chang et al., 1972).This polypeptide did not have the classical kinin sequence. It was called `leukokinin' and its precursor leukokininogen. In 1983, Okamoto and Greenbaum found that trypsin in high quantities released a kinin, `T-kinin' (T for trypsin-releasing) and that it constituted 70% of rat plasma kinins. This kinin was found to increase eight to ten fold in cases of acute inflammation (Okamoto and Greenbaum,1986).

The kinins and thus the kallikreins, in minute amounts, controlled essential physiological functions in the body. Excessive kinin formation was prevented by kininases and kallikrein inhibitors. Modern methods of biochemistry and molecular biology have provided us with a detailed knowledge of the kallikrein-kinin system and its components. The vital importance of this system for fundamental mechanisms in biochemistry, pharmacology, cell-biology which are of great interest and practical benefit to medicine has resulted in great involvement in research in this field.

1.2 Chemistry and Characterization of kinins

1.2.1 Bradykinin and its analogues

Plasma kinins are a [illegible] chain polypeptides which resemble bradykinin in [illegible] pharmacological properties (Webster,1966a).The kin[illegible]udes bradykinin, kallidin, methionyl-lysyl-bradykinin, glycyl-bradykinin, leukokinin, T-kinin, and other mammalian and non-mammalian kinins. Bradykinin is a nonapeptide with an aminoacid sequence of N,Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg,OH (Boissonass et al., 1963) and molecular weight of 1131 (Elliot et al.,1960b). Kallidin is a decapeptide having an additional N-terminal lysine residue (Pierce and Webster, 1961; Pless et al, 1962). Acidification of ox plasma to pH 2.5 for several days and subsequent neutralization yeilds a hendecapeptide methionyl-lysyl-bradykinin (Elliot et al.,1963). Two high molecular weight peptides have been isolated from incubates of human kininogen with an enzyme fraction of macrophages and polymorphonuclear leucocytes at pH 4.0 (Chang et al., 1972). Leukokinin M (macrophage enzyme mediated) has twenty five aminoacids and a molecular weight of 2826. Leukokinin PMN (PMN-cell enzyme mediated) has twenty one aminoacids and a molecular weight of 2416.

1.2.2 T-kinin (Ile-Ser-Bradykinin)

Trypsin in large quantities liberates T-kinin (T for trypsin-releasing) along with a small quantity of bradykinin from rat plasma (Okamoto and Greenbaum,1983a).The structure of the undecapeptide is Ile-Ser-Arg-Pro-Pro-Gly-Phe-Pro-Phe-Arg (isoleucyl-seryl-bradykinin) (Okamoto and Greenbaum, 1983b).The two protein precursers which release T-kinin in the presence of trypsin are T-kininogen I and II (Okamoto and Greenbaum 1983a; Okamoto and Greebaum ,1986.) Free T-kinin is released during an inflammatory response into the blood and inflammatory fluid (Barlas et al., 1986).

1.2.3 Other Mammalian kinins.

Werle and Preisser(1956) noticed that some bacterial proteases release a substance from milk which stimulates smooth muscle preparations, a peptide named colostrokinin. Later Guth found that calf saliva or sheep urinary kallikrein liberates colostrokinin from acid-heated bovine colostrum (Guth, 1959; Moriya et al.,1966). Submaxillary, urinary and pancreatic kallikrein, trypsin and chymotrypsin, liberate colostrokinin (Werle and Trautschold, 1960) although serum kallikrein is found to be inactive in these studies. The actions of this peptide are qualitatively similar to bradykinin, but relatively stronger in increasing capillary permeability. Its effects are partially destroyed by trypsin. The molecular weight of this kinin is 2000 and the N- and C- terminal aminoacids are different from those of bradykinin (Yamazaki and Moriya 1969).

1.2.4 Non-mammalian kinins.

Observations of the first nonmammalian kinin, wasp kinin, have been made by Schachter and Jaques. Substances of possible polypeptide nature were detected in venom of wasp (Polistes kinin), hornet and scorpion (Jaques and Schachter, 1954; Holdstock et al., 1957; Diniz and Moura Gonclaves,1960; Bhoola and Schachter, 1962). From the extract of venom of the wasp *Megascolia flavifrons* two kinins, Thr-6 bradykinin and Thr-6-bradykinin-Lys-Ala, have been identified. The bradykinin like effects on a number of vertebrate smooth muscle preparations have been explained by the action of these kinins (Yasuhara et al.,1987). The venom of Phoneutria nigriventor spider induces increase in vascular permeability in rabbit skin by activation of the tissue kallikrein kinin system of rabbit. The oedema induced by this venom is potentiated by the kininase II inhibitor, captopril and inhibited by B_2 receptor antagonists like Hoe 140 (Marangoni et al., 1993). Ornithokininogen has been isolated from chicken plasma. Its molecular weight is estimated as 74,000 and is found to have properties similar to those of mammalian high molecular weight kininogen in terms of aminoacid composition and susceptibility to plasma kallikreins. The structure is determined as Arg-Pro-Pro-Gly-Phe-Thr-Pro-Leu-Arg. It is similar to bradykinin except for substitution of Thr-6 and Leu-8 for Ser-6 and Phe8. It induces contraction of

