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CELL MEMBRANE STATUS AND CATIONS IN ESSENTIAL AND MALIGNANT HYPERTENSION

Rhian Touyz

A thesis submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, for the Degree of Doctor of Philosophy

Johannesburg, South Africa
1991

This is to certify that the thesis

"CELL MEMBRANE STATUS AND CATIONS IN ESSENTIAL AND MALIGNANT HYPERTENSION"

presented for the Degree of Doctor of Philosophy in Medicine to the University of the Witwatersrand is my own work, and has not been presented for a degree at any other University.

Unless otherwise stated, all the clinical and biochemical preparations were performed by myself.

Data for the illustrations have been adapted from the literature but the graphics are my own.

The studies in this thesis have been approved by the Human and Animal Ethics Committee of the University of the Witwatersrand. The study in Chapter 13 was also approved by the Animal Ethics Committee of the University of Potchefstroom.

R Touyz October 1991

Rhian M. Touyz

BSc (Hons), MSc (Med), MBBCh

Johannesburg

1991

ACKNOWLEDGEMENTS

Thanks and appreciation are expressed to Professor F.J. Milne for his encouragement, guidance and assistance so readily and generously afforded me during this study. To Professor N. Savage, my sincerest gratitude for her discussion and advice regarding the biochemical aspects of this project. I wish to thank Professor H. Seftel who introduced me to 'the world of hypertension' and who encouraged me to pursue this field of research.

Special appreciation and gratitude to Dr S.G. Reinach of the Biostatistics Department (Medical Research Council) for his expertise and invaluable help with respect to the statistical analysis used in this study.

Dr P.R. Marshall is thanked for breeding and handling the DOCA-salt SHR and Dr N. Butkow for his assistance with the spectrofluorometric measurement of platelet free calcium.

Professor A. Meyers is thanked for making laboratory facilities available to me.

In the superb preparation of this manuscript I am indebted to Ms J. Zock.

Thanks are expressed to the many patients and volunteers who so willingly participated in this study.

Sincerest thanks to my parents who gave me the opportunity to proceed thus far. Finally I would like to express my appreciation to my husband Louis and my children Joshua and Sarah for their endless support and encouragement during the preparation of this thesis.

ABSTRACT

Abnormal cell membrane function and altered cellular cation homeostasis have been implicated in the pathogenesis of essential hypertension. The role of these factors in the aetiology of malignant hypertension is unknown.

Cell membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities and cell membrane calcium binding were studied in platelets (which resemble vascular smooth muscle) and erythrocytes of black and white essential hypertensive patients and normotensive controls. Serum, platelet and erythrocyte concentrations of magnesium, calcium, sodium and potassium were also determined. In the black hypertensive group, platelet and erythrocyte membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were significantly depressed. Platelet sodium and calcium were significantly increased and serum magnesium, serum potassium, platelet magnesium and erythrocyte magnesium significantly decreased in the black hypertensive subjects. In the white hypertensive patients, platelet sodium and calcium were significantly raised and platelet magnesium significantly decreased. There were no significant differences for any of the ATPases studied between the white normotensive and hypertensive groups. Cell membrane calcium binding was altered in the black and white hypertensive subjects.

Cellular cation status and cell membrane ATPase activity

were also studied in black malignant hypertensive patients. Platelet and erythrocyte membrane Ca^{2+}-ATPase and Mg^{2+}-ATPase activities, erythrocyte membrane Na^{+}-K^{+}-ATPase activity and intracellular magnesium were significantly lower in the malignant hypertensive group compared to the black essential hypertensive group. Platelet calcium was significantly increased in the malignant hypertensive subjects.

The effects of magnesium supplementation on blood pressure in 10 week old Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were determined for 18 weeks. Oral magnesium supplementation retarded the development of hypertension in SHR.

Cell membrane function (muscle membrane Na^{+}-K^{+}-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities) and serum and erythrocyte magnesium, calcium, sodium and potassium concentrations were determined at various stages in the development of experimental malignant hypertension. Ten week old SHR and SHR treated with deoxycorticosterone acetate (DOCA) and 1% NaCl drinking water were studied weekly for 14 weeks. Malignant hypertension developed in the DOCA-salt SHR. In the premalignant phase serum and erythrocyte magnesium and muscle membrane ATPase activity were significantly lower in DOCA-salt SHR compared to SHR. During the late premalignant and malignant phases, intracellular calcium and sodium were significantly increased in the DOCA-salt SHR.

Data from this study suggest that black essential hypertensive patients have widespread magnesium changes with associated cell membrane ATPase depression and altered cell membrane calcium binding. The cellular changes in white hypertensive patients are due to other factors. These results suggest racial differences in cell membrane function in essential hypertension. In malignant hypertension the cellular abnormalities may be more severe than in benign hypertension. Magnesium supplementation may have a protective effect in the development of hypertension.

Portions of the work included in this dissertation have been published in the following papers:-

Touyz RM, Milne FJ. Some cellular events in hypertension - an overview. Cardiovasc J SA 1990; 1(3):163-171.

Touyz RM. Monovalent and divalent cations in the treatment of essential hypertension. Hosp Med 1991; XIII(1):27-29.

Touyz RM, Milne FJ. Cellular biochemical events in hypertension. J CME 1991; 9(1):43-55.

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CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

1.1 Definition and classification of hypertension

1.1.1 Definition of hypertension

Hypertension, one of mankind's most common diseases is the persistent elevation of arterial blood pressure. This concept was defined almost fifty centuries ago in the Chinese medical treatise attributed to Huang Ti (2698-2598 BC), where blood pressure was linked to diseases of the heart, kidneys and brain (Onesti et al, 1973). Blood pressure was first measured in 1733 when Reverend Stephen Hales performed his classic experiments on the horse (Willins and Keys, 1941; Backer, 1953). In the late 1800's, Akbar Mahomet at Guy's Hospital first described what is now termed 'essential hypertension' (Mahomet, 1881).

Elevated blood pressures are pressures that are abnormally high for the population. To speak of elevated arterial pressure implies that a normal baseline blood pressure is known. Sir George Pickering (Pickering, 1990) emphasised that there is no dividing line between normal and high blood pressure. Rather the relationship between arterial pressure and morbidity is quantitative:- the higher the blood pressure, the worse the prognosis. Blood pressure is a biophysical characteristic whose distribution shows no

natural subdivision into specific groups: it is determined by multifactorial variables. Some researchers believe that hypertension is a specific disease entity determined by monogenic inheritance where two groups exist - those whose pressures increase with age and those whose pressures do not increase with age (Tarazi and Ray, 1979). These divergent opinions formed the basis of the famous Pickering-Platt dispute (Swales, 1986). It is not generally accepted that hypertension is inherited polygenically.

Because of the problems that exist in defining hypertension, arbitrary levels have been used to indicate those individuals who have an increased risk of developing a morbid cardiovascular event, (left ventricular hypertrophy, myocardial infarcts, strokes and hypertensive encephalopathy) and/or those who will benefit from medical treatment. Despite the arbitrariness of dealing with specific values, there is a diagnostic, prognostic and therapeutic need for setting levels. The World Health Organisation has based its definition on consensus. In all populations, blood pressure values have a bell shaped distribution, skewed to the upper end. The discrete categories of 'hypertensive' and 'normal' levels of blood pressure are useful and practical simplifications, but it should be emphasised that it is a quantitative entity.

Most researchers and official bodies have defined elevated

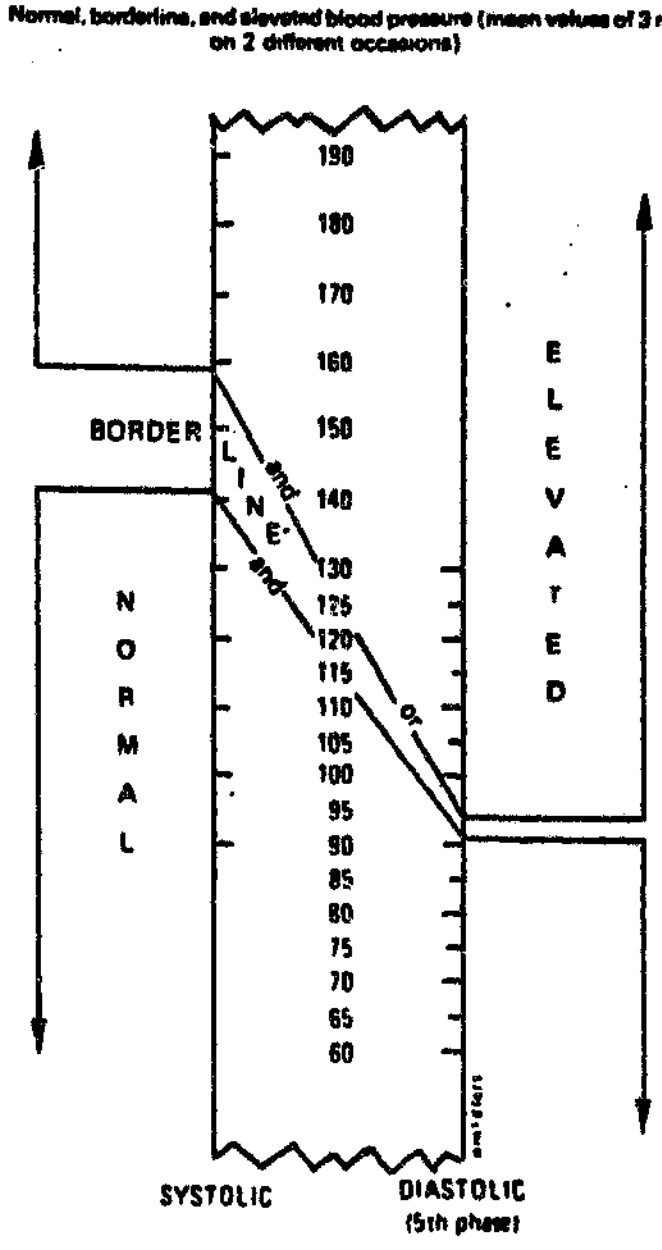


Figure 1.1: Normal, borderline and elevated blood pressure. (WHO 1978)

blood pressure as systolic greater than or equal to 160 mmHg and/or diastolic blood pressure (5th phase) greater than or equal to 95 mmHg, with normal systolic and diastolic blood pressures being less than 140 mmHg and 90 mmHg respectively (WHO, 1978). The pressure values between normal and elevated are defined as borderline (Figure 1.1). These levels apply to both sexes at any age.

1.1.2 Classification of hypertension

Hypertension may be classified according to type and degree.

1.1.2 (i) Classification by type

There are two major types of hypertension - essential (primary) and secondary. Essential hypertension is established by exclusion of identifiable secondary causes, and constitutes 90 to 95% of adult hypertension. The remaining 5 to 10% of hypertensive adults have secondary hypertension, where the cause is known and often reversible. Classification based on type of hypertension is presented in Table 1.1.

Table 1.1: Classification of hypertension based on type (Pickering, 1968; Tarazi and Ray, 1979).

A. Hypertension in which both systolic and diastolic pressures are raised

I. Essential hypertension
1. labile
2. established

II. Secondary hypertension: hypertension due to a known disease.

1. Diseases of the kidneys and urinary tract

i) Renal diseases - glomerulonephritis
- chronic pyelonephritis
- polycystic kidneys
- obstructive uropathy
- diabetic glomerulosclerosis
- interstitial nephritis
- connective tissue diseases
- renal tumours
- renal amyloidosis
- hereditary, radiation nephritis

ii) Renal arterial diseases - fibrous dysplasias
- atherosclerosis
- embolic obstruction
- traumatic obstruction or occlusion

iii) Compression of the kidneys - perinephritis
- perirenal haematoma

2. Endocrine hypertension

i) Catecholamine excess - phaeochromocytoma

ii) Steroids - mineralocorticoid excess - primary aldosteronism
- glucocorticoid excess - Cushing's disease

iii) Oral contraceptives

iv) Conditions associated with hypertension - thyrotoxicosis, myxoedema, acromegaly

3. **Neurogenic hypertension**
4. **Coarctation of the aorta**
5. **Hypertension of pregnancy**
   i) preeclampsia
   ii) eclampsia

B. Systolic hypertension in which the systolic pressure only is raised

1. **Increased stroke output of the left ventricle**
   - complete heart block
   - aortic incompetence
   - arteriovenous fistula
   - patent ductus arteriosus
   - Pagets disease of bone
   - thyrotoxicosis
   - fever
   - pregnancy
2. **Increased rigidity of aorta due to degenerative disease of the wall (atherosclerosis)**
3. **Decreased capacity of aorta in coarctation**

1.1.2 (ii) Classification by severity

Hypertension can be classified according to the severity of the elevated blood pressure into borderline, mild, moderate and severe. The arbitrary guidelines are presented in Table 1.2.

Table 1.2: Severity of hypertension

Severity	Diastolic BP	Systolic BP
Borderline	84-89 mmHg	128-146 mmHg
Mild	90-104 mmHg	147-159 mmHg
Moderate	105-114 mmHg	160-180 mmHg
Severe	> 114 mmHg	> 180 mmHg

The benign and malignant phases of hypertension

The benign phase usually occurs in older individuals in whom the pressures are not severe. The patients condition remains relatively stable for many years, and death if it is related to hypertension, is due to cardiac failure or stroke (Tarazi and Ray, 1979).

The malignant phase occurs at any age and in either sex. It is more common in the young than in the old and is characterised by severe hypertension, neuroretinopathy and by a rapid decline in renal function. Unless the blood pressure is reduced at an early stage, the condition is fatal within a few months or years. The causes of death include renal failure, left ventricular failure or cerebral haemorrhage. The pathological lesion of malignant hypertension is fibrinoid arteriolar necrosis (Houston, 1989) (See Chapter 12 for detailed definition of malignant hypertension).

1.2 Pathogenesis of hypertension

The pathogenesis of essential hypertension is multifactorial and highly complex. The mean systemic arterial pressure varies directly with the cardiac output and total peripheral resistance:-

> Mean systemic arterial pressure = cardiac output (CO) x total peripheral resistance (TPR).

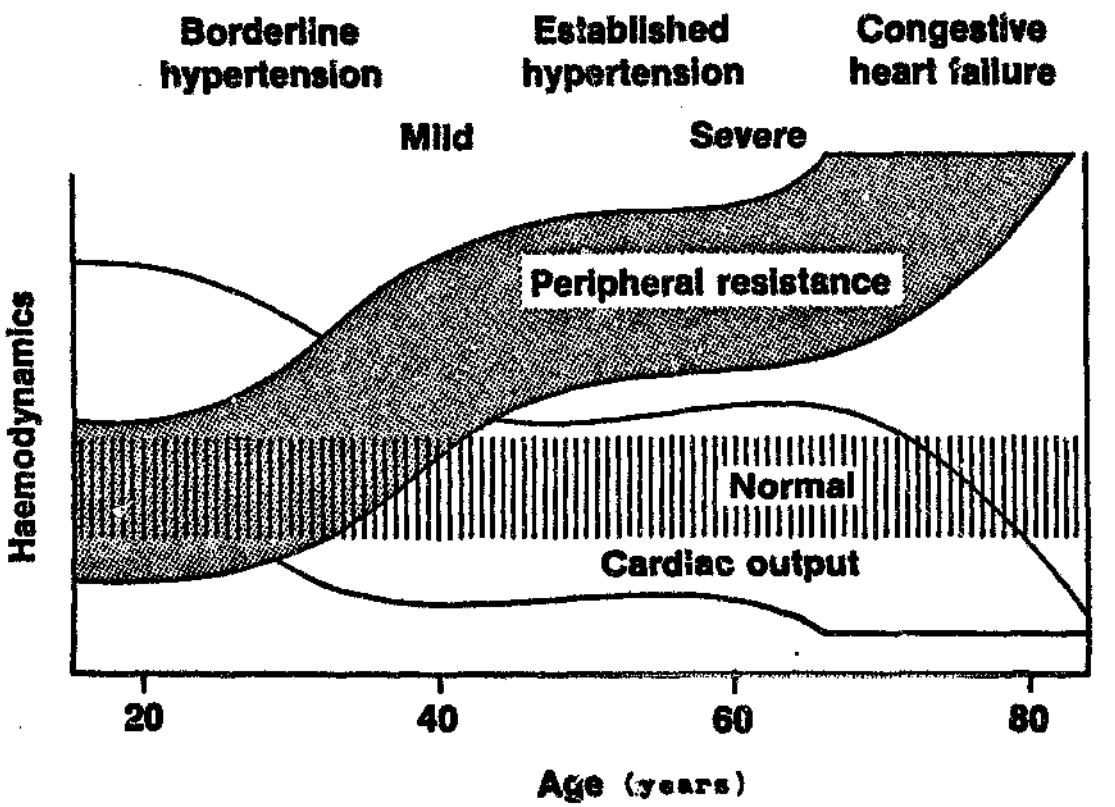


Figure 1.2. Changes in cardiac output and total peripheral resistance as hypertension progresses
(Eich 1962; Julius et al 1971; Frohlich 1987)

1.2.1 Cardiac output in hypertension

Cardiac output, the product of heart rate and stroke volume, is elevated in the early phases of hypertension and normalises in established hypertension (Frohlich, 1987) (Figure 1.2). The initial increase in cardiac output is associated with a normal or decreased total peripheral resistance (Julius et al, 1971). With time the arterioles constrict in an autoregulatory manner and peripheral resistance increases (Eich, 1962). The increase in peripheral resistance may result in a return of cardiac output to its original level. The initial elevation of blood pressure produced by the increase in cardiac output is now sustained by arteriolar vasoconstriction. Not all hypertensive patients exhibit this haemodynamic pattern. Werko and Lagerlof (1949) and Bolomey et al (1949) reported a group of hypertensive patients in whom the cardiac index was elevated with normal or low total peripheral resistance. Frohlich et al (1967) found cardiac output consistently higher in patients with defined renovascular hypertension.

Although these problems relating to the haemodynamics of hypertension have not been settled, a review of the data suggests that in established essential hypertension, cardiac output is normal with total peripheral resistance being the main determinant of increased blood pressure. In the earlier studies the hypertensive patients with low

peripheral resistance and increased cardiac output may not have had established essential hypertension.

1.2.2 Factors determining blood viscosity

Blood viscosity at a specific temperature depends on the plasma viscosity, cell concentration, cell aggregation and cell deformability (Chabanal and Chien, 1990). Many reports have documented that blood viscosity is increased in patients with essential hypertension compared to age-matched normotensive subjects (Letcher et al, 1983; Lorient-Roudaut et al, 1987; Weihmayr et al, 1987). Blood viscosity and blood pressure are significantly correlated (Chien, 1986). Factors contributing to the increased viscosity in hypertensive patients include elevated total plasma protein, increased fibrinogen (Letcher, 1983), increased haematocrit (Lowe et al, 1985), increased erythrocyte aggregation (Petralito et al, 1985) and functional abnormalities of erythrocytes (Orlov and Postnov, 1982; Cherubini et al, 1987).

The abnormalities in blood viscosity, together with alterations in cardiovascular function and structure, may play a role in the pathogenesis of hypertension.

1.2.3 Peripheral resistance

Increased total peripheral resistance is the major

haemodynamic feature of established essential hypertension. The importance of this was stressed by Page (1987). "If a single problem in the field of hypertension had to be given greatest priority, development and maintenance of increased peripheral resistance in hypertension would rank highest".

Peripheral resistance is regulated by many factors, but the most important of these is vascular smooth muscle tone and contractility.

1.2.3.1 Vascular smooth muscle in hypertension

1.2.3.1 (i) Smooth muscle growth

A universal feature of all arteries in hypertension is that their walls are thicker than normal. This reflects an increase in the synthetic function of the smooth muscle. Folkow (1982) demonstrated that the increased wall thickness contributes to the vascular hyper-reactivity of hypertension. The thicker wall confers a mechanical advantage of the vascular smooth muscle in its vasoconstrictor action. The biochemical and cellular mechanisms involved in vessel wall thickening are complex, but the process comprises both an increase in the number (hyperplasia) and size (hypertrophy) of the vascular smooth muscle cells (Seidel, 1987). Some cells may have an increased number of nuclei as well (Owens et al, 1981).