chicken smooth muscle and has strong hypotensive effect on the chicken (Kimura et al., 1988). Like birds, the snake *Bothrops jararaca* has been suggested to have its own kallikrein-kinin system.The venom causes a fall in the carotid artery pressure of the anaesthetised snake. This effect is tachyphylactic and is potentiated by captopril, a kininase II inhibitor. Incubation of *Bothrops jararaca* plasma releases a substance that produces hypotension and contraction of *Bothrops jararaca* uterus. Like mammalian kinins this substance is dialysable, thermostable in acid pH and is inactivated by chymotrypsin (Abdalla et al., 1989).

Cellular actions of kinins

The cellular actions of kinins are modulated by specific receptors. The kinin receptor stimulation causes activation of several second-messenger systems, such as arachidonic acid products, calcium, cyclic AMP, and cyclic GMP (Freay et al., 1989; Burch and Pasquale, 1990). Kinins also stimulate the secretion of renin from kidney (Beierwaltes and Carretero, 1986), release of vasopressin from the neurohypophysis (Baertschi et al., 1981) and secretion of catecholamines from the adrenal medulla (Staszewska-Barezak and Vane, 1967). The effects of kinins in biological fluids are very short lived because they are rapidly destroyed by kininases. The half-life of bradykinin and lys-braykinin in blood is estimated to be less than thirty seconds (McCarthey et al., 1965; Ferreira and Vane, 1967).

Kinin Receptors

The various physiological effects of kinins are mediated by specific receptors which are widespread and belong to two major categories B_1 and B_2 (Regoli and Barabe,1980). By activating B_1 and B_2 receptors and possibly by other mechanisms (Rhaleb et al.,1988) kinins exert a variety of biological effects. The two receptor hypothesis has been confirmed by data obtained with antagonists. Antagonists for B1 receptors were identified as des-Arg9-bradykinin and des-Arg 10-kallidin. B_2 antagonists were identified by replacing Pro7 with D-Phe in bradykinin (Vavrek and Stewart, 1985). Most of the well characterised actions of bradykinin and kallidin appear to be mediated by activation of the B_2 receptors. These effects include cell retraction, vascular smooth

muscle relaxation, contraction of visceral and bronchial smooth muscle and activation of peripheral afferent C type nerve fibres (Steranka et al., 1989). In contrast to B_2 receptors, B_1 receptors seem to be absent normally, and become evident in some pathological states, particularly in inflammmation and after exposure of the tissue to noxious stimuli (Regoli et al., 1981). The primary endogenous agonist for the B1 receptor is des-arg9-bradykinin, the metabolite that results from the hydrolysis of the C-terminal arginine of the kinin moity. Whereas the B_2 receptor has been cloned, the inflammation induced B_1 receptor has been eluding molecular biologists.

Recent findings suggesting the existence of new receptor types namely, B_3, B_4 and B_5 have been analysed critically, especially with reference to the criteria used for affirming their existence. The pharmacological characteristics of the receptor B_2 show differences between species, and two subtypes B_{2A} (rabbit, dog and possibly man) and B_{2B} (guinea pig, hamster, rat) have been proposed. A third receptor, B_3, has been proposed to explain the unusual behaviour of the trachea of guinea pigs to kinins. But its significance in humans is not known (Regoli et al., 1993).

T-kinin

The vascular responses of T-kinin are similar to bradykinin. It significantly increases the vascular permeability in a dose-dependant manner. Prostaglandin E_1, forskolin and the angiotensin converting enzyme inhibitor SQ 14225, potentiate the T-kinin induced plasma exudation. Hence T-kinin is suggested to have a major role in increasing vascular permeability associated with inflammation (Sugio and Greenbaum, 1988). The diminution of inflammatory response observed following thyroidectomy is suggested to be mediated at least in part by T-kinin. Thyroid hormones increase and hypothyroidism decreases the inflammatory responses. It has been demonstrated that T-kinin is greatly increased and bradykinin slightly increased after thyroidectomy and that T-kinin concentration in T-3 treated thyroidectomized animals is almost restored to control level. The ability of thyroidectomized animals to produce higher plasma T-kininogen compared to euthyroid animals when stimulated by laporotomy has been documented. There is a four

fold increase in T-kininogen production in the former (Bouhnik et al.,1988).