1.2.3.1 (ii) Vascular smooth muscle tone

Ruegg (1971) defined 'tone' as "economically maintained tension, calcium and energy dependent but not necessarily associated with tetanic (neurogenic or myogenic) activity". The economy of tension maintenance is inversely related to the speed of contraction - slow muscles are more 'tonic' than fast muscles. The tonic features are inversely related to myosin-ATPase activity and directly related to the length of the thick myosin filaments that contrast with a sliding filament mechanism. The tonic features of smooth muscle are due to modifications of this mechanism. Myogenic tone can be modified by nerves, hormones, ions, stretch and slow (generation) waves (Mellander and Johansson, 1968). The endothelium and its factors play an important role in modulating smooth muscle tone (Vanhoutte, 1988; Luscher, 1990). The primary mechanism regulating tone and peripheral resistance is contraction of the vascular smootn muscle and the basic determinant of myogenic contraction is calcium.

1.2.3.1 (iii) Calcium and vascular smooth muscle contraction

A number of mechanisms of vascular smooth muscle contraction have been described. Some of these mechanisms are dependent on intracellular calcium concentration while others are not. The major intracellular mechanisms of

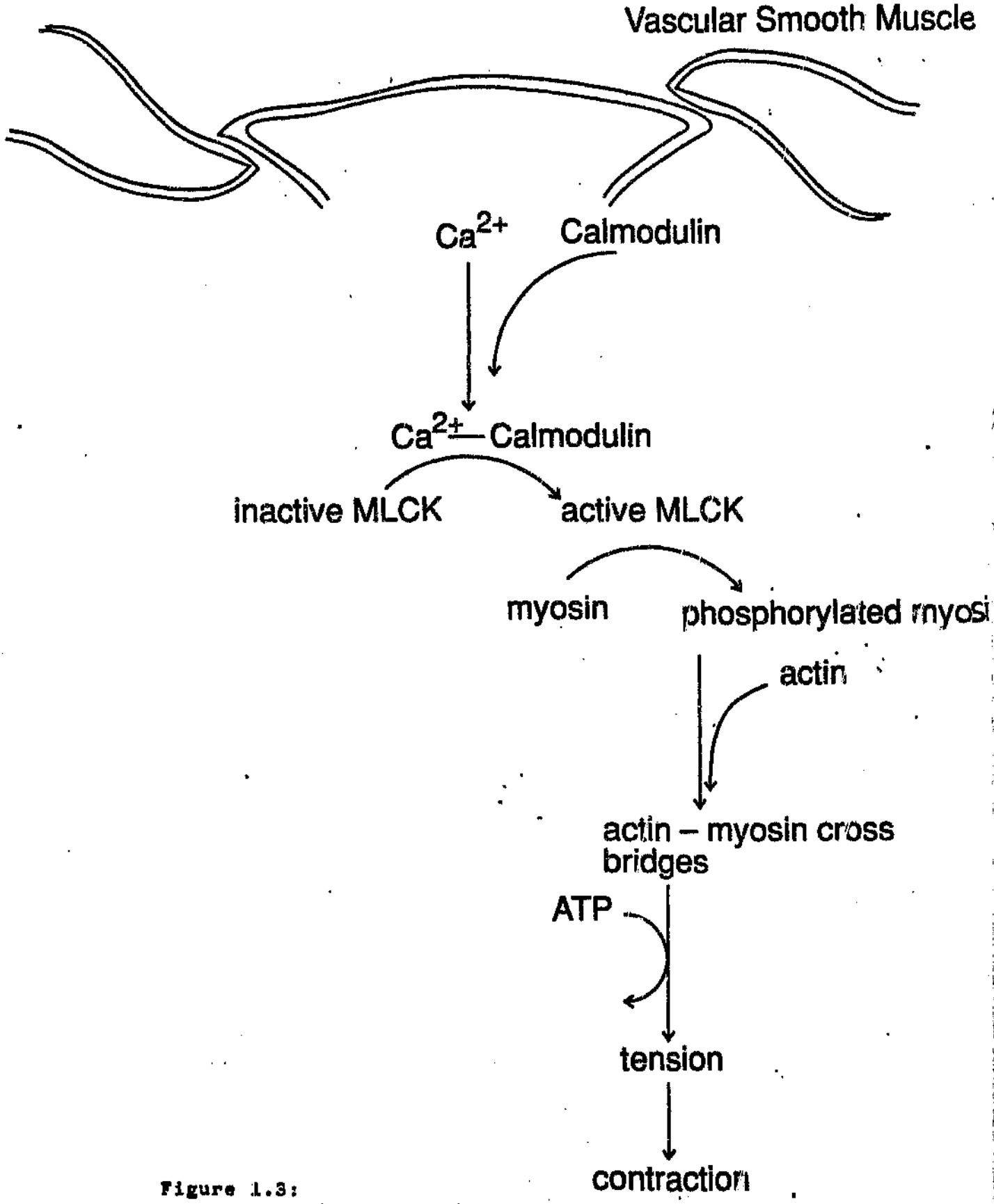


Figure 1.3:
Cascade of enzymatic reactions leading to vascular smooth muscle contraction. MLCK = myosin light chain kinase;

excitation-contraction coupling in vascular smooth muscle include:-

i) intracellular calcium concentration-dependent myosin light chain phosphorylation,

ii) intracellular calcium concentration-dependent regulation of contractile force by caldesmon, and

iii) regulation of actin-myosin interaction by the action of calponin and protein kinase C activation.

i) Myosin light chain phosphorylation

Within the smooth muscle cell, calcium stimulates phosphorylation of myosin, resulting in myosin-actin interaction, splitting of adenosine triphosphate (ATP) and muscle shortening (Khalil et al, 1987; Hartshorne, 1982) (Figure 1.3). Calcium first binds to the acid protein calmodulin, which has four calcium binding sites. At least three sites need to be occupied by a calcium ion before calmodulin will activate myosin light chain kinase (Bolton, 1986; Ishikawa and Hidaka, 1990). The calcium-calmodulin complex activates myosin light chain kinase, the enzyme responsible for phosphorylation (Kamm and Stull, 1985). This kinase can phosphorylate the myosin light chain in two ways - monophosphorylation at serine 19 (the usual way) or diphosphorylation at serine 19 and threonine 18 (Colburn et

al, 1988). Phosphorylation of serine 19 increases the activity of actin-activated myosin magnesium adenosine triphosphatase (Mg^{2+}-ATPase). The phosphorylated myosin forms cross bridges with actin, and the sliding of actin over myosin filaments by cross-bridge cycling, with ATP splitting, results in tension generation and consequent muscle contraction (Murphy et al, 1983). Dephosphorylation occurs by myosin light chain phosphatase (Murphy et al, 1983).

ii) Caldesmon

Caldesmon is a 145000 dalton actin-myosin stabilising protein. It binds to filamentous actin in the absence of calcium, resulting in inhibition of actin-myosin interactions. It binds to calmodulin in the presence of calcium, thereby removing the inhibition of actin-myosin interaction (Campbell, 1987). It can also inhibit the actin-activated myosin Mg^{2+}-ATPase in the presence and absence of calcium (Ngai and Walsh, 1987). Phosphorylation of caldesmon by a calcium-calmodulin-dependent kinase, reverses the inhibitory action of actin-activated myosin Mg^{2+}-ATPase (Ngai and Walsh, 1987).

iii) Calponin

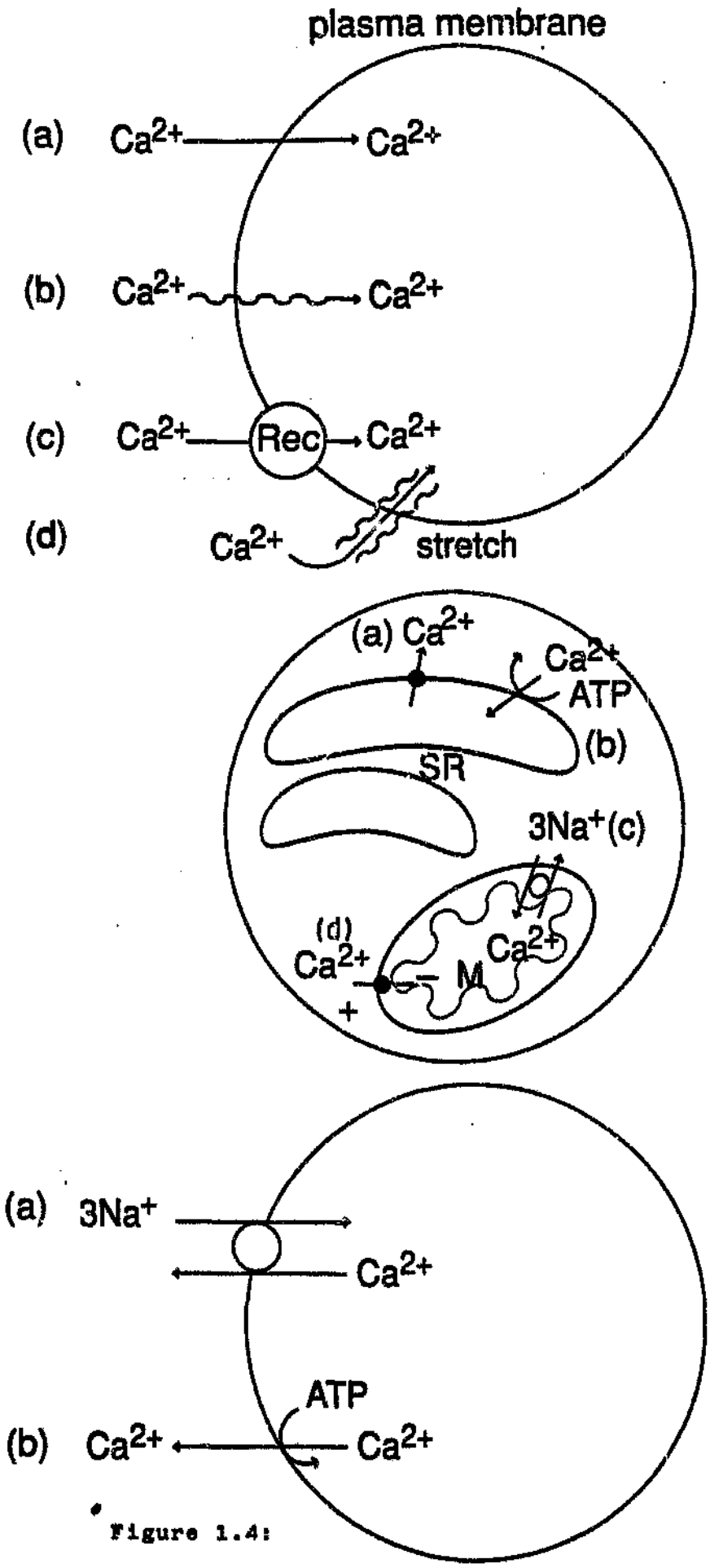
This is a basic calcium binding protein with a molecular weight of 34000 daltons. It is thought to play a role

either as a troponin T-like molecule or as a phosphoprotein in regulating the thin filament of smooth muscle (Takahashi et al, 1988). Calponin has an inhibitory effect on actin-activated myosin Mg^{2+}-ATPase activity in the presence of tropomyosin (Winder and Walsh, 1990). When phosphorylated, by protein kinase C or caldesmon kinase, the inhibitory effect of calponin on the actin-activated myosin Mg^{2+}-ATPase is abolished (Winder and Walsh, 1990). The exact role of this newly discovered protein needs to be elucidated.

Whatever the mechanism of contraction, calcium is a central and vital regulator of vascular smooth muscle contractility and a rise in intracellular calcium concentration is now regarded as one of the most important determinants of the tension developed by the contractile proteins (Morgan and Suematsu, 1990).

1.2.4 Vascular smooth muscle calcium metabolism

As the central ion in the control of vascular smooth muscle contraction, calcium may be involved in the pathogenesis of essential hypertension. An understanding of the general principles of cellular calcium metabolism and its relation to vascular smooth muscle contractility is pivotal to the pathophysiology of hypertension, because many cells, tissues and organs use calcium as a positive intracellular messenger.



i) Ca^{2+} entry into the cell

(a) steady Ca^{2+} influx

(b) Voltage dependent influx

(c) Receptor operated channel

(d) stretch activated channel

ii) Ca^{2+} mobilisation within the cell.

SR = sarcoplasmic reticulum

(a) IP_3 activated Ca^{2+} efflux

(b) SR Ca^{2+} – ATPase

M = mitochondria

(c) M. Na^{+} – Ca^{2+} exchange

(d) Ca^{2+} uniporter

iii) Ca^{2+} extrusion from the cell.

(a) Na^{+} – Ca^{2+} exchange

(b) Ca^{2+} – ATPase

Figure 1.4:

Pathways for the transport of calcium across cellular membrane

Many complex mechanisms are involved in maintaining serum levels of ionised calcium in the millimolar range. Similarly, complex systems maintain intracellular calcium concentrations at about 100 nM. Hence, a 10000-fold gradient of ionised calcium across the cell membrane is present and is functionally utilised by the cell as a mechanism of signal transduction. Cellular calcium homeostasis involves mechanisms that allow calcium to enter the cell, mobilise within the cell and exit from the cell.

1.2.4.1 Calcium entry into the cell

Smooth muscle cell membranes possess at least four different channels through which calcium enters the cell. These channels include the voltage gated channels, receptor-operated channels, calcium-leak channels and stretch activated channels.

i) Voltage-gated channels (potential-sensitive calcium channel) (Figure 1.4)

There are two components of voltage-gated calcium currents in vascular smooth muscle - one of which is activated by relatively weak depolarisations and inactivates rapidly while the other is activated by relatively strong depolarisations and inactivates slowly (Sturek and Hemsmeyer, 1986; Friedman et al, 1986). These two components are referred to as rapidly inactivating and

slowly inactivating. The two types of current may also be distinguished by their sensitivity to various organic calcium agonists and antagonists and their ability to conduct divalent cations. The slowly inactivating current is modulated by dihydropyridine agonists and antagonists and is more permeable to barium than to calcium. The rapidly inactivating current is insensitive to the dihydropyridine agents, and is equally or less permeable to barium than to calcium (Khalil et al, 1990).

The voltage-gated channels are opened by depolarisation of the cell following high extracellular potassium concentration. These channels are blocked by calcium channel antagonists (Bolton, 1986).

ii) Receptor-operated calcium channels (Figure 1.4)

Calcium and other ions enter the cell via these channels following activation of specific receptors by agonists. These are ATP operated channels and differ from voltage-dependent channels. They are insensitive to blockade by nifedipine and cadmium, they can be activated at negative holding potentials and they are rapidly desensitised by ATP (Benham and Tsien, 1987; Khalil et al, 1990). The agonist may induce a structural change in the receptor which then reveals a patent channel in the receptor molecule (Benham and Tsien, 1987).

iii) Stretch-activated channels (Figure 1.4)

As early as 1902, it was suggested that stretch is a stimulus for smooth muscle contraction (Bayliss, 1902). This mechanism is dependent on extracellular calcium. Stretch can stimulate calcium influx and it can open non-specific cation channels in smooth muscle membranes (Laher et al, 1987; Kirber et al, 1987). Although the exact mechanism of activation of the stretch-sensitive channels is unclear, the endothelium may play an intermediary role (Katusic et al, 1986).

iv) Calcium leak channel (Figure 1.4)

Calcium leak is defined as the calcium entry pathway that is not increased on stimulation of smooth muscle by depolarisation, agonists or stretch (Khalil et al, 1990). Increased hydrogen ion concentration and inorganic polyvalent cations (lanthanum, cobalt) can block most of the calcium leak (Van Breemen et al, 1972). The magnitude of the calcium leak into smooth muscle cells is large and is compensated by calcium sequestration into the sarcoplasmic reticulum and by extrusion into the extracellular space.

Activation of the voltage-gated and receptor operated channels allows an initial burst of calcium influx which then rapidly declines, leaving a residual steady-state

calcium inflow. The magnitude of this steady-state calcium influx is relatively small. The calcium leak pathway allows a continuous entry of calcium into the cell.

1.2.4.2 Intracellular calcium stores

Smooth muscle tissues contain about 1-2 mmol calcium per kilogram of cell (Van Breemen et al, 1966; Bolton, 1986). The free ionised calcium concentration within the cells is around 0,1 /umol calcium per kg of cell (Bolton, 1986). Recent studies, using fluorescent dyes, have reported intracellular free calcium concentrations as 100 nm (Erne et al, 1984). Since the ionised level changes with relatively little change in the bound calcium, the calcium stores within the cell are important and potential sources of calcium for contraction.

1.2.4.2 (i) Intracellular calcium may be ionised or non-ionised

Non-ionised calcium occurs in at least three forms:-

i) calcium bound to molecules
ii) calcium bound to compounds such as calmodulin
iii) calcium contained within membrane-bound structures, such as mitochondria, sacs of the sarcoplasmic reticulum and possibly the nucleus.

The calcium bound to molecules accounts for some of the

slowly exchangeable calcium in the cell. The membrane- and compound bound calcium is loosely bound and can dissociate quickly.

The non-ionised calcium constitutes an intracellular chemical buffer to changes in the free cytosolic ionised calcium.

1.2.4.2 (ii) Mobilisation of intracellular free calcium (Figure 1.4)

Studies have documented that in the absence of extracellular calcium or if calcium influx is inhibited, agonists can still produce a transient contraction of vascular smooth muscle (Somlyo et al, 1971; Saida and Nonomura, 1978). This indicates that calcium release from intracellular stores (sarcoplasmic reticulum and mitochondria) may contribute to the myogenic activation.

Calcium release from the sarcoplasmic reticulum can be triggered by two intracellular second messengers. These messengers are calcium and 1,4,5-inositol trisphosphate (IP_3) and calcium-induced calcium release from the sarcoplasmic reticulum.

1.2.4.2 (ii) a. Calcium induced calcium release

Khalil et al (1987) proposed that calcium release from the

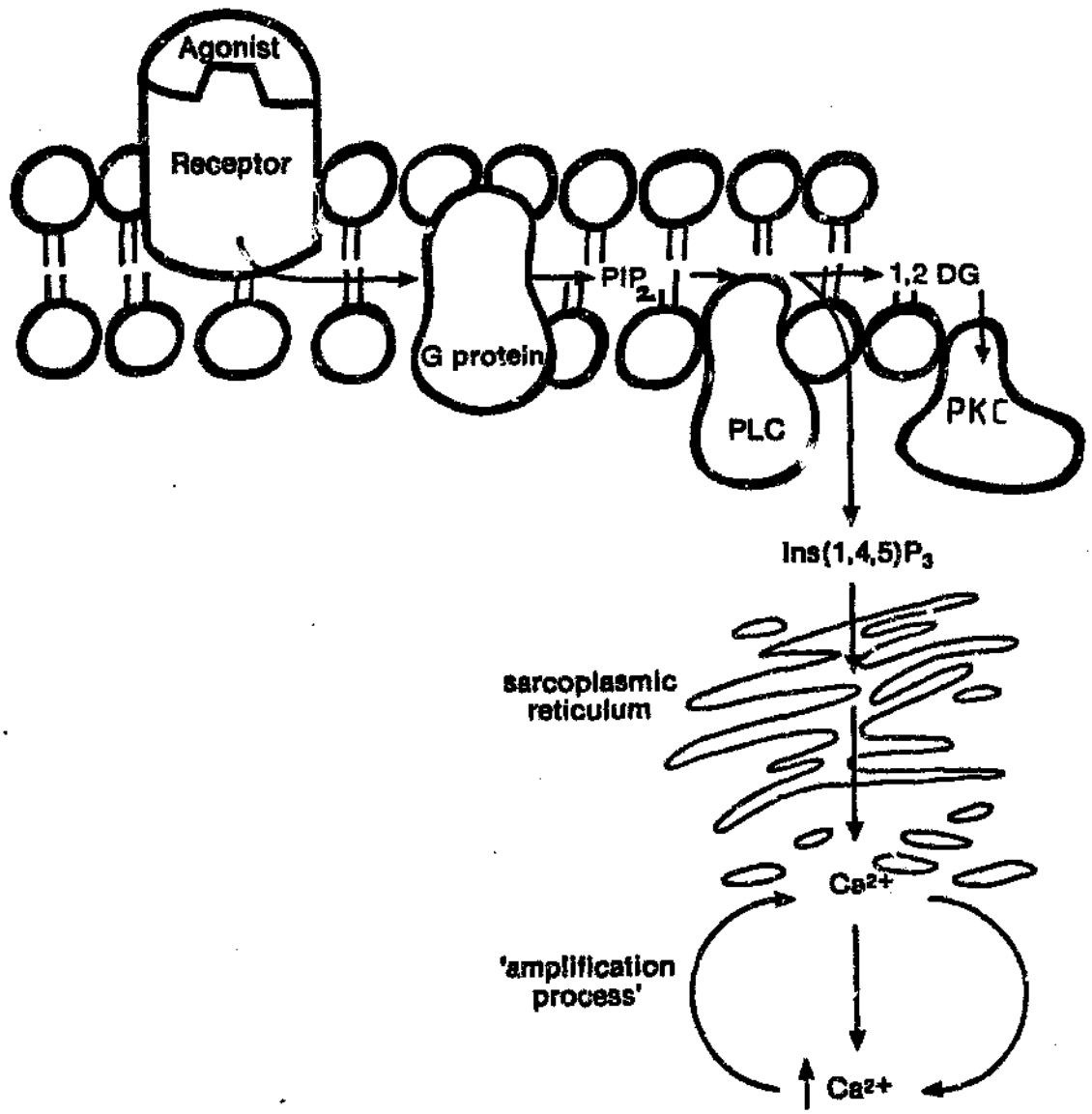


Figure 1.5. Calcium induced calcium release.

PLC= phospholipase C; PKC= protein kinase C; $Ins(1,4,5)P_3$ = inositol trisphosphate; PIP_2 = phosphatidyl inositol bisphosphate; 1,2 DG = 1,2 diacyl glycerol

sarcoplasmic reticulum is influenced by the level of cytoplasmic calcium. Calcium is mobilised from the sarcoplasmic reticulum by IP_3. The released calcium in turn stimulates further calcium release, thereby setting up an 'amplification or regenerative' process (Figure 1.5). The importance of this mechanism under physiological conditions is unclear.

1.2.4.2 (ii) b. Inositol trisphosphate-induced calcium release from the sarcoplasmic reticulum

The inositol lipids, a quantitatively minor fraction of membrane phospholipids, are located predominantly in the inner lamella of the plasma membranes. Phosphatidyl-inositol (Ptd-Ins) constitutes the major portion of these lipids. The phosphoinositide pathway, is regulated by specific proteins (Berridge, 1985). The pathway is stimulated when specific agonists or hormones bind to cell membrane receptors. These receptors (usually α_1 adrenergic type) are coupled to guanine nucleotide binding proteins (G protein) (Cockroft and Gomperts, 1985). The G protein is also coupled to a specific phosphodiesterase, phospholipase C (PLC), which cleaves the membrane bound lipid phosphatidyl-inositol 4,5-bisphosphate (Ptd-Ins 4,5 P_2) (by hydrolysis) into two compounds with different second messenger functions (Hanley and Steiner, 1989). The water soluble product inositol 1,4,5-trisphosphate (Ins 1,4,5 P_3) is released into the cytoplasm, whereas the hydrophobic

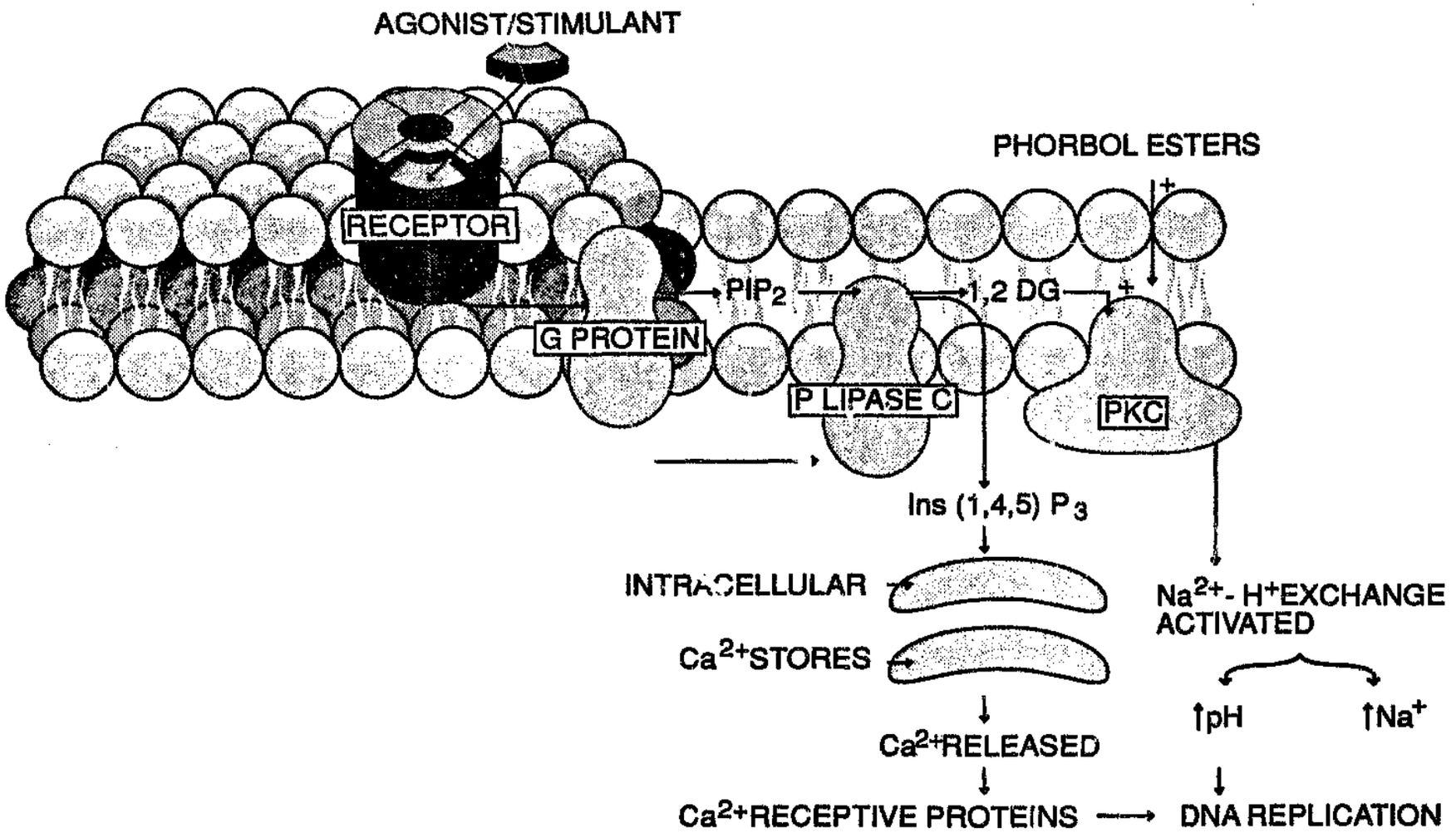


Figure 1.6;
Schematic representation of the major phosphoinositide lipids and their products of hydrolysis. P-lipase C = phospholipase C, PIP_2 = phosphatidylinositol 4,5 bisphosphate; 1,2 DG = 1,2 diacylglycerol, PKC = protein kinaseC; Ins (1,4,5) P3 = inositol 1,4,5 trisphosphate

product 1,2 diacylglycerol (1,2-DG) remains fixed to the plasma membrane (Figure 1.6).

Diacylglycerol has one primary function - it acts as a cofactor, with calcium, in activating an enzyme called protein kinase C (PKC) (Figure 1.6). Protein kinase C is localised in the interior of the cell membrane until free diacylglycerol is liberated in response to an external stimulus linked to the G-protein and phospholipase C (Nishizuka, 1986). Protein kinase C controls the activity of cell membrane Ca^{2+}-Mg^{2+}-ATPase as well as the influx of calcium through voltage dependent channels (Rickard and Sheterline, 1985; Deriemer et al, 1985). This system may also influence Na^{+}-K^{+}-ATPase, Na^{+}-H^{+} exchange and membrane adenylate cyclase activity (Simmons et al, 1986; Berridge, 1986). Studies have documented that phorbol esters can activate PKC and smooth muscle contraction with no detectable increase in cytoplasmic calcium (Danthuluri and Deth, 1984; Jiang et al, 1986).

Inositol trisphosphate, the other second messenger, is freely diffusible in the cytoplasm, and does not appear to activate or inhibit any cellular enzymes. Instead, IP_3 acts as a calcium ionophore, i.e. it causes the sarcoplasmic reticulum to release its stored calcium into the cell resulting in free intracellular calcium levels to increase (Hanley and Steiner, 1989) (Figure 1.6). The release of calcium from non-mitochondrial organelles occurs

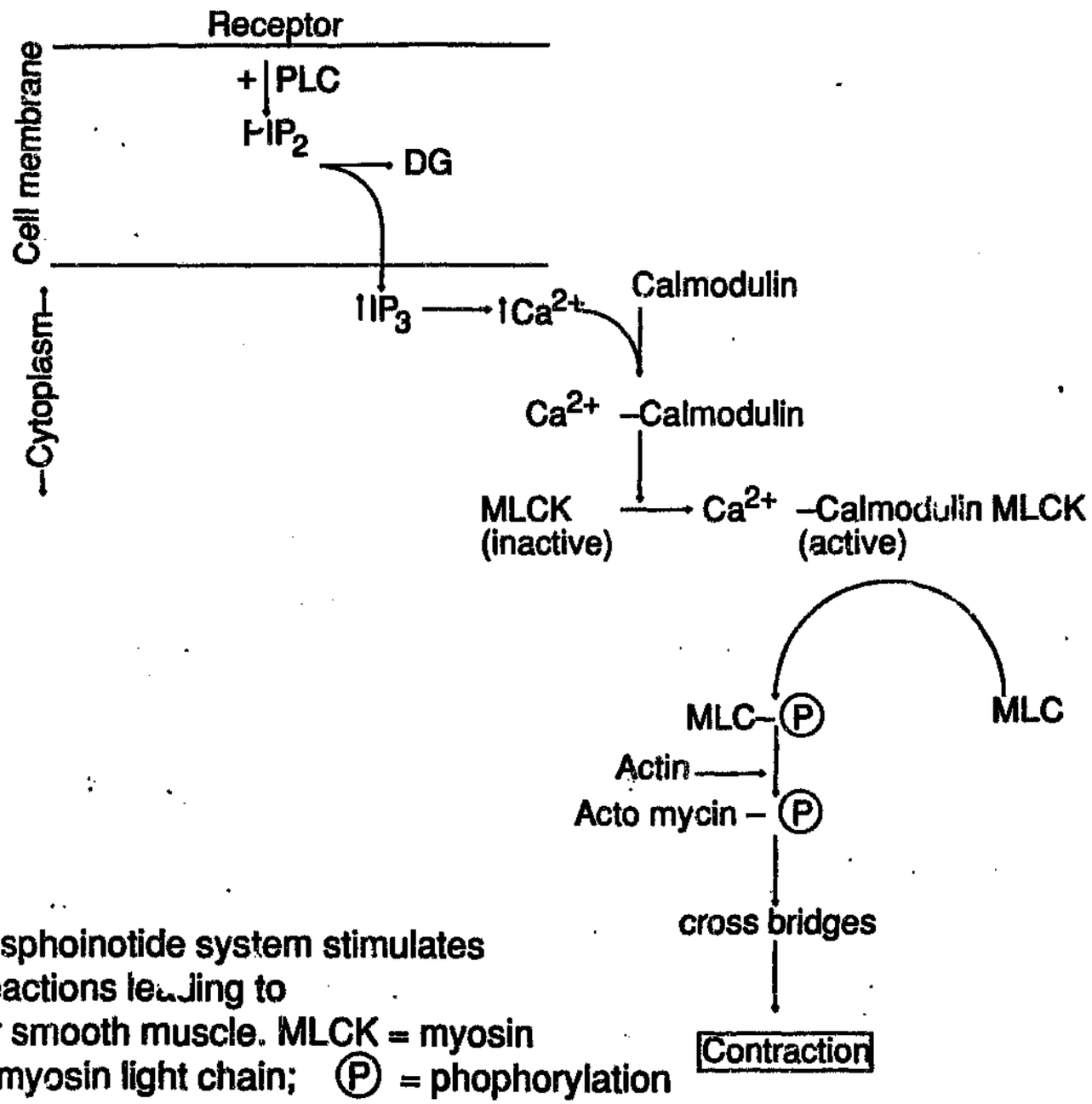


Figure 1.7:

Activation of the phosphoinotide system stimulates a cascade of enzymatic reactions leading to contraction in the vascular smooth muscle. MLCK = myosin light chain kinase; MLC = myosin light chain; Ⓟ = phophorylation

via a guanidine nucleotide regulatory mechanism (Streb et al, 1983; Gill et al, 1986).

In smooth muscle cells, the released intracellular calcium binds to calmodulin causing light chain phosphorylation and contraction (Somlyo et al, 1985) (Figure 1.7). The calcium-calmodulin complex by different mechanisms, can also result in hydrolysis of cAMP and stimulation of neurotransmitter release (Hanley and Steiner, 1989). The inositide messenger molecule is deactivated in two ways; it can be dephosphorylated to inositol 1,4-bisphosphate (Ins 1,4 P_2) or it can be phosphorylated in the 3 position to produce inositol 1,3,4,5-tetrakisphosphate (Ins 1,3,4,5, P_4) (Storey et al, 1984; Batty et al, 1985). Ins 1,4 P_2 is further metabolised to inositol 1-phosphate or inositol 4-phosphate and then to inositol (Storey et al, 1984). Ins 1,3,4,5 P_4, is dephosphorylated to produce Ins 1,3,4 P_3.

In acidic environments, PLC activation may release inositol 1:2 cyclic 4,5-trisphosphate rather than IP_3 (Williamson, 1986; Berridge, 1986). This cyclic phosphate has specific functions, one of which is to release calcium in platelets and pituitary cells (Wilson et al, 1985).

The hormones or agonists that bind to the cell receptors which activate the phosphoinositide pathway include angiotensin II, serotonin, noradrenaline and antidiuretic hormone (Heagerty et al, 1987; Griendling et al, 1989).

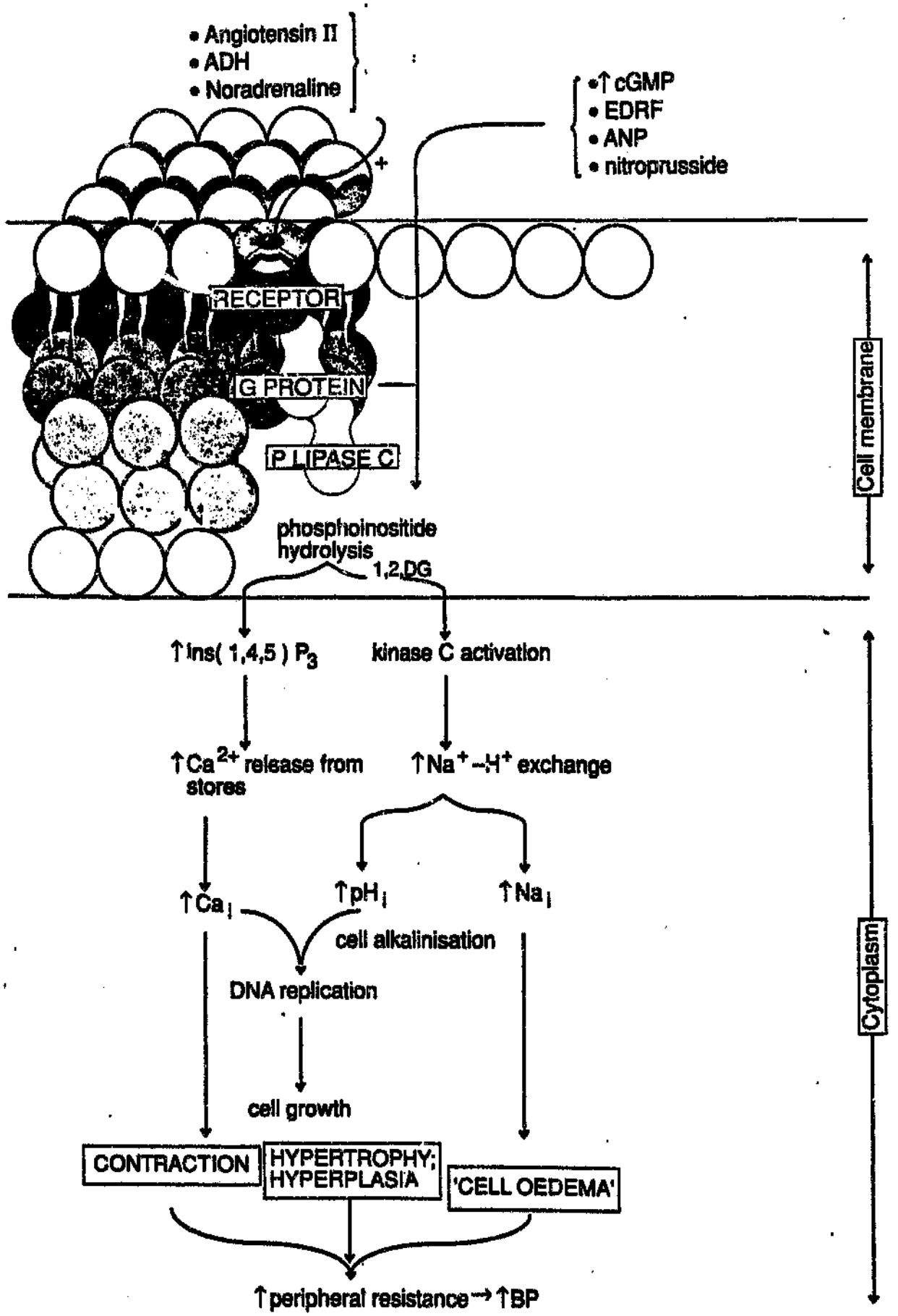


Figure 1.8:

Cellular functions mediated by phosphoinositide lipids in vascular smooth muscle. ADH = antidiuretic hormone; EDRF = endothelial derived relaxing factor; ANP = atrial natriuretic peptide; Rec = receptor; + = stimulation; – = inhibition; i = intracellular.

The effects of phosphoinositide stimulation in vascular smooth muscle cells are presented in Figure 1.8. Nitroprusside, atrial natriuretic factor (ANF) and endothelial derived relaxing factor (EDRF) all inhibit phosphoinositide hydrolysis by increasing intracellular hydrolysis and increasing intracellular guanosine monophosphate (GMP) (Rapoport and Murad, 1983).

1.2.4.3 Cytoplasmic calcium removal and homeostasis

The plasma membrane plays a major role in the removal of excess cytoplasmic calcium. Two plasmalemmal mechanisms have been described: the Ca^{2+}-ATPase pump and the Na^{+}-Ca^{2+} exchange carrier. In addition to these plasma membrane events, two other intracellular membranes determine the cytoplasmic calcium concentration. These are the sarcoplasmic reticulum membrane and the mitochondrial membrane. Both of these membranes have pump-leak systems which actively take up calcium from and passively leak calcium into the cytoplasm.