The gene for T-kininogen is not present in human. However, Wunderer et al., (1986) have isolated T-kinin from the ascites fluid of patients with ovarian carcinoma indicating the presence of a protein carrying the T-kinin molecule in the cancer cells.T-kinin has been identified in various human malignant exudates (Wunderer et al., 1990). Yet, it is not known whether the leukokininogen and leukokinin-H released by cathepsin D are the components of the T-kinin system, which has been reported by Wunderer (Grebow et al.,1979; Roffman and Greenbaum,1979). T-kinin probably acts through kinin B_2 receptors in the rat uterus. While the potency of T-kinin is similar to bradykinin the affinity of T-kinin to the receptor is ten-fold lower than that of bradykinin. However, the D-isomer of T-kinin has an affinity greater than that of T-kinin and is more potent in causing contraction. The data from binding studies on T-kinin analogues suggests that the substitution of hydroxyproline for Pro-5 together with the D-configuration at Ile-1 and/or Ser-2 may be useful in the development of T-kinin antagonists (Gao et al., 1993)

Recent studies indicate that in the rat kidney, T-kinin is converted into bradykinin, probably by aminopeptidase M. Kininase I inhibitor, Mergetpa does not affect the conversion while the kininase II inhibitor, BPP 9a increases the proportion of T-kinin converted to bradykinin (Viera et al.,1994).

1.3. Kinin-forming enzymes.

The kinin forming enzymes are also known as kininogenases or kallikreins. They have been regarded as a group of trypsin-like serine proteases that liberate an extremely biologically active peptide, kinin, from plasma kininogens (Webster, 1970). The kininogenases include plasma and tissue kallikreins and T-kininogenases.

Kallikreins

The kallikreins have been separated into two classes - those derived from glandular sources, named tissue kallikrein and those derived from plasma named plasma kallikrein which differ in their molecular

weight, origin, biochemical properties and biological actions on plasma kininogens. All known plasma kallikreins are inhibited by soybean trypsin inhibitor (SBTI) (Werle and Maier, 1952; Bhoola et al., 1960),whereas the tissue kallikreins are not (Back and Steiger, 1968). In addition, the plasma kallikrein, in most species except the guinea pig, releases kinin only from high molecular weight kininogen (Yano et al., 1967), while tissue kallikrein releases kinin from low and high molecular weight kininogen.

1.3.1 Plasma kallikrein.

The kallikreins of all mammalian plasma is seen in the form of an inactive precursor, prekallikrein. It is also known as Fletcher factor, because the deficiency of prekallikrein was first noticed in a patient named Mr. Fletcher (Wuepper, 1973a). Prekallikrein is a single chain glycoprotein synthesised in the liver. It is a member of single gene code (Seidel et al., 1989). It is present in plasma as a complex bound to high molecular weight kininogen (Mandle et al., 1976; Mandle and Kaplan, 1977). Plasma prekallikrein can be activated by Hageman factor and high molecular weight kininogen is converted to active bradykinin (Mandle et al., 1976; Kaplan et al., 1992). Inactive Hageman factor is activated by kallikrein through a positive feedback mechanism (Cochrane et al., 1973). Activated Hageman factor and plasma thromboplastin antecedent (factor XI) circulate bound to high molecular weight kininogen. Inactive factor XI is, therefore, converted to active factor XI_A through high molecular weight kininogen to participate in the intrinsic coagulation pathway (Ratnoff et al, 1961). Prekallikrein can also be activated by acetone (Kraut et al., 1928); caseine (Werle, 1936), trypsin (Werle et al., 1955) and negatively charged surfaces such as glass, kaolin, fused alumina and celite (Margolius, 1963).

In addition to its role as an intermediate in bradykinin generation (Wuepper,1972, 1973b; Weiss et al., 1974; Seito et al., 1974) kallikrein is found to be required for normal functioning of coagulation pathway and plasma fibrinolytic pathway, and chemotaxis (Kaplan and Austen 1972a; Kaplan et al., 1973). Prekallikrein has been identified as the factor (Wuepper, 1972, 1973a) genetically deficient in members of the Fletcher family (Hathaway et al., 1965). It may also function as the co-

factor in the fibrinolytic pathway (Wuepper, 1973b) along with Hageman factor (Iatridis and Ferguson, 1961, 1965; Niewiarowski et al., 1959), the plasminogen proactivator (Kaplan and Austen, 1972b) and plasminogen.

1.3.2 Plasma kallikrein inhibitors

A system in the body having as potent a biological activity on the kininogenase-kinin system requires a regulating mechanism that limits the concentration of the active principles. The inhibition of the proteolytic enzymes by extract of plants (plant .inhibitors) or animal tissues (natural inhibitors) was first recognized by Frey et al., (1930) when he injected blood contaminated urine into anaesthetised dog and found that it was completely inactive on the blood pressure. The general characteristics of the reactions of the proteinase inhibitors with the enzymes are: (1) pH-dependant forming of an enzymatically inactive complex with the enzyme at a definite molar ratio, (2) rapid equilibrium of the reaction, and (3) finally complete recovery of activity of the enzyme after splitting the complex.

Plasma kallikrein is rapidly inactivated by Chloride inhibitor which inactivates both plasma kallikrein and Hageman factor. Further a_2 macroglobin, antithrombin III and a_1 antitrypsin have been identified as plasma kallikrein inhibitors (Fritz et al., 1979). The salivary gland secretion of medicinal leech is found to contain plasma kallikrein inhibitor activity which is capable of blocking the amidolytic activity of the enzyme in an irreversible manner (Baskova et al., 1988).

1.3.3 Tissue kallikreins

Kallikreins derived from glandular sources are generally termed tissue kallikreins. Tissue kallikreins are kinin serine proteases which release kallidin from kininogen substrate (Pierce and Webster, 1961).The occurrance and distribution of tissue kallikrein genes have been evaluated (Drinkwater et al., 1988). Human pancreatic and renal kallikreins possess identical aminoacid sequences and no more than three closely related genes (Baker and Shine, 1985; Schedlich et al., 1987), while in the mouse the kallikrein group of serine proteases consist of twenty four highly homologous genes (Evans et al., 1987). In human, tissue kallikrein gene family comprises the hRkall, hGK-1 and PAS.

These are clustered on the long arm of chromosome 19q13-3-13-4 (Evans et al., 1988; Morris, 1989; Clements, 1994). In mouse and rat kallikrein activity is associated with the highly conserved members of a large multigene family located on chromosome seven (Richards et al., 1982; Mason et al., 1983; Schedlich et al., 1988).

Structure and Specificity

Tissue kallikreins isolated from different exocrine and endocrine glands indicate their initial synthesis as 37,000 or 30,000 dalton prepropeptides which are converted into a 38,000 dalton active form by proteolytic processing and glycolysation. The active kallikrein is capable of binding to an inhibitor to form a 92,000 dalton inhibitor complex. (Chao et al, 1986). Although,they are physico-chemically, enzymatically and immunologically identical, tissue kallikreins perform different functions at different anatomical sites depending on the electrolyte and endocrine control (vanLeeuwen et al.,1984).

Sources

Tissue kallikreins have been detected in pancreas (Frey et al., 1968),gastro-intestinal mucosa (Zeitlin and Smith, 1973; Seki et al.,1972), salivary glands (Bhoola and Ogle, 1966) and renal cortex (Nustad, 1970).They have also been identified in uterus (Marlis et al., 1982), and rabbit foetal placenta (Weerasinghe and Gadsby, 1992).

Pancreatic kallikrein.

All mammalian species so far investigated contain pancreatic tissue kallikrein. In the pancreas, tissue kallikrein is found at the level of rough endoplasmic reticulum and mainly in the zymogen granules of pancreatic acinar cells. They are not found in the cento-acinar cells of duct cells (Amouric et al., 1982). Human pancreatic kallikrein is stable at pH 8 and is rapidly inactivated at pH 2.6. It is a glycoprotein with a molecular weight of 35,000. The enzyme is inhibited by aprotinin but not by ovomucoid, soybean trypsin inhibitor or limabean inhibitor (Amouric and Figarella, 1980).

Urinary kallikrein.

It has been reported that urinary kallikrein is derived mainly from renal cortex (Nustad,1970). Man excretes about 200-210kallikrein units per day (Frey et al.,1968). Although, urinary kallikrein has been reported to be derived mainly from renal cortex, its secretion into the urine is not the exclusive mechanism of excretion of tissue kallikrein into urine. A second mechanism, the renal transfer of endogenous tissue kallikrein from blood into urine can contribute to the urinary kallikrein (Fink and Schleuning, 1982).

Cardiac tissue kallikrein

Tissue kallikrein has been detected in rat cardiac tissue. In addition, the mRNA which codes for glandular kallikrein has been found in the ventricles (Nolly et al., 1992).

Functions

In addition to their classical role as bradykinin-releasing enzymes tissue kallikreins are known to be involved in the post translational modification of protein hormones and growth factors (Weerasinghe and Gadsby, 1992). Recently, the possibility of tissue kallikreins being powerful modulators of blood pressure has been proposed. Transgenic mice overexpressing human tissue kallikrein had been developed.The mice had significantly lowered blood pressure. Administration of aprotinin, a potent tissue kallikrein inhibitor restored the blood pressure back to normal (Wang et al., 1994).