1.2.4.3 (i) Plasma membrane Ca^{2+}-ATPase (Figure 1.4)

This is an ATP, magnesium dependent pump that transports calcium out of the cytoplasm (Nicholls, 1986). It is stimulated by calmodulin, inhibited by vanadate and is insensitive to ouabain (distinction from Na^{+}-K^{+}-ATPase) (Rapp, 1981; Popescu, 1984). The Ca^{2+}-pump is the major

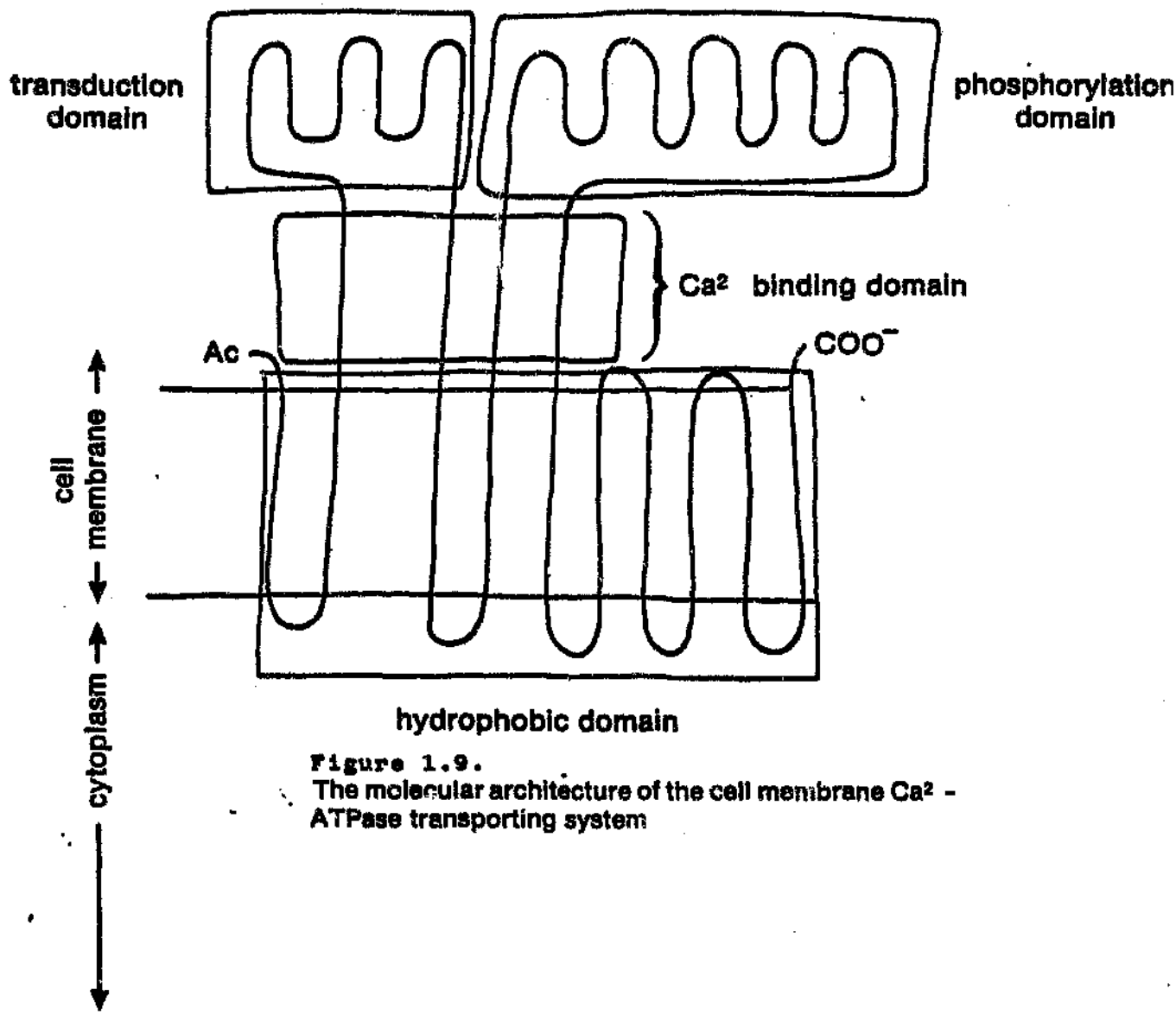


Figure 1.9.
The molecular architecture of the cell membrane Ca^2 - ATPase transporting system

mechanism for calcium extrusion in vascular smooth muscle (Brading and Lategan, 1985). The molecular architecture of the cell membrane Ca^{2+}-ATPase pump is presented in Figure 1.9.

1.2.4.3 (ii) Sodium-calcium exchange (Figure 1.4)

This mechanism provides an alternative pathway in the plasma membrane through which excess intracellular calcium is driven against a large calcium gradient (Morel and Godfraind, 1985). It occurs in many cell types, including vascular smooth muscle (Reuter et al, 1973). The process is driven by the transmembrane sodium and calcium ionic gradients and the membrane potential. At least three sodium ions are exchanged for each calcium ion, so generating a net current in the direction of the sodium movement (Blaustein and Hamlyn, 1984). In the past the Na^{2+}-Ca^{2+} exchange was assumed to be a predominantly Ca^{2+}-efflux pathway. It is now clear that depending upon the membrane potential, ionic gradients of sodium and calcium and the relative importance of intracellular calcium, this pathway may contribute to either calcium extrusion or calcium influx. In cardiovascular diseases, especially hypertension, the Na^{+}-Ca^{2+} exchange process may be a cause of increased intracellular calcium (Blaustein and Hamlyn, 1984). The Na^{+}-Ca^{2+} transport mechanism is dominant in cardiac muscle (Brading and Lategan, 1985).

1.2.4.4. Calcium transport systems of the sarcoplasmic reticulum and mitochondria

1.2.4.4 (i) The Ca^{2+}-ATPase pump of sarcoplasmic reticulum

Ca^{2+}-ATPase is the main protein component of the sarcoplasmic reticulum. A hydrophillic portion extends from the cytoplasmic surface of the membrane, and a hydrophobic core is inserted into the membrane bilayer (Allen, 1980) (Figure 1.9). Sarcoplasmic reticulum membranes accumulate 2 moles of calcium per mole of ATP hydrolysed (Hasselbach, 1964).

1.2.4.4 (ii) The calcium-release channels of sarcoplasmic reticulum

In muscle, the rate of calcium release is almost three times higher than that of calcium uptake. Opening of the channels requires low concentrations of cytoplasmic calcium, which explains the phenomenon of calcium-induced calcium release (Carafoli et al, 1989; Carafoli et al, 1990). The channel is activated by micromolar concentrations of nucleotides such as ATP and inositol trisphosphate and is inhibited by high concentrations of magnesium and/or calcium (Carafoli et al, 1990). The IP_3-sensitive channels are different to those stimulated by ATP. Sarcoplasmic reticular calcium release is the main regulator of the cytosolic free calcium concentration.

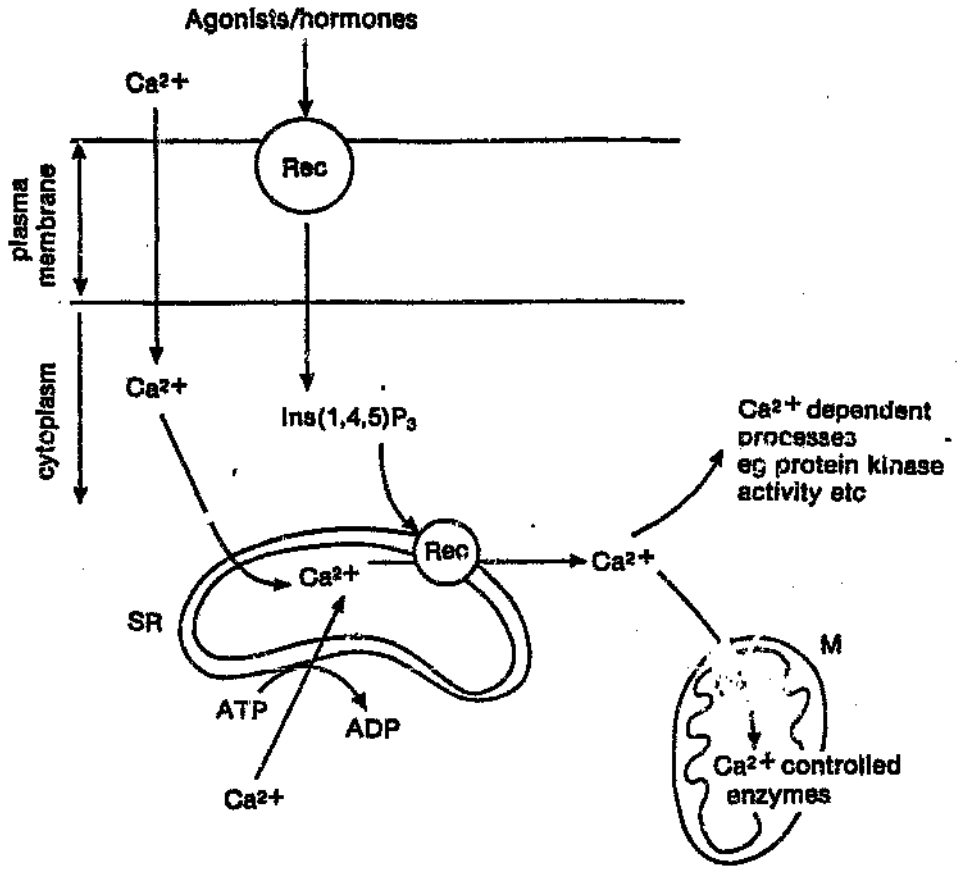


Figure 1.10:
Calcium mobilisation by intracellular stores

SR = sarcoplasmic reticulum; M = mitochondria; $Ins(1,4,5)P_3$ = inositol(1,4,5)trisphosphate

Ca^{2+} is taken up by the SR and mitochondria. $Ins(1,4,5)P_3$ stimulates Ca^{2+} release into the cytoplasm. Cytoplasmic Ca^{2+} activates Ca^{2+} dependent processes and some of the Ca^{2+} is taken up by the mitochondria, increasing mitochondrial Ca^{2+} and stimulating intra-mitochondrial enzymes.

(Figure 1.10).

1.2.4.4 (iii) The mitochondrial Ca^{2+}-uniporter

Mitochondria from many cell types, including vascular smooth muscle, can actively transport calcium across the inner membrane into their matrix (Nicholls and Akerman, 1982) (Figures 1.4; 1.10). The energy for this active transport is the electrical potential generated across the inner membrane during oxidation of metabolic substrates by the respiratory chain or during hydrolysis of ATP (Carafoli et al, 1990). The exact function of the mitochondrial calcium transport system is unclear.

In summary, calcium is the major second messenger that regulates vascular smooth muscle contraction. Calcium enters cells via different calcium channels and is released from the intracellular stores - particularly the sarcoplasmic reticulum, by stimulation of the phosphoinositide system. Calcium is extruded from vascular smooth muscle cells mainly via an active Ca^{2+}-pump and less importantly by a sodium-calcium exchange mechanism. Under pathological conditions, calcium enters the cell by the sodium-calcium exchange system.

1.2.5 The role of magnesium in vascular smooth muscle contraction

Magnesium, the second most abundant intracellular cation, plays an important role in vascular smooth muscle contraction. It is essential for the regulation of contractile proteins, sarcoplasmic reticular membrane transport of calcium, it is a vital cofactor in ATPase activities and in the metabolic regulation of energy-dependent cytoplasmic and mitochondrial pathways (Altura and Altura, 1990).

1.2.5.1 Regulation of magnesium in vascular smooth muscle cells

1.2.5.1 (i) Magnesium concentration

The exact mechanisms of vascular smooth muscle magnesium metabolism are not clearly understood. The total magnesium content of vascular smooth muscle is about 10-35 mmol per kilogram dry cell weight (Palaty, 1971; Somlyo et al, 1979). Almost half of this can be depleted in magnesium free solutions (Palaty, 1971). The free cytoplasmic magnesium concentration in vascular smooth muscle is about 0,1 mM to 4,0 mM (Palaty, 1971; Hess and Weingart, 1981). In erythrocytes, the free magnesium concentration is 261 $\pm$ 9.8 μM (Resnick et al, 1984).

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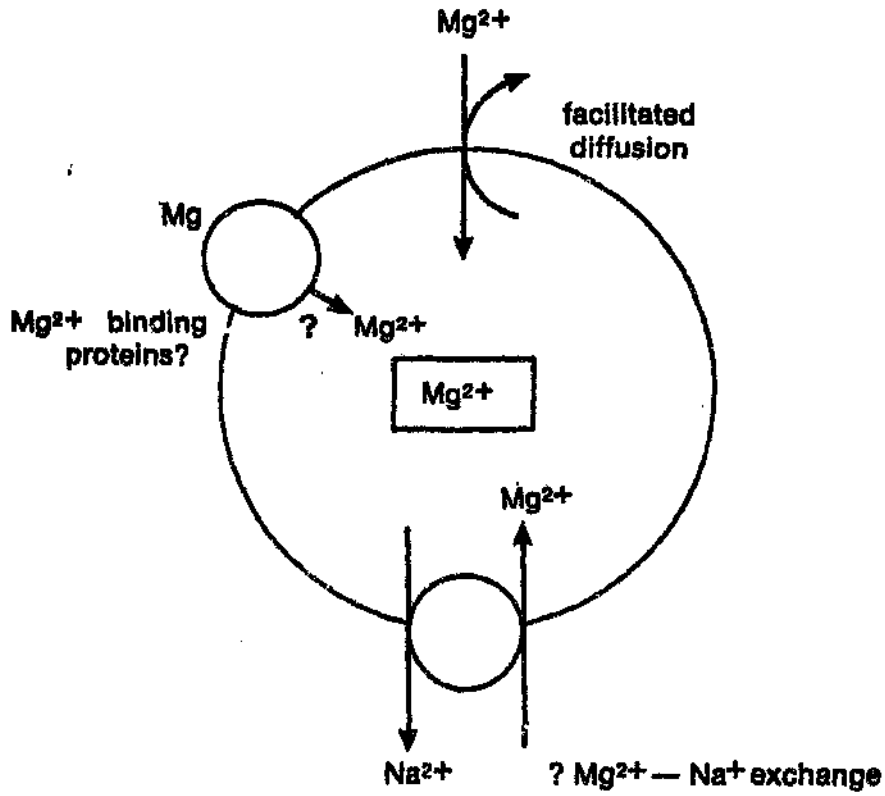


Fig. 1.11 Hypothetical mechanisms for transmembrane magnesium transport

1.2.5.1. (ii) Intracellular magnesium distribution

Most of the intracellular magnesium is complexed. It may be chelated by adenine nucleotides and citrate (Palaty, 1974) or it may be bound to proteins:- 1 magnesium ion per G-actin monomer on F-action (Bogucka, 1976; Gunther et al, 1986a; 1986b). Similar to the calcium-calmodulin complex, there may be a 'magmodulin' which binds to intracellular magnesium (Walker and Duffus, 1983). Within the cell, magnesium is compartmentalised in the nuclei, mitochondria and sarcoplasmic reticulum (Gunther et al, 1986).

1.2.5.1 (iii) Magnesium transport across cell membranes

Figure 1.11

1.2.5.1 (iii) a. Magnesium influx into the cell

Uptake of magnesium by the cell seems to occur by facilitated diffusion (Gunther and Vormann, 1984). The energy required by this mechanism is provided by the cell metabolism (Palaty, 1971).

1.2.5.1 (iii) b. Magnesium transport across the mitochondrial membranes

Isolated mitochondria from vascular smooth muscle show energy-linked magnesium transport (Sloane et al, 1978). Active transport is bidirectional, and both magnesium

influx and efflux are blocked by inhibitors of mitochondrial respiration (Sloane et al, 1978).

1.2.5.1 (iii) c. Magnesium extrusion from the cell

Since the intracellular magnesium concentration is less than one fifth of the extracellular concentration, active extrusion of magnesium from the cell must take place for this gradient to be maintained. The energy for outward movement of magnesium against a concentration gradient is generated through an inward movement of sodium (Palaty, 1971). Vitamin B6, Vitamin D, insulin and taurine may increase intracellular magnesium concentrations, whereas adrenaline may decrease the levels (Durlach and Durlach, 1984).

1.2.5.2 Effects of magnesium on vascular tone

Mg^{2+}-ATP is the substrate of actomyosin ATPase and of all the transport ATPases (Aikawa, 1981). It is a vital cofactor for many enzymes, it regulates intracellular calcium homeostasis and it stabilises cell membranes (Bara, 1988). These metabolic processes are all important in the maintenance of vascular smooth muscle tone.

Lowering the magnesium concentration of isolated animal and human vessels induces rapid contractile responses and potentiates the action of various neurohumoral constrictor

agents, including angiotensin II, adrenergic amines, eicosanoids, serotonin and cations such as potassium and calcium (Altura and Altura, 1981; Altura et al, 1983; Altura and Altura, 1985). The greater the deficit in extracellular magnesium concentration, the greater the vascular contraction and the greater the potentiation of constrictor agents. The potentiation of vasoactive agents in the presence of magnesium deficiency may be related to altered receptor binding affinity of the agents (Somlyo et al, 1966; Altura and Altura, 1985).

1.2.5.3 Effects of magnesium on vascular smooth muscle calcium

Specific membrane magnesium sites control and regulate intracellular concentration, distribution, influx and efflux of calcium in vascular smooth muscle. Magnesium binds competitively with calcium on the outer cell membrane (Bara, 1988). It alters receptor interactions and inhibits the slow inward current of calcium (Witteman and Grobbee, 1990). Also the Ca^{2+}-ATPase pump responsible for calcium extrusion from the cell is dependent on magnesium (Turlapaty and Altura, 1980). By altering vascular smooth muscle calcium metabolism, magnesium may play an important role in the regulation of vascular tone, contractility and reactivity.