1.3.4 Tissue kallikrein inhibitors

Tissue kallikreins have been regulated by a number of plasma serine protease inhibitors. All substrate analogues occurring within the sequence Ser386-Pro-Phe-Arg-Ser-Val-Gln392 from bovine kininogen are tested for inhibitory activity. The heptapeptide Ac-Ser-Pro-Phe-Arg-Ser-Val-Gln-NH2 is the most effective inhibitor. The N- terminal seryl residue (P4) and the prolyl residue (P3) slightly improve the

inhibitor action. The phenylalanine residue at P2 appears to have a more pronounced effect. The arginyl residue at P1 and seryl residue at P2 appear to be the most important ones in the inhibitory sequence. The valyl residue at P2 and C-terminal glutaminyl residue improve the inhibition.Almost eighty percent of the binding energy of the inhibitors comes from the core sequence Phe-Arg-Ser which occurred between P2 and P1 (Deshpande and Burton, 1992).Inhibitors of kallikrein were first described in many bovine organs such as lung, pancreas and kidney (Kraut et al, 1930). They inhibit tissue kallikrein and a number of other proteases including trypsin (Vogel and Werle, 1973). Another kallikrein binding protein has been identified in the serum, lung and liver of rats. This inhibitor specifically binds to active tissue kallikrein and not to inactive tissue kallikrein and plasma kallikrien. Protein C inhibitor, a relatively nonspecific heparin-dependant serine protease inhibitor present in plasma and urine inhibits the amidolytic activity of tissue kallikrein by formation of PCI- tissue kallikrein complex. This inhibitor may be identical with kallikrein binding protein (Ecke et al.,1992). Kallistatin another member of the serpin (serine proteinase inhibitor) family inhibits human tissue kallikrein by formation of an equimolar complex and by generation of a small carboxyl terminal fragment from the inhibitor due to cleavage at the reactive site by tissue kallik-rein (Zhou et al., 1992).

1.3.5 T-kininogenases

T-kinin is released by acid proteinases of granulomatous tissue in rats with Carrageenin induced inflammation. Two types of acid proteinases seem to be responsible for kinin release. One is identified as Cathepsin-D. Cathepsin-D does not release T-kinin alone but T-kinin containing peptides, T-kinin Leu (Sakamoto et al., 1988) and Met-T-kinin as well (Sakamoto et al.,1987). T-kininogenases like enzyme has been found in rat and mouse submandibular glands and in rat peritoneal white cells. This enzyme belongs to a group of structurally similar yet distinct kallikrein like serine proteases. The purified protein has an apparant molecular weight of twenty-eight thousand. It is kininogenase and not esterase activity that can be enhanced ten-fold in the presence of dithiothreitol (Barlas., 1987).

1.3.6 T-kinin inhibitors

The esterolytic activity of the enzyme is inhibited by soybean trypsin inhibitor and Ap[illegible] whereas lima bean and ovomucoid trypsin inhibitors stimul[illegible]is enzyme is localized at the granular convoluted tubu[illegible] cells in rat submandibular glands (Xiong et al., 1990). Studies on substrate specificity, pH optimum and agents which inhibit T-kininogenase activity indicate that this enzyme is different from plasma and tissue kallikreins, cathepsin D and other known kininogenases. Thus, the rat seems to have a complete T-kininogenase-T-kinin system (Barlas et al., 1989).

1.4 Plasma Kininogens.

1.4.1 a & b High Molecular Weight kininogen and Low Molecular Weight kininogens

Kininogens are extremely versatile proteins which serve a plethora of distinct biological roles and their functional diversity is reflected by a complex multidomain structure. They have multifunctional domains; an aminoterminal domain for cysteine proteinase inhibitor, a bradykinin moity and in the case of high molecular weight kininogen a carboxyl terminal domain for a cofactor of blood coagulation (Kitamura., 1987). Plasma kininogens include low molecular-weight kininogen, high molecular-weight kininogen (Jacobsen,1966a, 1966b; 1966d,) and T-kininogen (Okamoto and Greenbaum, 1983a).

Structure and Specificity

Two kinds of kininogens with different susceptibilities to plasma kallikrein have been found in the plasma of human beings, dogs, rabbit, rat and guinea pig. Kininogen I a glycoprotein with molecular weight 76000 containing 12.6% carbohydrate and 581 aminoacid residues is called high molecular weight kininogen. Kininogen II also a glycoprotein with molecular weight 48000 containing 19.8% carbohydrate and 356 aminoacid residues is termed low molecular weight kininogen (Yang et al.,1971). Pierce, (1970), purified and

distinguished them from each other by DEAF-Sephadex A-50 chromatography and polyacrylamide gel electrophoresis. Plasma kallikrein releases kinin from high molecular weight kininogen by sephadex G-200 gel filtration. Human low molecular weight kininogen is a single chain glycoprotein of molecular weight 68,000 which is converted to a two- chain protein by limited proteolysis with tissue kallikrein to form a heavy chain (molecular weight 62,000) and a light chain (molecular weight 4000). Human high molecular weight kininogen represents a single chain glycoprotein of molecular weight 114,000, which is split into two chains of similar size (H-chain molecular weight 58,000 and L-chain, molecular weight 62,000) by limited proteolysis with tissue kallikrein. Purified kininogens readily form self-aggregates ranging from dimer to hexamer (HMW kininogen) and from dimer to decamer (LMW kininogen) respectively (Mori and Nagasawa., 1981).

Sources of kininogens.

The primary, though not the exclusive source of kininogen are the parenchymal cells of the liver(Chao et al., 1988). Immunohistochemical studies have detected kininogens in the acinar cells of rat submandibular glands (Chao et al., 1988), and in distal tubular and upper collecting duct cells of human kidney (Proud et al., 1981). Presence of kininogen in human platelets has been reported (Schmaier et al., 1986). Yet, it is not known whether kininogen originates from these cells or is taken up from plasma derived body fluids. Immunoreactive kininogen has been localised in the principal cells of collecting ducts and tissue kallikrein has been detected in the connecting tubule cells, segment of the nephron preceding the cortical collecting ducts. This anatomical relationship is in accordance with studies that described intermingling of principal cells and connecting tubule cells where connecting tubules merge into cortical collecting ducts in the human nephron. The relationship between cells that contain tissue kallikrein and its substrate, kininogen, suggest that kinins can be generated in the lumen of distal cortical segments of human nephron.

At least four types of kininogen mRNAs exist in rat liver. Two of them are low molecular weight kininogen mRNA and high molecular weight kininogen mRNA, while the other two are T-kinin encoded mRNA. The two T-kininogen mRNAs dramatically increase after

induction of acute inflammation and T-kininogen turns out to be identical with alpha 1 major acute phase protein.

Functions of kininogens.

Kininogens function as the large precursor molecules to small vasoactive peptides, the kinins. Kinins are released from the single chain kininogens of molecular weight 68000 (low molecular-weight kininogen) and 110,000 (high molecular weight kininogen) via limited proteolysis with specific kininogenases like kallikreins. High molecular weight kininogen and prekallikrein are among the large number of proteins that are involved in the cascades that initiate and propagate blood coagulation. In concert with Hageman factor and factor XI they trigger the intrinsic blood coagulation via the contact activation pathway (vanIwaarden and Bouma,1987). Kininogens are potent inhibitors of cysteine proteinases (Sueyoshi et al.,1985). Structural analysis indicates that the inhibitor domains of kininogen are similar to the low molecular weight cysteine proteinase inhibitors of the stefin and cystanin type. In addition, it has been proposed that the kininogens arise from cystatins by gene triplication (Muller- Esterl et al., 1985).

1.4.1c T-kininogen

T-kininogen is a unique protein forming the substrate for T-kinin,a potent pharmacological mediator of inflammatory response (Okamoto and Greenbaum, 1983a). Unlike high molecular weight kininogen and low molecular weight kininogen, T-kininogen is an acute phase protein that makes up ninety percent of plasma kininogen content following an injection of Carrageenin. This work is confirmed by demonstrating that the mRNA of T-kininogen in rat liver increases following an inflammatory challenge while mRNA of other kininogens does not (Kagayama et al.,1985).It is a thiol protease inhibitor and can inhibit a variety of enzymes including Cathepsin B (Okamoto and Greenbaum, 1983b). It is not a substrate for plasma and tissue kallikreins but it serves as a substrate for thiol activated kininogenases present in rat and mouse submandibular glands (Oh-Ishi et al., 1982). It is synthesised in the liver in enormous quantities following an inflammatory response (Barlas et al., 1987). It is found to be present in normal concentrations in Brown-

Norway Katholeik strain of rats (Sueyoshi et al., 1985). Its plasma level is reduced following administration of anti-inflammatory drugs (Barlas et al., 1986).The female rats have a higher plasma level of T-kininogen than males.Lactating females and new born have very high circulating levels.