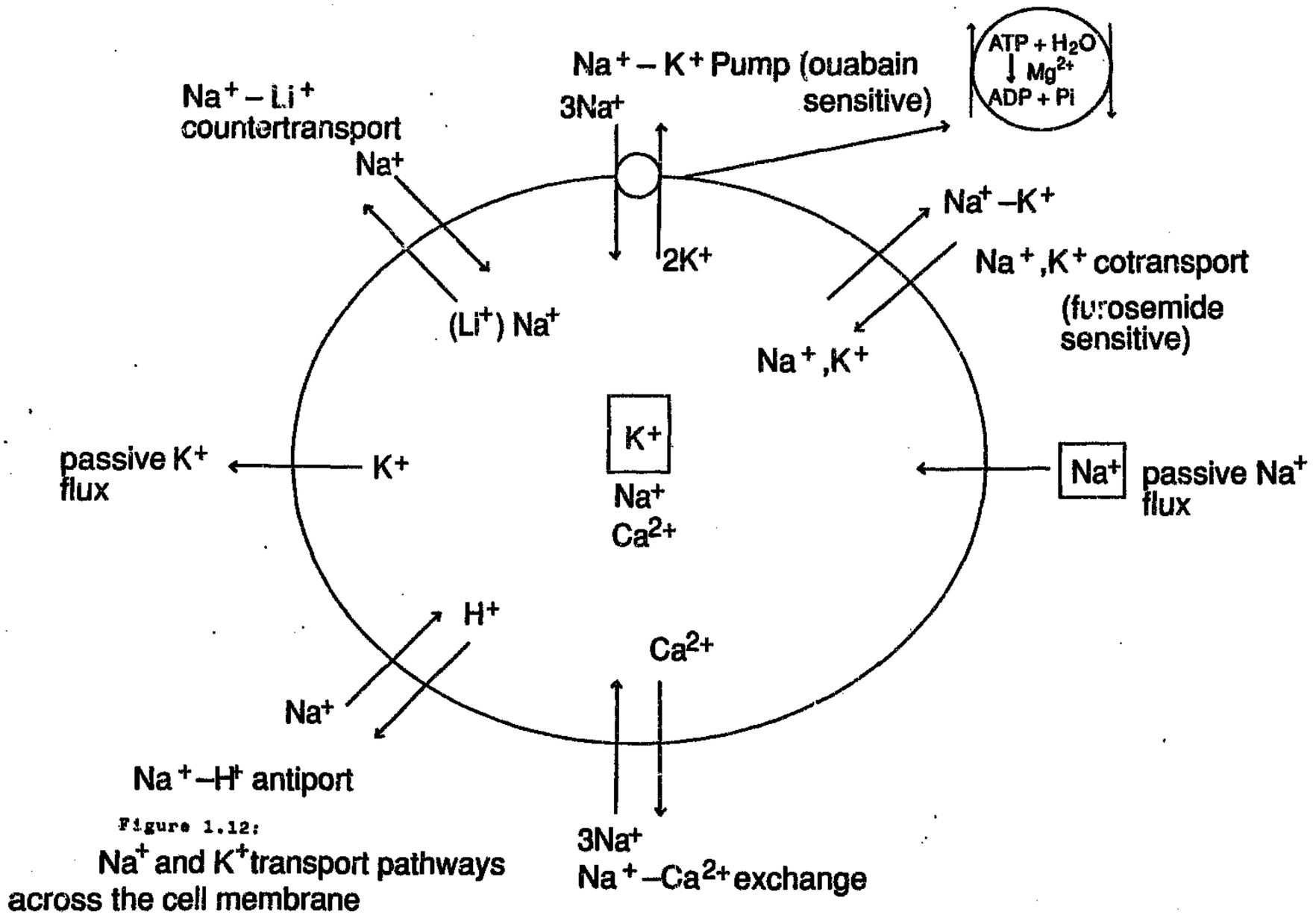


Figure 1.12:
Na^+ and K^+ transport pathways across the cell membrane

1.2.6 Sodium, potassium and vascular smooth muscle

At the cellular level, the ions do not function in isolation. Sodium, potassium, magnesium and calcium interact with each other and abnormalities in cellular regulation of one cation may affect regulation of the others. Calcium and magnesium are directly involved in vascular smooth muscle contraction and ultimately peripheral resistance. Sodium and potassium may influence peripheral resistance by altering calcium regulation, by modulating cellular water content and by maintaining electrochemical gradients.

1.2.6.1 Cellular regulation of sodium and potassium (Figure 1.12)

Intracellular sodium concentrations depend on the rate of sodium entry into the cell and the ability of the sodium pump to extrude it. Cytosolic potassium levels are determined by the rate of potassium pumped into the cell. Sodium-potassium transport mechanisms across cell membranes may be ouabian-sensitive or ouabain insensitive.

1.2.6.1 (i) Ouabain-sensitive transport

The sodium-potassium pump (Na^+-K^+-ATPase) mobilises sodium out of and potassium into the cell against concentration gradients. This is the best defined sodium transport

system and is quantitatively the most important (Jorgensen, 1986). The Na^+-pump is inhibited by ouabain (Jorgensen, 1986). In erythrocytes, at physiological levels of intracellular sodium, the pump transports sodium out of the cell at a rate that is proportional to the intracellular level (Hilton, 1986). Very small changes in cellular sodium concentration around physiological levels exert a large effect on Na^+-K^+-ATPase (Lasker et al, 1985). The relationship begins to break down when the intracellular sodium increases above the physiological range, as the pump approaches its maximum rate of activity (Garay and Garrahan, 1973). In leukocytes, the relation between the intracellular sodium concentration and its efflux by means of the pump is linear up to levels twice the normal value (Hilton, 1986).

1.2.6.1 (ii) Ouabain insensitive transport
(Figure 1.12)

a) Passive sodium flux (uniport)

This is the undirectional movement of sodium ions through membrane channels down a concentration gradient from the extracellular fluid (Hilton, 1986).

b) Sodium-potassium cotransport (Na^+-K^+ symport)

The Na^+-K^+ symport transports sodium and potassium

simultaneously across the cell membrane (Wiley et al, 1974). This pathway depends on chloride and is electrically neutral, as it carries sodium, potassium and chloride into and out of the cell in a stoichiometry of 1:1:2 (Ch'pperfield, 1980). The Na^+-K^+ cotransport pathway is sensitive to furosemide and bumetanide (Wiley et al, 1974; Tokushige et al, 1986). It exists in many cell types, including vascular smooth muscle (Chipperfield, 1986; Tokushige et al, 1986). In human erythrocytes under basal conditions, the net transport of ions through the Na^+-K^+ cotransport is in an outward direction (Brugnara, 1986). In renal epithelium, endothelium and vascular smooth muscle cells, the net transport is in an inward direction (Owen, 1984). The Na^+-K^+ symport is regulated by cyclic adenosine 3'5'-monophosphate (cAMP) (inhibits the system) and cyclic guanosine 3'5'-monophosphate (cGMP) (stimulates the system) (Palfrey et al, 1980; O'Donnel and Owen, 1986). The system is also ATP dependent (Chipperfield, 1986).

c) <u>The sodium-lithium (Na^+-Li^+) countertransport</u>

The Na^+-Li^+ countertransport is the fraction of lithium efflux that depends on the presence of external sodium (Canessa et al, 1980; Brugnara et al, 1983). Under physiological conditions (i.e. in the absence of lithium), the erythrocyte Na^+-Li^+ exchange mediates 1:1 Na^+ = Na^+ with no net effect on the intracellular sodium level

(Canessa et al, 1980; De la Sierra et al, 1988). The physiological function of Na^+-Li^+ countertransport is unknown. It is not involved in ionic regulation at the cellular level. Erythrocyte Na^+-Hi^+ countertransport may be the counterpart of the Na^+-Li^+ antiport in other cells or the two transport systems may be independent entities (Aronson, 1982; Canessa et al, 1987).

d) The sodium-hydrogen exchange (Na^+-H^+ antiport)

The plasma membrane Na^+-H^+ exchange is an ubiquitous system that is driven by the sodium and hydrogen gradients across the cell membrane. Amiloride and its analogues block the Na^+-H^+ antiport (Seifter and Aronson, 1986). This transport system plays an important role in many physiological processes including i) the response to mitogens and growth factors; ii) activation of platelets and leukocytes; iii) cellular sodium volume and pH regulation; iv) response to vasoactive agents and v) sodium reabsorption at the renal proximal tubules level (Grinstein et al, 1985; Grinstein and Rothstein, 1986; Seifter and Aronson, 1986). The Na^+-H^+ antiport is activated by protein kinase C and by increased intracellular calcium concentrations (Huang et al, 1987; Berk et al, 1987).

e) Sodium-calcium exchange

As discussed in section (1.2.4.3 ii), this transport

mechanism can exchange three or more sodium ions for each calcium ion, so generating a net current in the direction of the sodium movement (Blaustein, 1977). It depends on the membrane potential, the intra- and extracellular concentrations of sodium and calcium, and it can operate in either direction across the cell membrane (Aviv, 1988).

Abnormalities in these transport systems may be associated with increased vascular smooth muscle contractility and increased cell size, both of which will ultimately affect peripheral resistance and blood pressure.

1.3 Cellular changes in essential hypertension

The underlying pathogenetic lesion in essential hypertension may be related to defective cell membrane transport and altered intracellular ion regulation. Postnov and Orlov (1984) proposed a widespread abnormality in the ion transport function of the cell membrane and changes in its ultrastructure and physico-chemical properties resulting in insufficient membrane control over intracellular calcium (the 'membrane defect' or membranopathy). The significant consequence of this membrane defect is an increased cytoplasmic calcium concentration (Postnov, 1990). Mechanisms that may be responsible for the intracellular calcium overload in hypertension include altered cell membrane calcium binding and calcium influx, increased intracellular calcium

mobilisation and decreased calcium efflux. A number of these defects have been described in human hypertension.

1.3.1 Intracellular calcium concentrations

Intracellular calcium studies have been facilitated by the use of fluorescent dyes which are released within the cell, and which fluoresce in a manner quantitatively related to the cytoplasmic free calcium concentration (Tsien, 1980; Tsien, 1982). Using fluorescent dyes, intracellular calcium content has been reported to be raised in platelets, lymphocytes and erythrocytes from essential hypertensive patients and spontaneously hypertensive rats (Bruschi et al, 1985; Cooper et al, 1987; Erne et al, 1984; Zidek et al, 1982; Papageorgiou and Morgan, 1990; Morgan and Suematsu, 1990). Erne et al (1984) demonstrated a significant correlation between platelet cytosolic calcium concentration and blood pressure. Furthermore, intracellular platelet calcium concentration normalises following antihypertensive therapy (Erne et al, 1984; Lenz et al, 1985). Some studies have failed to show changes in intracellular free calcium levels in human and experimental hypertension (Nabika et al, 1985; Bing et al, 1986a). In white hypertensive patients, total erythrocyte calcium was unaltered (Bramley et al, 1986). These conflicting data could be related to different populations, cell models and techniques studied. Intracellular calcium concentrations may be elevated in certain models of hypertension and in

specific subgroups of human essential hypertension.

1.3.2 Cell membrane calcium binding

Calcium binds to many components of the cell membrane and is a function of both the membrane lipids and proteins (Duffy and Schwartz, 1973). Bound calcium has important effects on cell membrane function. It influences the activity of the potential operated channels, it controls calcium influx and it acts as a cell membrane stabiliser (Romero, 1976; Hurwitz et al, 1982). Calcium binds to the inner and outer sides of the cell membrane. The outer side of the membrane is exposed to ionic calcium concentrations three times greater than the inner aspect and both low and high affinity binding are important. The inner side of the cell membrane is exposed to ionic calcium concentrations of approximately 1 μmol/l and high affinity binding is important here. Cell membrane calcium binding may be altered in hypertension.

Animal and human studies have suggested defective calcium binding to the outer membrane of cells from hypertensive rats and patients. Most studies have reported decreased calcium binding (Losoter et al, 1977; Postnov et al, 1979). Bhalla et al (1978), however, demonstrated increased calcium binding in the aorta of spontaneously hypertensive rats.

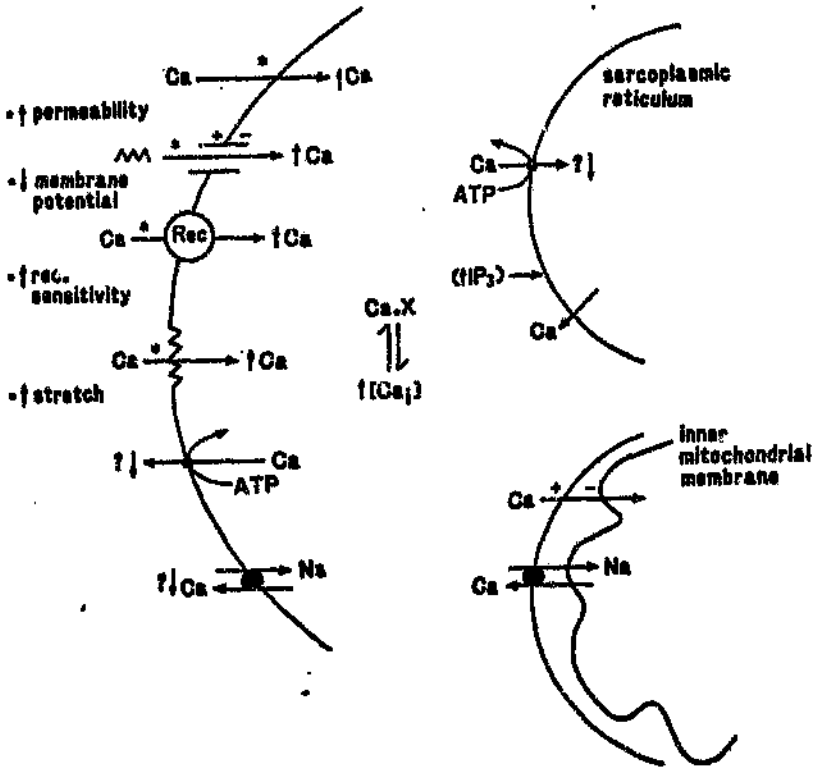


Figure 1.13. Alterations in calcium transport in essential hypertension.

Decreased calcium binding to the inner aspect of the cell membranes of hypertensive subjects has been demonstrated in erythrocytes, adipocytes and hepatocytes (Devynck et al, 1981a; Friedman, 1983a; Robinson, 1984; Postnov and Orlov, 1985; Cirillo, 1990). Since these defects are similar in 3 week old and adult spontaneously hypertensive rats, the cell membrane abnormality is probably genetic in origin (Devynck et al, 1981b).

The functional consequences related to defective calcium binding may be increased activity of the potential operated channels with a resultant increased calcium influx.

1.3.3 Calcium influx

Bound calcium may influence the activity of potential operated channels. In a variety of cell types, calcium bound to the inner membrane increases potassium conduction with resultant hyperpolarisation of the cell (Romero, 1976; Isenberg, 1977). Defective calcium binding, as in essential hypertension, would favour depolarisation with resultant activation of potential operated channels (Robinson, 1984). Experimental and human studies have demonstrated partial depolarisation of platelet and synaptosome plasma membranes (Kravtsov et al, 1983; Buhler and Resink, 1988). Cell membrane depolarisation and activation of potential operated channels results in increased basal calcium influx and consequently increased

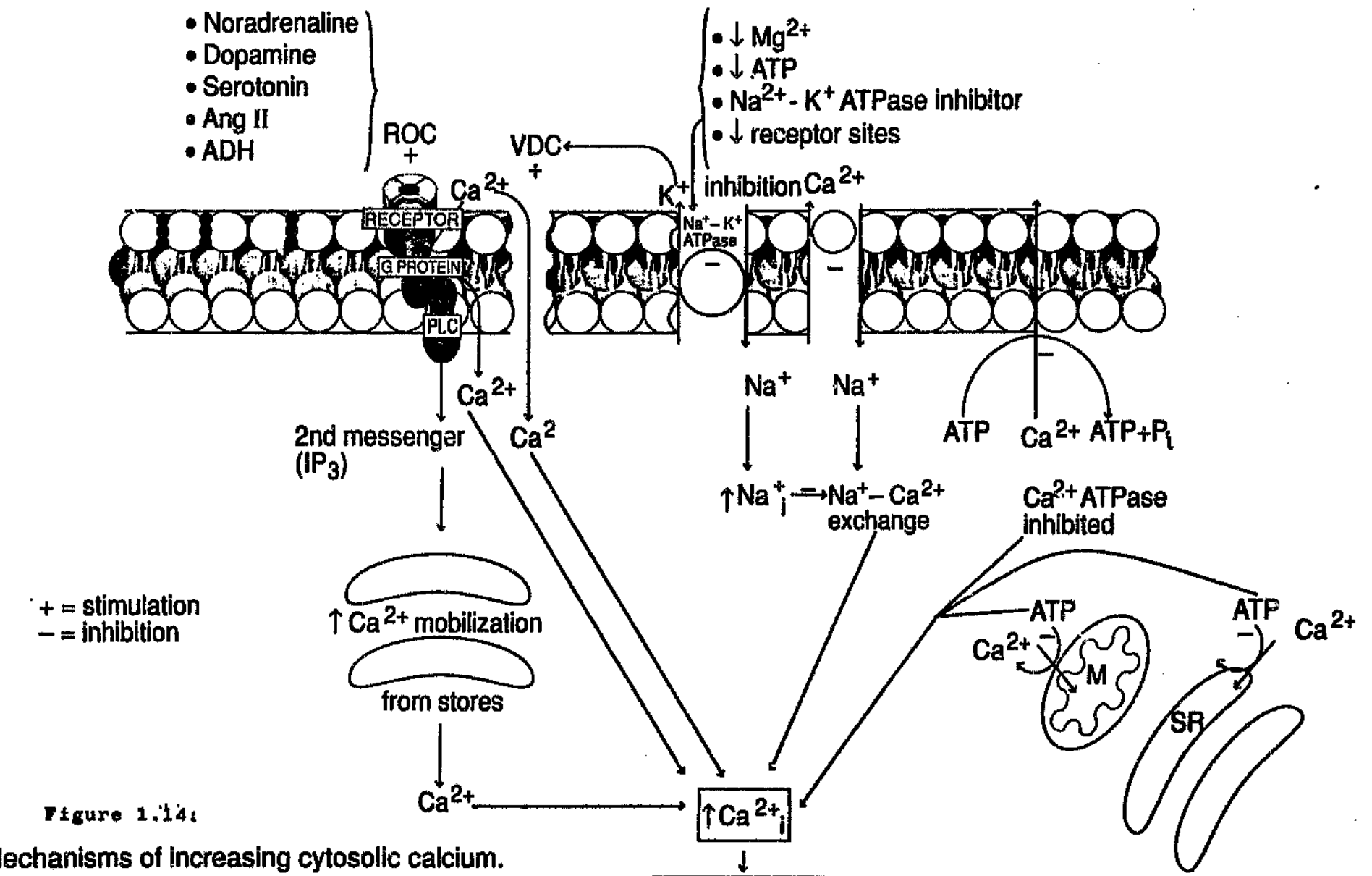


Figure 1.14:

Mechanisms of increasing cytosolic calcium.
VDC = Voltage dependent channel; PLC = phospholiphase C,
Ang II = angiotensin II ROC = receptor operated channel
SR = sarcoplasmic reticulum; M = mitochondrian; IP_3 = inositoltrisphosphate
Pi = inorganic phosphate

cytosolic calcium (Figure 1.13).

Enhanced calcium entry into cells in essential hypertension may be related to an increased response to vasoactive agonists (Buhler et al, 1986). The vasculature of hypertensive patients is more sensitive to vasoactive agents than normotensive subjects (Friedman, 1983b). This hyperresponsiveness of cells may be genetically determined (Nara et al, 1984).

1.3.4 Intracellular calcium mobilisation - the phosphoinositide system and hypertension

The generation of phosphoinositides plays a central role in the regulation of transmembrane ionic fluxes and, via diacylglycerol, mediates control of cell pH, cell differentiation and cell growth (Heagerty and Ollerenshaw, 1990). Phosphoinositide metabolism is also implicated in the regulation of calcium influx and efflux across the plasma membrane, membrane fluidity, membrane calcium binding and membrane Ca^{2+}-ATPase and adenylate cyclase activities (Michell, 1975; Allan, 1982; Marche, 1985). Alpha-$_1$-adrenoceptors, vasopressin, endothelin and angiotensin II receptors are directly coupled to phosphoinositide turnover, while α_2-adrenoceptors are coupled to adenylate cyclase inhibition (Michell and Kirk, 1981; Dostal et al, 1990). Evidence is accumulating to suggest that phosphoinositide metabolism is altered in

essential hypertension resulting in increased intracellular calcium concentrations (Figure 1.14). This defect may provide a link between abnormal monovalent and divalent ion fluxes, membrane lipids, intracellular calcium and blood pressure.

In erythrocyte membranes from hypertensive patients the phosphoinositide lipid content was low with an increased rate of P^{32} incorporation into Ptd Ins 4,5-P_2 (Marche et al, 1985). These findings and others have demonstrated an increased rate of phosphoinositide turnover (Tremblay et al, 1990). This signalling system is enhanced in erythrocytes from normotensive offspring of hypertensive patients (Riozzi et al, 1987).

Recent studies examining the phosphoinositide system in platelets have demonstrated that phospholipase C is enhanced and phosphatidylcholine turnover is increased in spontaneously hypertensive rats and hypertensive humans (Koutouzov et al, 1987; Marche et al, 1990). SHR studies have demonstrated that Ins 1,4,5 P_3 is produced in excess in resistance vessels, and PLC activity is increased in the aorta (Durkin et al, 1988; Ollerenshaw et al, 1988; Uehara et al, 1988; Durkin et al, 1990). Enhanced phosphoinositide metabolism results in increased intracellular calcium mobilisation, raised cytosolic calcium, smooth muscle contraction and ultimately increased peripheral resistance with elevation in blood pressure.

In addition to its calcium mobilising effects, phosphoinositide hydrolysis may influence blood pressure by its effects on the vascular media. Activation of Na^+-H^+ exchange by autonomic hyperactivity or phosphoinositide stimulation (by vasoconstrictor agonists such as angiotensin II, noradrenaline, vasopressin) results in cell alkalinisation with cell growth and proliferation (Rozengurt, 1986) (Figure 1.14). In spontaneously hypertensive rats (SHR), thymocyte Na^+-H^+ exchange is increased (Feig et al, 1987). Also, the basal intracellular pH in SHR resistance vessels is more alkaline than in Wistar Kyoto (WKY) vessels (Izzard and Heagerty, 1989). This growth promoting effect only occurs when a tissue is stimulated excessively for a long time (Berridge, 1987a; 1987b).

Stimulation of the phosphoinositide signalling system initiates and maintains contraction in vascular smooth muscle cells. This system also provides a means for cell proliferation. In essential hypertension, phosphoinositide hydrolysis is increased resulting in intracellular calcium elevation, increased Na^+-H^+ exchange, cell alkalinisation and consequent DNA synthesis. In vascular smooth muscle these cellular activities manifest as enhanced contraction, hypertrophy and hyperplasia - all basic hallmarks of essential hypertension.

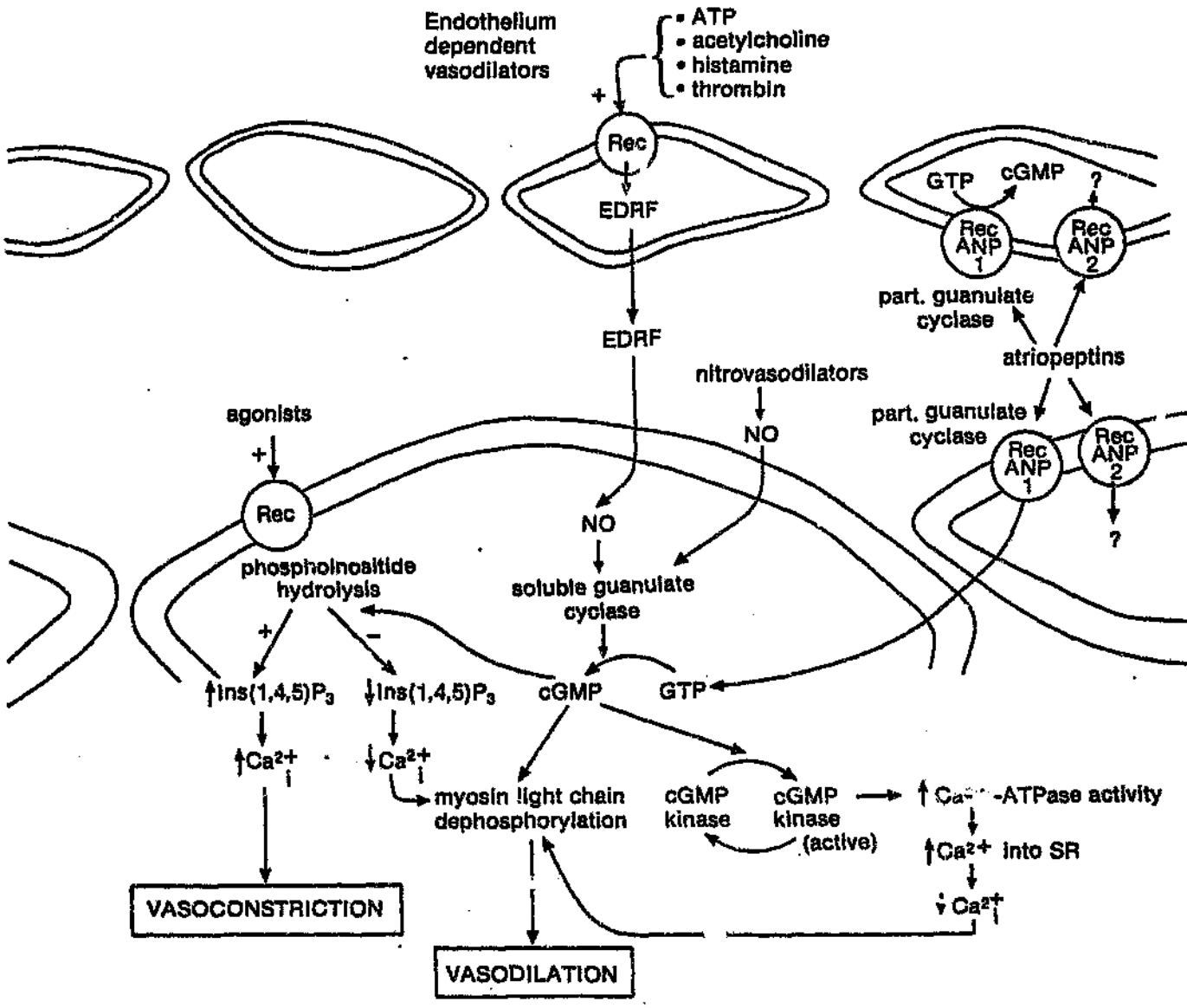


Figure 1.15: Interaction of endothelial factors, phosphoinositides, G proteins and calcium in vascular smooth muscle contraction and dilation

EDRF = endothelial derived relaxing factor; Rec = receptor; NO = nitric oxide; $Ins(1,4,5)P_3$ = inositol 1,4,5 trisphosphate; part. = particulate; ANP = atrial natriuretic peptide; — = inhibit; + = stimulate; i = intracellular; ↑ = increase; ↓ = decrease.

NO, derived from EDRF and nitrovasodilators, activates soluble guanulate cyclase resulting in increased cGMP. cGMP activates cGMP dependent protein kinase which alters phosphorylation of smooth muscle proteins. Increased cGMP causes myosin light chain dephosphorylation and increases activity of Ca^{2+}-ATPase resulting in decreased cytostolic Ca^{2+} and decreased myosin light chain phosphorylation. cGMP also inhibits phosphoinositide hydrolysis. Atriopeptins activate particulate guanulate cyclase via ANP1 receptors. The function of ANP2 receptors is unknown.

1.3.5 Cyclic nucleotides, calcium and hypertension (Figure 1.15)

Cellular calcium metabolism is influenced not only by phosphoinositide stimulation, but also by cyclic nucleotides (Namm, 1982). Cyclic nucleotides are involved in cellular events leading to contraction or vasodilation, hormone secretion (catecholamines, renin), ion transport and cell proliferation (Hamet and Tremblay, 1990). The major cyclic nucleotides include cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Cyclic AMP is generated intracellularly by a membrane-bound enzyme-adenylate cyclase, via a cyclasing reaction in the presence of magnesium (Hamet and Tremblay, 1990). Cyclic-AMP-dependent protein kinase is the best characterised target enzymes of cAMP (Walsh et al, 1968). This enzyme translates the cAMP message into specific cellular functions. Cyclic AMP promotes calcium uptake into the tubular system, increases calcium influx, prevents calcium-calmodulin activation of myosin light-chain kinase and inhibits phospholipase C hydrolysis of phosphatidylinositol (Rasmussen and Goodman, 1977). The nett result of adenylate cyclase stimulation is to inhibit an increase in intracellular calcium.

Cyclic GMP is also synthesised by a cyclasing reaction from a triphosphate substrate, GTP, in the presence of magnesium, by an enzyme guanulate cyclase (Figure 1.15).

This enzyme exists in two forms, soluble and particulate guanulate cyclase (Murad et al, 1979). It is activated by nitric oxide free radicals (Murad et al, 1979). Cyclic GMP activity is expressed via cGMP-dependent protein kinase. A number of vasodilators act via cGMP. Nitroprusside, nitrate, nitroglycerin, nitric oxide and related substances promote the formation of nitric oxide free radicals and have been termed 'nitrovasodilators'. Endothelium-dependent dilators (endothelial derived-relaxing factor-EDRF) and atrial natriuretic factor (ANF) also act via cGMP (Ballerman and Brenner, 1985; Furchgott, 1984; Marshall and Kontos, 1990). The activation of guanylate cyclase and the accumulation of cellular cGMP results in phosphoinositide inhibition, decreased protein kinase activity, myosin light chain dephosphorylation and decreased intracellular calcium by enhancing Ca^{+}-ATPase activity (Murad et al, 1987). These cellular events lead to vascular smooth muscle relaxation and consequent vasodilation. Both cAMP and cGMP are degraded by cyclic nucleotide phosphodiesterases.

A number of abnormalities relating to the cyclic nucleotides have been described in essential hypertension. Plasma and tissue levels of cAMP and adenylate cyclase activity are increased only in the early phases of hypertension. Once hypertension is established, there is progressive diminution of cAMP concentrations and adenylate cyclase activity (Resink et al 1986; Hamet and Tremblay, 1990). Alterations of cAMP and cGMP-dependent protein

kinase activity have been demonstrated in cardiovascular tissues from SHR (Bhalla et al, 1980). In SHR, at the age when hypertension is established, protein kinase activity and its response to cAMP stimulation is decreased (Coquil and Hamet, 1980; Prashad, 1985). Phosphodiesterase activity has been reported to be increased, decreased or remain unchanged in the aorta and heart of SHR (Hamet et al, 1990; Bahner et al, 1987). Although several anomalies in cyclic nucleotide metabolism in SHR and essential hypertension have been described, the exact role of the cyclic nucleotide system in the pathogenesis of hypertension remains unclear.

1.3.6 Calcium efflux

The two major calcium extrusion transport mechanisms, Ca^{2+}-ATPase and Na^{+}-Ca^{2+} exchange may play a role in the raised intracellular calcium in essential hypertension. Increased cytosolic calcium should result from decreased activity of the Ca^{2+}-ATPase pump or from inhibition of the Na^{+}-K^{+}-pump which results in intracellular sodium accumulation with decreased Na^{+}-Ca^{2+} exchange (Figures 1.13, 1.14).

Studies examining erythrocyte and platelet membrane Ca^{2+}-ATPase in essential hypertension have demonstrated abnormal activity (Lin et al, 1985; Vincenzi et al, 1986; Resink et al, 1986). However, there is no agreement concerning the nature of the abnormality, whether Ca^{2+}-ATPase activity is

increased, decreased or unchanged in hypertension, and whether the enzyme is calmodulin dependent or independent. Findings of reduced Ca^{2+}-ATPase activity in erythrocytes of essential hypertensive patients are different to those derived from platelets. Erythrocytes from hypertensive individuals have decreased V_{max} for basal and calmodulin-stimulated Ca^{2+}-ATPase as well as reduced affinity of the enzyme to calcium (Postnov et al, 1984; Vincenzi et al, 1986). Resink et al, however, reported that basal and calmodulin-stimulated V_{max} values for Ca^{2+}-ATPase are higher in patients with essential hypertension (Resink et al, 1986). Takaya et al (1989) suggested that defective calcium pump activity is associated with low-renin hypertension. This was confirmed by De la Sierra et al (1990) who reported a negative correlation between the apparent dissociation constant for internal calcium and plasma renin activity. This group demonstrated that plasma membrane Ca^{2+}-pump abnormalities are not ubiquitous in essential hypertension, but rather occur in a subgroup of hypertensive patients (De la Sierra et al, 1990).

Other factors besides intracellular calcium and sodium may influence Ca^{2+}-ATPase activity. Magnesium, a vital cofactor for all ATPases, is lower in erythrocytes of hypertensive patients compared to normotensive controls (Resnick et al, 1984; Touyz et al, 1989). Erythrocyte magnesium depletion may contribute to decreased erythrocyte Ca^{2+}-ATPase activity in hypertension. To date, there is no

data regarding platelet magnesium levels in essential hypertension.

Decreased calcium efflux due to depressed Ca^{2+}-ATPase activity may be an important cause of intracellular calcium overload in hypertension. The extent of participation of the Na^{+}-Ca^{2+} exchange in calcium regulation in non-cardiac cells is unclear.

1.3.7 Cellular sodium and hypertension

Raised intracellular sodium has been repeatedly demonstrated in hypertension. The accumulation of excess intracellular sodium in the wall of vessels may result in vasoconstriction or exaggerated vasoconstrictor responses to agonists. These effects may be due to a number of mechanisms including:-

i) increased sodium-binding capacity of the paracellular matrix of arteries resulting in tissue oedema with exaggerated intraluminal bulging, decreased vessel diameter and increased peripheral resistance (Feigl et al, 1963; Friedman and Friedman, 1967).

ii) altered transmembrane sodium gradient which is determined by the extracellular sodium content (Simon, 1990). The increase in transmembrane sodium transport may potentiate vasoconstrictor and trophic responses

to agonists whose mode of action includes the stimulation of sodium influx (Harris and Palmer, 1972; Mendoza et al, 1980; Berczi and Simon, 1988).

iii) Sodium-linked noradrenaline transport. The active transport of noradrenaline uptake and release from nerve endings is driven by the electrochemical energy derived from the transmembrane sodium gradient (De Champlain et al, 1968). The rate of uptake of the transported molecule varies directly with the extracellular sodium concentration, whereas the efflux is enhanced by an increase in the intracellular sodium content (Bogdanski and Brodie, 1969). In the absence of a sodium gradient, noradrenaline transport is abolished.

iv) Sodium-linked transport systems. Several sodium-linked transport systems in vascular smooth muscle whose activity may be altered by changes in the transmembrane sodium gradient, include Na^{+}-Ca^{2+} countertransport and Na^{+}-H^{+} exchange (Aviv and Lasker, 1990).

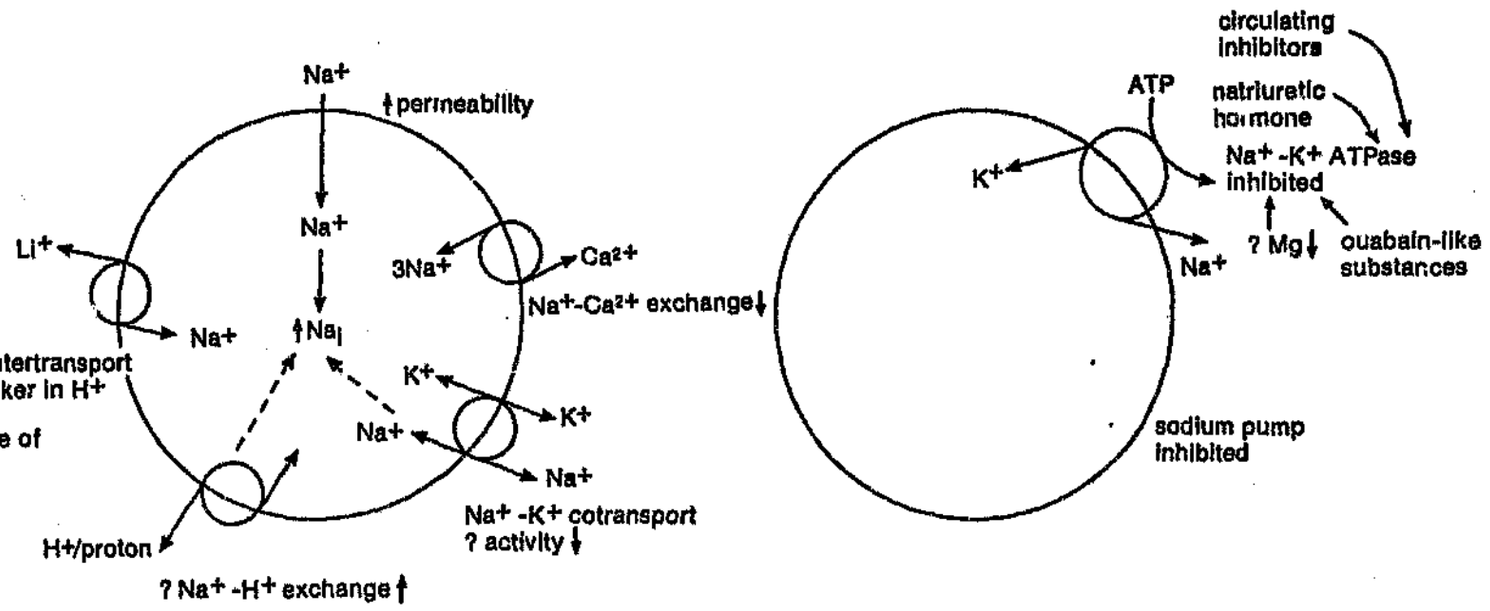
v) Volume expansion and fluid overload (Haddy et al, 1978; Haddy et al, 1980).

Increased intracellular sodium in hypertension may be due to altered sodium transmembrane transport systems.

1.3.8 Intracellular sodium in hypertension

Most studies examining cellular sodium in hypertension have been performed in circulating blood cells, particularly erythrocytes and leukocytes. Data relating to erythrocyte sodium in essential hypertension are conflicting (See Section 8.1.1.1 iii). Studies examining intracellular sodium under experimental conditions that resemble in vivo situations have revealed that erythrocyte sodium is decreased and intracellular potassium increased in hypertensive patients (Trevisan et al, 1983; Svensson et al, 1984; Simon and Engel, 1987).

Unlike erythrocytes, more consistent findings of elevated sodium in experimental and human hypertension have been reported in leukocytes and lymphocytes in particular (Furspan and Bohr, 1985; Bing et al, 1986a; Heagerty et al, 1986). This may be related to the consistent findings of decreased maximal sodium-pump activity in these cells. A recent study however reported no significant differences in the isotopically exchangeable intracellular sodium concentration between lean and overweight hypertensives compared with normotensive controls (Ng et al, 1990).



(a) Ouabain insensitive Na^+ transport

(b) Ouabain sensitive Na^+ transport

Figure 1.16:

Cellular sodium-transport systems in essential hypertension

Na^+ = sodium; K^+ = potassium; Mg^{2+} = magnesium; Ca^{2+} = calcium; H^+ = hydrogen
Li^+ = lithium; ↓ = decreased; ↑ = increased; i = intracellular

1.3.9 Abnormalities in sodium transport in hypertension (Figure 1.16).

1.3.9.1 The sodium pump (Na^+-K^+-ATPase) (Figure 1.16b)

Under steady-state conditions, the sodium pump reflects the rate of sodium entry into cells. Failure of this pump results in progressive intracellular sodium accumulation with resultant increased myocardial contractility and vasoconstriction and enhanced responsiveness of blood vessels to vasoactive agents (Haddy et al, 1978). Both increased and decreased erythrocyte Na^+-K^+-ATPase activity have been described in hypertension (Parker and Berkowitz, 1983; Rahman et al, 1986; Ringel et al, 1987; Syme et al, 1990). In these studies relatively small populations of subjects who have been arbitrarily divided into normotensive and hypertensive groups were studied. When a large group of subjects were not categorised as hypertensive or normotensive, the systolic, diastolic and mean blood pressure levels were indirectly correlated to erythrocyte Na^+-K^+-ATPase activity and directly related to intracellular sodium (Rygielski et al, 1987). Na^+-K^+-ATPase activity was negatively correlated to cytosolic sodium concentration. A recent in vivo study reported increased sodium-potassium pump activity and potassium efflux in skeletal muscle of SHR (Syme et al, 1990). This could represent increased potassium efflux via calcium-activated potassium channels or as part of cell volume

regulation secondary to increased Na^+-H^+ antiporter activity. Studies examining leukocytes in essential hypertension, have consistently demonstrated decreased Na^+-K^+-ATPase activity (Edmonson et al, 1975; Chien and Zhao, 1984; Boon et al, 1985).