1.5. Fate of kinins

1.5.1 Kininases

Kininases are peptidases that inactivate kinins. Bradykinin is susceptible to degradation by a variety of endo and exopeptidases. The main enzymes which metabolize bradykinin are kininase II or angiotensin converting enzyme and kininase I or carboxy-peptidase N (Erdos, 1990). Kininase I family comprises of kininase I-CPN and kininase I-CPM. Other kininases include aminopeptidase P, maprin, endopeptidase 24.15, prolyl endopeptidase, neutral endopeptidase, carboxypeptidase M and diamidase. These peptidases are widely distributed in various tissues and cells in the body and their subcellular locations vary as well. As bradykinin is inactivated when any of its peptide bonds are cleaved all these enzymes qualify as potential kininases in vivo (Seidgel, 1992).The relative importance of each of these peptidases in controlling the kinin levels varies with species, type of biological fluid and tissue site of formation of the peptide.In human the circulating bradykinin is primarily regulated by kininase I-CPN but on endothelial surface, particularly in the pulmonary vascular bed, by kininase II. In contrast the most potent kinin-degrading enzyme in rat plasma is kininase II (Ishida et al,1989).

Circulating carboxypeptidase-N and membrane bound carboxypeptidase -M cleave the C- terminal arginine to produce des-arg9-bradykinin. Angiotensin converting enzyme or kininase II cleaves two inner peptide bonds producing completely inactive metabolites. Aminopeptidases remove the amino terminal arginine and in addition a neutral endopeptidase, that occurs both in circulation and tissues, probably degrades the molecule into even smaller peptide fragments. Two additional endopeptidases designated as kininase A and B have been identified in the synaptic membranes of rabbit brain (Camargo et al., 1973).

1.6 Role of kinins.

1.6.1 Physiological actions of kinin.

Kinins are present as large precursor molecules originating in the liver and contained in high concentrations in the circulating blood. They are released only when plasma or tissue kallikreins are activated by various stimuli. Kinins are released locally to act as autocoids and to be rapidly inactivated either at the site of release or in circulating blood or the kininases of lung. Some of its physiological functions are:

Vasodilation.

In animals and man, bradykinin is one of the most potent vasodilator substances known. The hypotensive effect of kinins are attributed to the peripheral vasodilation and may be the result of a complex mechanism. Kinin receptors are found in the endothelium, smooth muscle membrane, autonomic nerve terminals and in other cells such as mast cells, blood cells and fibroblasts. When activated, these receptors may act directly to modify the cell membrane function or indirectly by promoting the release of other endogenous agents which change the membrane functions and produce vasodilation. B2 receptors have been demonstrated to be present in endothelia where they promote the release of endogenous vasodilators, endothelium derived relaxing factor and prostacycline which act by reducing the tonus of the neighbouring smooth muscle ((D'Orleans et al., 1985: Cahille et al., 1988). However in some vessels, for instance, the renal artery of dogs B1 and B2 receptors appear to be present in the smooth muscle fibre, acting directly by promoting the formation of prostaglandins, which inhibit smooth muscle tone (Rhaleb et al,1988).

Vascular permeability.

In peripheral vessels, kinins act at the capillary and venous level by increasing vascular permeability. The site of action appears to be the endothelial cells which contract in response to kinins and in this way increase the vascular permeability (Haddy et al., 1970).

Venoconstriction.

Kinins act at the venous level by stimulating the venous smooth muscle. Isolated large veins from several animal species have been shown to be sensitive to kinins and to contract in response to bradykinin (Regoli and Barabe, 1980). Receptors for kinins, both B_1 and B_2 types appear to be present in smooth muscle rather than in endothelia, since venous endothelium does not play any major role (DeMay and Vanhoutte, 1982: Seidell and La Rochalla, 1987).These effects are extremely important since kinins are among the endogenous agents involved in the response of tissues to inflammatory stimuli and in allergic reactions (Regoli, 1985; Baumgarten et al., 1985).

Release of endogenous agents.

A variety of endogenous agents are activated by kinins. Synthesis and release of prostaglandins are stimulated by kinins (Nasjletti, and Malik, 1979). Kinins release prostaglandins by activating B_1 and B_2 receptors (Rhaleb et al., 1988). With regard to histamine release, kinins particularly kallidin and B2 receptor antagonists , have been found to be potent histamine releasers in the rat (Johnson and Erdos, 1973; Devillier et al., 1985). This appears to be a nonspecific phenomenon common to peptides containing positively charged aminoacids and is probably not due to activation of receptors.