Reduced pump activity, volume expansion and hypertension appear to be causally related through a circulating Na^+-K^+-ATPase inhibitor (See Section 9.1.1.1 ii). Haddy (1978) proposed that sodium pump activity in hypertension is suppressed in low renin, salt dependent, volume-expanded forms of hypertension. The volume expansion acts in part to raise pressure by generating the production of a 'cardiac glycoside' like factor (Haddy, 1980). Depressed Na^+-K^+-ATPase activity in hypertension may also be related to a decreased Na^+-K^+ pump number (Ringel et al, 1987) and possibly to decreased intracellular magnesium concentration.

1.3.9.2 Ouabain insensitive sodium transport in hypertension (Figure 1.16a)

1.3.9.2 (i) The Na^+-Li^+ countertransport

Sodium-lithium countertransport in erythrocytes is increased in some patients with essential hypertension (Canessa et al, 1980; De la Sierra et al, 1988; Laurenzi and Trevisan, 1989). The activity of this transporter is

related to the presence of a family history of hypertension (Canali et al, 1981; Carr et al, 1989; Carr et al, 1990a). Its increased activity in normotensive individuals may represent a marker for a predisposition to hypertension (Carr et al, 1990a). In the general population, there is a continuous and bimodal distribution pattern of the Na^+-Li^+ counterpart (Boerwinkle et al, 1986). Subjects in the upper mode of the distribution curve have an altered sodium transport pathway that has been related to the development of hypertension (Boerwinkle et al, 1986). The prevalence of the increased Na^+-Li^+ counterpart in the hypertensive population depends on racial and ethnic factors (Canessa et al, 1984; Bunker et al, 1987). In addition to the genetic factors, a number of environmental variables affect Na^+-Li^+ countertransport. These include hypokalaemia hypercholesterolaemia, diabetes, exercise, renin status, pregnancy and oral contraceptives (Worley et al, 1982; Brugnara et al, 1983; Beuckelmann et al, 1985; McDonald et al, 1990; Carr et al, 1990b).

The pathogenetic role of the Na^+-Li^+ countertransport in essential hypertension is unknown. It may be a genetic marker of hypertension or it may represent Na^+-H^+ exchange in cells other than erythrocytes (Weder, 1986; Duhm and Behr, 1987).

1.3.9.2 (ii) Sodium-hydrogen exchange

The Na^+-H^+ exchanger has many functions, but one of the most important is the sodium uptake pathway on the apical membrane of the proximal tubule epithelial cell (Van Dyke and Ives, 1988). It therefore plays a vital role in sodium transport in the proximal tubule. Although it has been proposed that the erythrocyte Na^+-Li^+ exchanger represents Na^+-H^+ exchange in other cells, there are a number of differences between these systems. Na^+-H^+ exchange is amiloride sensitive whereas Na^+-Li^+ exchange is resistant to this agent (Kinsella and Aronson 1981; Kahn, 1987). The data now suggest that the renal Na^+-H^+ exchanger and the erythrocyte Na^+-Li^+ countertransport system are not identical, but may represent different modes of function of the same or related transport systems (Ives, 1989; Ives, 1990).

Increased Na^+-H^+ exchange activity has been demonstrated in cultured vascular smooth muscle cells from SHR and in platelets from hypertensive subjects (Livine, 1987; Berck et al, 1989). Mechanisms which increase Na^+-H^+ exchange activity include cell pH, cell sodium concentration, growth factors, vasoconstrictors (angiotensin II, vasopressin, endothelin), hormones (thyroid and glucocorticoids), osmotic agents and environmental factors (Grinstein and Rothstein, 1986; Van Dyke and Ives, 1988; Cogan, 1990). Whether increased Na^+-H^+ exchange in essential hypertension

is a primary abnormality in hypertension or due to the factors indicated above is unclear. Whatever the mechanism of the increased activity of this transport system, it has several effects on vascular smooth muscle which may be important in the development and maintenance of hypertension. These include: increased cell sodium, increased cytosolic pH and increased cell volume. According to the Blaustein hypothesis, increased intracellular sodium elevates cytosolic calcium by its effects on the Na^+-Ca^{2+} exchanger (Blaustein, 1977). Cell alkalinisation stimulates DNA synthesis with increased cell growth and differentiation (Frelin et al, 1990). Increased cell volume results in decreased vessel diameter with consequent increased peripheral resistance.

1.3.9.2 (iii) Sodium-calcium exchange

Sodium-calcium exchange activity in smooth muscle membranes appears to be very low, less than 1% of that found in cardiac myocyte membranes (Morel and Godfraind, 1984). This may be due to loss of activity during preparation, or Na^+-Ca^{2+} exchange may not be abundant in vascular smooth muscle. The role (if any) of this transport system in essential hypertension is unknown.

1.3.9.2 (iv) Sodium-potassium cotransport

Data have demonstrated altered Na^+-K^+ cotransport in

essential hypertension. Early studies demonstrated a lower V_{max} for the outward component of the Na^+-K^+ cotransport in erythrocytes of hypertensive patients compared to normotensive controls (Garay et al, 1980; Cusi et al, 1981). Subsequent studies reported a higher V_{max} for the influx and efflux or basal efflux of erythrocyte Na^+-K^+ cotransport of hypertensives (Wiley et al, 1984; Weder et al, 1987). A recent report has shown a negative correlation between Na^+-K^+ cotransport and blood pressure in a general male population (Hajem et al, 1990). These conflicting results may be related to technical differences. Genetic factors may explain the altered Na^+-K^+ cotransport in hypertension. The presence of low or high effluxes in erythrocytes from hypertensives is associated with similar findings in their normotensive, first degree relatives (Smith et al, 1984).

1.3.9 2 (v) Passive sodium inflow

In essential hypertensive patients, the increased intracellular sodium may be due to enhanced sodium 'leak' or increased cell membrane permeability to sodium. This has been demonstrated in erythrocytes and lymphocytes (Pederson et al, 1983; Linjnen et al, 1984).

The extensive literature on cellular sodium and its transport in essential hypertension has demonstrated that the topic is complex and not entirely resolved. What can

be concluded is that in essential hypertension, leukocyte sodium is increased, most probably due to abnormal sodium pump activity. Whether erythrocyte sodium pump, sodium-potassium cotransport, sodium-hydrogen exchange and sodium-calcium exchange mechanisms are altered in hypertension are unresolved. Genetic factors are important, but not exclusive, determinants of both sodium-potassium cotransport and sodium-lithium countertransport. The sodium-lithium system appears to be genetically linked to hypertension. Whether these sodium transport abnormalities are directly involved in the pathogenesis of hypertension remains unknown.

1.3.10 Sodium-calcium interactions

Special relationships exist between cellular calcium metabolism and sodium homeostasis. The link between these two cations may be important in hypertension. Two hypotheses relating to sodium and calcium interactions in the aetiology of hypertension have been proposed. The first is based on the premise that an abnormality in sodium metabolism is the primary defect and that defective calcium regulation at the cellular level is a secondary event. The second hypothesis is that the primary abnormality in essential hypertension is in cellular calcium regulation and that changes in cellular and systemic sodium metabolism are due to altered cellular calcium homeostasis (Aviv, 1988; Aviv and Lasker, 1990).

The first hypothesis was based on the fact that hypertension occurs because of the inability of the kidneys of susceptible subjects to excrete 'excess' salt (Dahl et al, 1960; de Wardener and MacGregor, 1982; Blaustein and Hamlyn, 1984). This results in the secretion of 'natriuretic' hormone which elevates blood pressure. This hypothalamic factor inhibits Na^+-K^+-ATPase in many cell types and organs (Iwaoko et al, 1991). Its secretion is stimulated by extracellular volume expansion. Inhibition of Na^+-K^+-ATPase in the renal tubules results in natriuresis with 'normalisation' of the extracellular fluid volume (Blaustein and Hamlyn, 1984). The non-specific hypothalamic inhibitor also inhibits the sodium pump in other cells, including neurons and vascular smooth muscle cells (Blaustein and Hamlyn, 1984; Iwaoka et al, 1991). In these cells, decreased Na^+ pump activity results in increased intracellular calcium. Two mechanisms are involved:-

i) voltage gated channel activation by membrane depolarisation (Haddy and Pamnani, 1980).

ii) decreased Na^+-Ca^{2+} exchange because of a reduced transmembrane sodium gradient (Blaustein, 1977; Blaustein and Hamlyn, 1984).

Increased vascular smooth muscle calcium would result in vascular contraction and increased cytosolic calcium in

adrenergic neurons enhances neurotransmitter release.

The second thesis is based on findings that Na^+-H^+ activity is increased and intracellular calcium is raised in platelets of essential hypertensive patients (Erne et al, 1984; Rosskopf et al, 1991). Stimulation of cytosolic calcium mobilisation also induces Na^+-H^+ antiport activity (Aviv and Lasker, 1990). This results in increased sodium entry and accelerated pump activity (Little et al, 1986). In the renal proximal tubules this is expressed as augmented sodium reabsorption (Harris et al, 1986). Consequently, salt sensitivity may be a manifestation of these alterations (Kurtz and Morris, 1990). In the juxta glomerular cells, raised cytosolic calcium and activation of the Na^+-H^+ exchange results in decreased renin secretion (Fray et al, 1977).

The calcium Na^+-H^+ antiport link also plays a role in the hyperplastic/hypertrophic features of hypertensive vascular smooth muscle cells (Schwartz and Ross, 1984). Vasoactive agents, growth factors and serum exert their cellular effects by increasing cytosolic calcium and stimulating the Na^+-H^+ antiport (Owen and Villereal, 1983). The relationship between raised cytosolic calcium and hyperactivity of the Na^+-H^+ antiport may be the common factor for the enhanced vasoactive response, the growth process of vascular smooth muscle and salt sensitivity, which are all characteristic features of essential

hypertension.

1.3.11 Cellular 'growth' in essential hypertension

A number of growth characteristics are typical of cells in SHR and essential hypertensive patients. These include the hypertrophy and hyperplasia of vascular smooth muscle cells, the proliferation of sympathetic terminals supplying the arterial bed, erythrocytosis and increased platelet number in SHR (Scott and Pang, 1983; Bruschi et al, 1986; Lever, 1986; Postnov, 1990). The growth potential and membrane abnormalities of these cells in essential hypertension can be attributed to a combination of genetics, tissue growth factors and hormones (Rozengurt, 1986; Schelling et al, 1991). Cells have the intrinsic ability to proliferate (Yamori et al, 1984). The altered expression of cellular oncogenes (proto-oncogenes) could explain part of the cellular membrane abnormalities described in hypertension (Postnov, 1988). The abnormally functioning proto-oncogenes (c-myc, c-ras and c-fos) may intervene in the phosphoinositide signal transduction pathway, thereby activating protein kinase C, (Jove and Hanafusa, 1987; Postnov, 1990). It has recently been hypothesised that an increase in cytosolic free calcium concentration triggers the expression of proto-oncogenes, which in turn directs the characteristic increase in protein synthesis (Marban and Koretsune, 1990).

1.3.12 Summary

The investigation of cellular abnormalities in human hypertension has relied on cells other than those from the vascular smooth muscle itself. Since platelets have characteristics in common with smooth muscle cells, they are a suitable model for investigation of hypertension and many studies now use platelets as the cell study model. The cellular abnormalities that have been described in essential hypertension include altered Na^+-K^+ pump activity, elevated intracellular Na^+, abnormal Na^+-Li^+ countertransport and raised intracellular Ca^{2+}. Since intracellular Ca^{2+} is an important determinant of vascular smooth muscle contractility, changes in cytosolic Ca^{2+} levels will ultimately affect peripheral resistance and blood pressure. The increased Ca^{2+} may be due to the opening of membrane voltage or hormone dependent Ca^{2+} channels or to the mobilisation of Ca^{2+} from intracellular storage sites. The second messenger for the release of intracellular Ca^{2+} is Ins (1,4,5) P3 and its site of action is the sarcoplasmic reticulum. Many vasoactive hormones which may participate in the development or maintenance of hypertension exert their contractile effect through production of Ins (1,4,5) P3. Ins (1,4,5) P3 is raised in many cell types in hypertensive patients. Inhibition of Ins (1,4,5) P3 by increased cGMP results in vasodilation. Three groups of vasodilators produce their effects through increasing cGMP synthesis. These are the

nitrovasodilators, ANF and the endothelium dependent vasodilators - which stimulate EDRF (nitric oxide) synthesis. In hypertension, where the vascular wall is abnormal, there is impairment of endothelium-dependent relaxation and endothelium-dependence contraction becomes prominent. The constricting response predominates despite the continuous release of EDRF. Altered proto-oncogene expression may result in cell membrane abnormalities and potentiated cell growth in essential hypertension.

Proto-oncogene expression, growth factors, endothelial function, phosphoinositide metabolism and cellular handling of cations are interdependent. Modification of these factors may account for the multifaceted membrane abnormalities associated with essential hypertension. Whether the abnormalities found in the cells studied also occur in vascular smooth muscle have yet to be determined.

1.4 Essential hypertension - causes and associations

Hypertension arises from interactions between genetic and environmental factors. These factors exert their effects via physiological control mechanisms which are themselves interrelated.

1.4.1 Biological factors

1.4.1.1 Genetic factors

The greatest single determinant of high or low pressure is probably genetic. Black and white family studies have demonstrated associations between blood pressures of siblings and between parents and children as young as a few days old (Zinner et al, 1971; Murphy, 1973; Zinner, 1985; Ibsen et al, 1980). Correlations between spouses are less consistent and none are seen between parents and recently adopted children (Sackett et al, 1975; Biron and Mongeau, 1978). Identical twin blood pressures correlate more strongly than those of non-identical twins (Feinleib, 1975). Whether or not the higher prevalence of hypertension in blacks is genetically determined is unclear. The phenotypic expression of a genetic tendency to elevated blood pressure only operates in the presence of appropriate environmental stimuli.

1.4.1.2 Effects of age

Arterial pressure is related to height, weight and age. Multiple regression analysis shows that age has a larger independent effect than any other measure of development (Beresford and Holland, 1973). In all races and in all societies, pressures rise through childhood and adolescence until physical maturity is reached. Thereafter, in

westernised societies, pressure increases with age (Hart, 1980). In small, isolated, poor and economically undeveloped communities, there is no rise of blood pressure with age (Akinkugbe, 1972). The best predictor however of the role of blood pressure acceleration is the pressure already attained i.e. the higher the pressure, the more rapidly it will increase with age (Hart, 1980). The rise in pressure with age is multifactorial. Age includes an accumulation of environmental effects and the effects of genetically determined (and sex linked) ageing processes in the body (Henry and Cassel, 1969).

1.4.1.3 The adrenergic drive

The association between stress, autonomic response and hypertension has been extensively studied and Osterfield and Sheketle (1967) concluded that acute psychological stress may initiate sudden and transient elevations of blood pressure in some individuals. There is no evidence however that this is a maintaining cause.

1.4.1.4 Body fat and obesity

The positive relationship between body fat and blood pressure levels has been consistently demonstrated in cross-sectional and longitudinal population studies from early childhood to old age (Beilin, 1987). This relationship is not restricted to the obese, but is

continuous throughout the entire range of fat distribution (Arkwright et al, 1982). The Framingham study, carried out over 16 years, demonstrated that each 10% weight gain was associated with a 6,5 mmHg increase in systolic blood pressure (Ashley and Kannel, 1974). Alterations in body weight in childhood, adolescence or early adult life are related more strongly to blood pressure increases than the initial or final weight (Kannel et al, 1967). Since fat adolescents usually become fat adults, children who have higher blood pressures with high fat levels are more likely to become hypertensive adults.

1.4.2 Dietary factors

1.4.2.1 Sodium and potassium

The important role of sodium and potassium and their relations to volume control and vascular smooth muscle activity in blood pressure regulation are undoubted (see Section 1.3.7), but the role of dietary sodium and potassium in determining blood pressure levels in the community is unclear. Most within population studies of dietary sodium in relation to blood pressure have shown no association or even an inverse correlation (Staessen et al, 1981; Kesteloot et al, 1984b). In some positive studies, the effects of sodium disappear after adjusting for body weight. The conflicting data that has been presented in the literature may be due to the fact that subsets of the

population may be salt sensitive and others salt resistant in terms of blood pressure change. It appears that sodium restriction to about 70 mmol/24 hr is of value in hypertensives and may reduce the need for drug therapy (Beilin, 1987).

Interactions between effects of dietary sodium and potassium may be important in view of their potentially opposing and interacting effects on blood pressure. The blood pressure lowering effects of potassium supplements in hypertensive subjects appears to depend on a high sodium intake (Smith et al, 1988) (see Section 8.1.1).

1.4.2.2 Magnesium and calcium

Magnesium sulphate is an effective antihypertensive agent when administered intravenously (Singh et al, 1989). Epidemiological and clinical studies associating magnesium intake and blood pressure levels are however conflicting (Resnick et al, 1985; Cappuccio et la, 1985; Touyz et al, 1989). Low dietary calcium intake may be associated with high blood pressure (see Sections 8.1.1.3 i and 8.1.1.4 i). Clinical trials involving calcium supplements have been negative (Zoccali et al, 1984; Cappuccio et al, 1987). Further, larger scale, randomised placebo-controlled trials are needed in normotensive and hypertensive subjects.

1.4.2.3 Alcohol

The effects of alcohol on blood pressure are seen in both sexes of all races studied and with different types of alcoholic beverages (Beilin, 1987). The effect is age dependent and appears to be independent of, but additive to obesity and the use of oral contraceptives (Arkwright et al, 1982; Beilin and Puddey, 1984). A cause and effect relationship has been confirmed by randomised trials in both normotensive and treated hypertensive individuals (Puddey et al, 1985). It is still unclear whether the dose-response curve relating the amount of alcohol consumed to blood pressure levels is linear throughout the drinking range, or J-shaped (Beilin, 1987; Klatsky, 1986). The exact mechanism by which alcohol increases blood pressure is unknown.

1.4.2.4 Other dietary factors

Vegetarians have lower blood pressures and less of an increase with age compared to the general population of meat eaters despite a high sodium intake (Rouse and Beilin, 1984). In a tightly controlled study comparing the distribution of blood pressure in Seventh Day Adventist Vegetarians and Morman omnivores, Rouse and Beilin (1983) showed that the prevalence of hypertension was only 2% in the vegetarians compared to 10% in the meat eaters. Dietary constituents that may account for these differences

include the absence of fish, meat and poultry, higher consumption of magnesium, calcium, potassium and vitamin C and a lower intake of satured and mono-unsatured fats, cholesterol, total protein, iron and vitamin B_{12}.

Other nutritional factors that may have an effect on blood pressure include dietary fibre and marine oils (Burr et al, 1985; Anderson and Tietyen-Clark, 1986; Knapp et al, 1989; Wing et al, 1990). Also increasing the polyunsatured to satured fat ratio may lower blood pressure (Puska et al, 1983). However, the independent effects of fibre and fats on blood pressure are still unproven.

1.4.3 Behaviourial and psychological factors

1.4.3.1 Physical activity

Regular physical exercise may have a protective effect on blood pressure elevation (Seals and Hagberg, 1984; Jennings et al, 1986). Jennings et al (1986) demonstrated that in mild hypertensive subjects, systolic blood pressure fell 10-12 mmHg after 3 weekly sessions for 4 weeks of 65-70% maximal exercise lasting 45 minutes each.

1.4.3.2 Psychological factors

Epidemiological studies of blacks and whites have demonstrated that in high stress communities, a tendency to

hold anger in when provoked (suppressed hostility) was associated with higher blood pressure and a greater prevalence of documented hypertension compared with those who reported an 'anger-out' coping style (Harburg et al, 1979). Associations between hypertension and Type A/B personalities have also been reported (Smyth, 1990).