Bradykinin and Smooth muscle

At extremely low concentrations bradykinin produces contraction and relaxation of smooth muscle in the gastro-intestinal and urogenital tract. At the target organ, bradykinin interacts with discriminator proteins of the plasma membranes and triggers, via changes in certain membrane functions, its biological responses. The binding to the discriminator makes specific demands on the nonapeptide. The binding results from an angular confirmation which existed in the solution. The complete sequence is responsible for the specific confirmation. Consequently, the biological activity of partial sequences is low. The bradykinin induced changes in enzyme activity are connected with other effectors like

prostaglandins and calcium ions. The calcium ion dependance of the effect of bradykinin on guinea pig ileum and rat uterus indicates the importance of these ions as additional second messengers. Bradykinin stimulates the influx of calcium ions into the ileum. It has been found to be ineffective if no extracellular calcium is available (Paegelow et al., 1979; Innis et al.,1981).

1.6.2 Pathological conditions

Diabetes Mellitus

Diabetes mellitus is associated with abnormal renal haemodynamics and renal electrolyte metabolism. Renal kallikrein and its kinin products have a role in renal haemodynamic regulation (Levy et al, 1977). Kallikrein containing cells of the rat and human are adjacent to the glomerular afferent arteriole. Intact glomeruli have specific sites for kinins. Kinins can stimulate eicosanoid production by both afferent arterioles and glomerular mesangial cells. Hence ,kallikrein-kinin system may participate in the tubulo-glomerular feedback mechanisms that regulate glomerular function. Kinins can also cause changes in electrolyte and water transport in renal tubules and other transporting epithelia. Development of systolic hypertension and reduced urinary kallikrein have been reported in diabetes. Early alterations of renal kallikrein kinin system in diabetic states are suggested to contribute to the development of hypertension in diabetes (Pellufo et al., 1992).

Hypertension and cardiac hypertrophy

Kinins are potent vasorelaxant peptides which cause an important contribution to blood pressure homeostasis (Sharma, 1989; 1988a).The functions of kinins in relation to blood pressure regulation are vasodilation, reduction of peripheral resistance and regulation of sodium excretion (Adetuyibi and Mills, 1972). An intrarenal hormone system has been suggested to control water and electrolyte excretion and thus participate in blood pressure regulation (Carretero and Scicli, 1981). Alterations in tissue structure appear in the myocardium in hyertensive states including a remodelling of intramyocardial coronary arterioles and a disproportionate accumulation of fibrillar collagen within their

adventitia and neighbouring interstitial space. These expressions of fibrosis appearing in hypertensive hypertrophic heart are linked with renin-angiotensin system. Bradykinin receptors have been identified in the myocardium. A nonendothelial tissue angiotensin converting enzyme whose binding density is marked in the matrix of heart valves, adventitia and sites of fibrosis has been identified. This enzyme may be responsible for regulating concentration of angiotensin II and bradykinin that govern fibroblast collagen turnover. The cardioprotective effect of bradykinin by inhibition of noradrenaline liberattion mediated by B_1 receptor activation has also been suggested (Chahine et al., 1993).

Inflammation

Kinins are peptide mediators which are produced early in and throughout an inflammatory episode. They are capable of producing all of the observed cellular effects such as vasodilation, venoconstriction and endothelial cell retraction that result in vascular leak and pain. Kinins also have the capacity to release transmiitters from nerve terminals (substance P), stimulate synthesis of cytokines, induce formation of prostaglandins and release endothelium derived releasing factor from endothelial cells. Hence many of the inflammatory and vascular disorders involve kinins in their pathogenesis.

1.7 Antihypertensive agents

Angiotensin converting enzyme inhibitor

Angiotensin converting enzyme inhibitors are established in the treatment of hypertension.The hypotensive effects of these inhibitors like captopril are due to reduction of the peripheral resistance and increased blood flow to the organs. Captopril acts by inhibition of ACE enzyme itself. Some of the antihypertensive effects occur through non angiotensin II mediated mechanisms like decreased kinin degradation leading to enhanced production of vasodilators, arachidonic acid metabolites (Smith et al., 1993). Earlier reports indicating an increase of plasma kinin and decrease in plasma angiotensin are available (Iimura, Shimamoto, 1989).Renal kallikrein kinin system has a contributing role in the hypotensive effect of captopril (Dessi-Fulgheri et al., 1993).

There have been conflicting reports about effects of angiotensin converting enzyme inhibitors on insulin sensitivity and glycemic control. Studies with captopril have been shown to increase insulin sensitivity and that this is due to enhanced glucose uptake into skeletal muscle. Hence, a modest effect of angiotensin converting enzyme inhibitors that they enhance insulin mediated glucose uptake. The mechanism of this effect is likely to be a combination of increased muscle blood flow, local renin-angiotensin system blockade and elevated kinin levels (Donnelley, 1992). Recent reports indicate that the improvement in insulin sensitivity produced by ACE inhibitor is kinin dependant and not on angiotensin II antagonism (Tomiyama et al, 1994).