1.4.3.3 Environmental stress

The term 'hypertension' itself implies a disorder initiated by social and personal stress beyond the limits of tolerance. High blood pressure is the disease of high pressure societies and many studies have shown higher pressures in urban migrants than in rural populations of origin (Akinkugbe, 1969; Sever et al, 1980; Poulter et al, 1984). Of the environmental factors related to hypertension, socioeconomic status has received the most attention. This variable has provided a consistent association between social status and blood pressure levels among both whites and blacks (Syme et al, 1974; Dyer et al, 1976; Seedat et al, 1982). The Hypertension Detection and Follow-up Program (HDFP) Cooperative Group examined the relationship between education and hypertension prevalence and showed that education was inversely related to hypertension in both race and gender groups (HDFP, 1977). This inverse education-hypertension correlation, however, is not uniformly evident across all ages. As age increased, educational status became less predictive of

blood pressure levels (HDFP, 1972; HDFP, 1977).

Reasons for the high prevalence rates of hypertension among lower socioeconomic groups remain unresolved. Possibly, factors which contribute to high blood pressure may be more prevalent in the lower socioecoromic status communities, for example:- inadequate nutrition, social instability, poor housing etc. However, not all subjects of lower socioeconomic status develop hypertension. Other biological and psychosocial variables must interact with the factors of low socioeconomic status to make some people more susceptible to developing high blood pressure.

Socioecological stress, social disorganisation, and low socioeconomic status may contribute to the differences of essential hypertension between blacks and whites (see Section 1.5 for details regarding hypertension in blacks).

1.4.4 Summary

Although essential hypertension has been defined as a progressive rise in arterial blood pressure occurring with age for which there is no obvious underlying aetiology a number of causes, associations and predictors have been defined (Horan and Lenfant, 1990). Within Westernised communities the major causes of increased blood pressure appear to be related to excess body fat, regular alcohol consumption, dietary factors, physical activity and

socioeconomic status. Lipid abnormalities and altered glucose tolerance are also associated with hypertension (Weber et al, 1991). A combination of these factors with an underlying genetic predisposition to developing hypertension is probably the major determinant of blood pressure in individuals in particular and populations in general.

1.5 Hypertension in blacks

Epidemiological, clinical and pathophysiological differences have been demonstrated when black and white hypertensive groups are compared. These differences may have important therapeutic and prognostic implications.

The prevalence of hypertension in black populations is disproportionately higher than that in whites in urbanised areas of Africa, the United States and in the West Indies (Schneckloth, 1962; Akin Kugbe, 1972; HDFP, 1979; Aubert and Blount, 1990). Hypertension occurs at an earlier age in blacks and is usually more severe than in whites (Joint National Committee for the Detection, Evaluation and Treatment of High Blood Pressure, 1988). The higher incidence and severity of hypertension in blacks has been associated with more congestive cardiac failure, left ventricular hypertrophy and end stage renal disease (Saunders, 1987). Factors that may be responsible for these differences include genetics, environmental variables

(dietary and socioeconomic differences) and pathophysiologic mechanisms.

1.5.1 Genetic factors

High blood pressure in blacks is due to genetic factors modified by environmental variables (Grim et al, 1980; Check, 1986). American blacks with darker skin tend to have higher blood pressures than those with lighter skin colour (Harburg et al, 1978; Tyroler and James, 1978). These factors however may be associated with low socioeconomic status and other environmental variables (Tyroler and James, 1978). The differences between black and white hypertension are not simply due to genetics and other variables must play a role.

1.5.2 Behavioural and social factors

Behavioural factors such as anger-coping e (suppressed hostility) and John Henryism (an active, effortful stress coping style or hard work and determination against overwhelming odds), and social factors such as low socioeconomic status, socioecological stress, lack of social support, and the processes of acculturation may all contribute significantly to the higher prevalence and increased severity of hypertension in blacks (Harburg et al, 1979; James, 1985; Anderson et al, 1989).

1.5.3 Dietary factors

Different dietary factors have been implicated in the aetiology of hypertension in blacks and whites.

1.5.3 (i) Sodium

The exact role of excessive sodium consumption in the aetiology of hypertension remains unclear (Pickering, 1981). Some studies have reported high dietary sodium intake among urban blacks (Desor et al, 1975; Karp et al, 1980). Others however have suggested a lower, rather than a higher intake than whites (Grim et al, 1980; Frisancho et al, 1984). Blacks may not ingest more dietary sodium than whites, but they appear to excrete less sodium than whites for a specific amount of sodium ingested. Hence, even at low levels of sodium consumption, the pressor effects of sodium may be greater for blacks than for whites (Saunders, 1987).

1.5.3 (ii) Potassium

A high sodium to potassium ratio as well as low levels of potassium intake are associated with hypertension (Frisancho et al, 1984). Blacks consume significantly less potassium and have a higher dietary sodium:potassium ratio compared to whites (Grim et al, 1980; Frisancho et al, 1984). These factors may be important in the black-white

hypertension differences.

1.5.3 (iii) Calcium

Low levels of calcium intake may be associated with hypertension. Blacks have a higher dietary sodium:calcium ratio compared to whites (Langford et al, 1980). Also, calcium supplementation appears to be effective in lowering blood pressure in hypertensive blacks (Zemel et al, 1986). Thus, low dietary intake of calcium may be important in the pathogenesis of black hypertension.

1.5.3 (iv) Magnesium

There is no reported data in the literature examining magnesium intake in black and white hypertensive groups. Since potassium rich diets are also rich in magnesium, it may be suggested that if blacks have a low potassium intake they probably also have a low magnesium intake.

In summary, potassium (and possibly magnesium) intake is lower, the sodium - potassium ratio is higher and the sodium - calcium ratio is greater in blacks compared with whites. These factors may be important in the pathogenesis of black hypertension.

1.5.4 Pathophysiology of hypertension in blacks

1.5.4.1 Renal function

Two aspects of the renal system have been extensively studied in black hypertension. These include the renin-angiotensin system and sodium metabolism.

1.5.4.2 Low renin hypertension

As a group, black hypertensives have lower plasma renin levels than white hypertensives (Wisenbaugh et al, 1972; Gillum, 1979; Freis et al, 1983). The differences in renin profiles between the two groups appears to be age related. Hypertensive subjects, both black and white, under 30 years have high or normal renin levels, whereas in black hypertensives older than 50 years, there is a high incidence of low-renin hypertension (Brunner et al, 1943). Grim et al (1980) reported that black hypertensives younger than 40 years had significantly higher plasma renin levels than patients older than 40 years.

Suppressed renin activity in black hypertensives may be due to defects in the normal homeostatic response to renin release, to changes in renal blood flow, to altered plasma volume and to a greater salt retaining capacity (Saunders, 1987). It has also been suggested that atrial natriuretic factor may suppress renin in the presence of salt retention

(Check, 1986). Resnick et al (1983) reported an association between low renin essential hypertension and decreased concentrations of serum ionised calcium. Although recent reports have shown altered cellular calcium handling in black hypertensives, it has not yet been demonstrated that low renin or black hypertensives have raised intracellular calcium levels compared to normal-to-high renin or white hypertensives. Data with respect to plasma renin status in black hypertension is not consistent, and some reports have failed to identify differences in renin activity between black and white hypertensive subjects (Kaplan et al, 1976; Luft et al, 1977).

1.5.4.3 Renal sodium excretion

Blacks have a higher blood pressure increase at lower levels of sodium loading and a greater retention of sodium compared to whites (Saunders, 1987). The enhanced capacity for renal reabsorption of dietary or intravenous loads may be related to the kallikrein-kinin system. Activity of this system, via secretion of renal kallikrein and ultimately kinin, produces both natriuresis and renal vasodilation (Warren and O'Connor, 1980). A deficiency in the natriuretic vasodilatory renal kallikrein-kinin system may result in a higher incidence of low-renin hypertension associated with sodium retention in blacks (Warren and O'Connor, 1980; Grim et al, 1980). During unrestricted

sodium consumption, urinary kallikrein was greater in white normotensives compared to black normotensives, black hypertensives and white hypertensives (Levy et al, 1977). When sodium intake was restricted, all groups showed increased urinary kallikrein excretion, but the increase was blunted in the black hypertensive subjects (Levy et al, 1978).

Despite the suppression of aldosterone and stimulation of ANF (triggered by an expanded plasma volume), there is excessive sodium retention in hypertensive blacks (Saunders, 1987; Sowers et al, 1988). Besides the kallikrein-kinin involvement, the excess retention of sodium may be due to the lower total renal blood flow and higher renal vascular resistance observed in blacks compared with whites who have similar blood pressure levels (Levy et al, 1978; Frohlich et al, 1984). Defective stimulation of dopamine (a vasodilator with natriuretic properties) may also influence the sodium retention in black hypertension (Sowers et al, 1988). Blacks have an exaggerated decrease in glomerular filtration rate which may also contribute to the racial differences in sodium excretion (Luft et al, 1980).

1.5.4.4 Plasma volume

Results from studies examining blood volume in black and white hypertension have been conflicting. Some reports

have demonstrated increased plasma volume in black hypertensive subjects, while others have not found racial differences (Chrysant et al, 1979; Messerli et al, 1979; Dunn et al, 1983). Since the measurement of plasma volume is relatively imprecise, it may be difficult to demonstrate subtle racial differences that may be present.

1.5.4.5 Salt sensitivity

Luft et al (1979a) examined the effects of volume expansion (150 mg sodium per day and intravenous infusion of normal saline) and volume contraction (10 mg sodium per day and three 40 mg doses of furosemide) on blood pressure in blacks and whites. Following volume expansion, blacks excreted significantly less urinary sodium than whites. When blacks and whites consumed equivalent amounts of sodium, the black subjects showed consistently and significantly higher blood pressure than whites (Luft et al, 1979b). Blacks may be more susceptible to the blood pressure effects of sodium (i.e. they are more salt sensitive), compared to whites. This salt sensitivity in both normotensive and hypertensive blacks is due to the enhanced renal reabsorptive capacity for sodium and is manifested by excessive elevation in blood pressure following a high salt intake (Sowers et al, 1988). The pressor response may be related to the enhanced sodium retention as well as to the interaction of sodium with other blood pressure controlling factors (Dustan and Kirk,

1988). The higher dietary Na^+:K^+ ratio and increased urinary Na^+:K^+ in many black hypertensive subjects may be associated with the increased occurrence and severity of hypertension and salt sensitivity in blacks (Saunders, 1987). Hence the salt sensitivity could also be related to low dietary potassium intake.

1.5.4.6 Obesity

The greater prevalence of hypertension in blacks may be partly due to the greater prevalence of obesity in this group, especially among females (Stamler et al, 1978; Bunker et al, 1990). Obesity is positively correlated with blood pressure in African and American blacks (HDFP, 1977; Seftel et al, 1980; Seedat et al, 1982; M'buyamba Kabangu et al, 1986). Two large epidemiological American studies, however, reported that the relationship between blood pressure and weight was not as strong among blacks as it was in whites (Boyle et al, 1967; Tyroler et al, 1975). Hence, although the relationship between obesity and hypertension among blacks needs to be resolved, obesity in black females is associated with an increased risk for hypertension in this group.

1.5.4.7 Haptoglobin protein

There may be a genetic difference in the blood protein haptoglobin between black and white hypertensive subjects.

Studies have revealed that salt sensitive individuals were more likely to be homozygous for one haptoglobin genotype, while salt resistant subjects were predominantly homozygous for the other (Check, 1986). The importance of these differences is unclear.

1.5.4.8 Neurogenic factors

Hyperactivity of the sympathetic nervous system may play a role in the aetiology of hypertension (Abboud, 1982). Studies however have failed to report differences in noradrenaline levels between black and white hypertensive and normotensive groups (Lichtman and Woods, 1967; Sever et al, 1976; Sever et al, 1979).

1.5.4.9 Cell membrane cation transport defects

A number of differences in cellular ion regulation between blacks and whites have been identified. Erythrocyte studies have shown that blacks have lower Na^+-K^+-ATPase activity, Na^+-K^+ cotransport and Na^+-Li countertransport compared to whites (Aviv and Gardner, 1989). Some blacks have an accelerated sodium turnover that is related to enhanced activity of the Na^+-K^+ exchanger system (Kuriyama et al, 1988). Nakamura et al (1989) demonstrated that fibroblasts from blacks exhibit hyperresponsiveness to serum stimulation as compared with fibroblasts from whites. This enhanced responsiveness was associated with an

increased magnitude of intracellular calcium transients and accelerated calcium efflux.

These cellular factors may contribute to the accumulation of intracellular sodium and to the enhanced sodium reabsorption by the proximal tubules observed in blacks (Hall, 1990). If similar differences occur in vascular smooth muscle cells and renal tubular epithelium, the predisposition of blacks to salt-sensitive forms of essential hypertension can be explained.

1.5.5 Hypertension in South African blacks

Donnison reported in 1929 that

> 'Over two years at a native hospital in the South of Kavirondo district of Kenya, during which period approximately 1 800 patients were admitted, no case of raised blood pressure was encountered .....'

Today, over fifty years later hypertension is one of the most common cardiovascular diseases in South Africa, with one in four adult black patients with cardiac failure suffering from high blood pressure. Seedat (1981) reported that twenty-five percent of urban Zulus are hypertensive. By contrast, hypertension appears to be rare in rural black populations (Sever, 1980). According to Seftel (1973) after violence and accidents, hypertension and its complications are the most common cause of death among

Witwatersrand blacks. This significant increase in black hypertension may be attributable to the process of urbanisation. With environmental changes or acculturation there is a rise in blood pressure. The factors in the acculturation process which are responsible for the elevation of pressure include social stresses (alcoholism, violence, illegitimacy, overcrowding), obesity and high salt intake (Seftel et al, 1980).

The relationship between cations and hypertension in the black South African has recently provoked interest. Some studies have reported that urbanised black hypertensives have a low serum potassium which could be attributed to a high starch intake, habitual traditional purgation and low intake of proteins and vegetables (Sever et al, 1980; Mokhobo, 1982). Rural communities (where the incidence of hypertension is low) on the other hand, have high potassium intakes, usually through high fruit and vegetable consumption. Augmented salt intake has been implicated as a cause for the increased incidence of hypertension in city blacks (Seftel et al, 1980). It has however been shown that in Johannesburg blacks the salt intake is not excessive and that their blood sodium levels are not significantly raised compared to Johannesburg whites. Another study reported that dietary sodium and urine sodium/creatinine ratio were significantly raised in urban blacks, but there was no within-population relationship between either dietary sodium or urine sodium/creatinine

ratio and blood pressure (Cohen et al, 1982).

Little data are available about magnesium, calcium and blood pressure with respect to hypertension in South Africa. Results of a recent study have shown that in a group of hypertensive Johannesburg black male labourers, there is a significantly lower concentration of serum and erythrocyte magnesium and serum calcium concentrations when compared to their normotensive counterparts (Touyz et al, 1989).

Although sodium and potassium (and recently magnesium and calcium) intakes have been associated with the increased prevalence of hypertension in the urban black community, it is important to realise that urban and rural populations differ from each other in many other ways besides dietary intakes. Primitive individuals are smaller in size, do not become obese with age, are more physically fit and are genetically more homogeneous. Although little attention has been attributed to these differences it is likely that these are influencing factors with regard to the prevalence of hypertension.

Whatever the mechanisms of essential hypertension in the black South African, the condition is reaching epidemic proportions and early recognition and treatment of the disease is essential.

1.5.6 Summary

There is consistent and convincing evidence that hypertension and some of its complications are common in blacks in America, the United Kingdom, the West Indies and in urban Africa. In contrast, black subjects in rural Africa have little or no hypertension. The process of urbanisation is associated with major dietary changes, excessive salt intake, obesity and increased psychosocial stress. These factors in addition to altered regulatory mechanisms controlling blood pressure and a genetic predisposition to developing high blood pressure, are probably the major determinants of hypertension in blacks.

CHAPTER 2

HYPOTHESIS

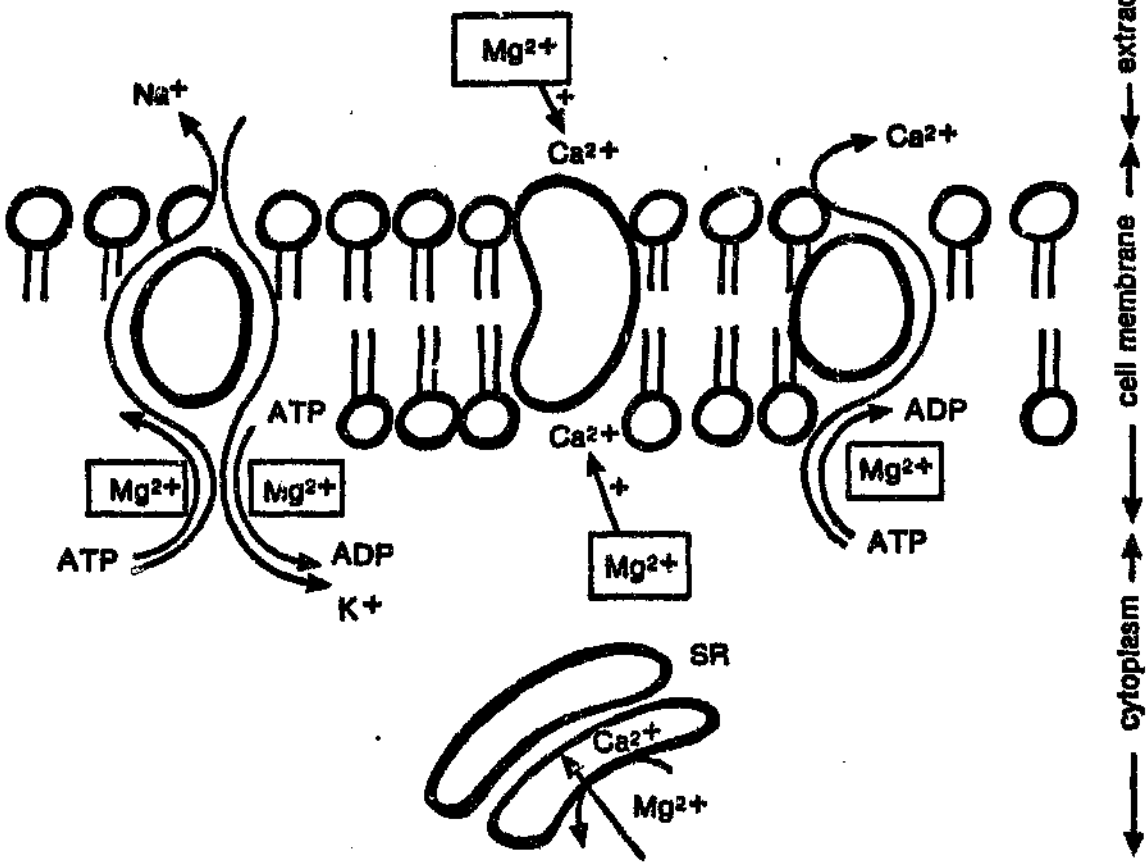


Figure 2.1.

Normal state. Mg^{2+} stabilises the cell membrane, stimulates Ca^{2+} binding and influences Na^{+}, K^{+} and Ca^{2+} transmembrane transport. SR = sarcoplasmic reticulum

2. HYPOTHESIS

1. The major determinant of increased blood pressure is increased total peripheral resistance.
2. Total peripheral resistance is influenced by vascular smooth muscle tone and contractility.
3. Vascular smooth muscle activity is determined by the intracellular calcium concentration. Increased intracellular calcium results in increased contractility.
4. Peripheral resistance is also influenced by the luminal diameter of the vessel.
5. Intracellular sodium concentration and water content may affect the vessel lumen. Increased intracellular sodium is associated with cell oedema, vessel wall engorgement, decreased lumen size and increased peripheral resistance.
6. In essential hypertension, intracellular sodium and calcium concentrations have been found to be increased.
7. The exact mechanisms responsible for these biochemical changes in hypertension are unknown.
8. Since cellular sodium, potassium and calcium metabolism are closely related to magnesium metabolism, abnormalities in magnesium homeostasis will affect the other cations.

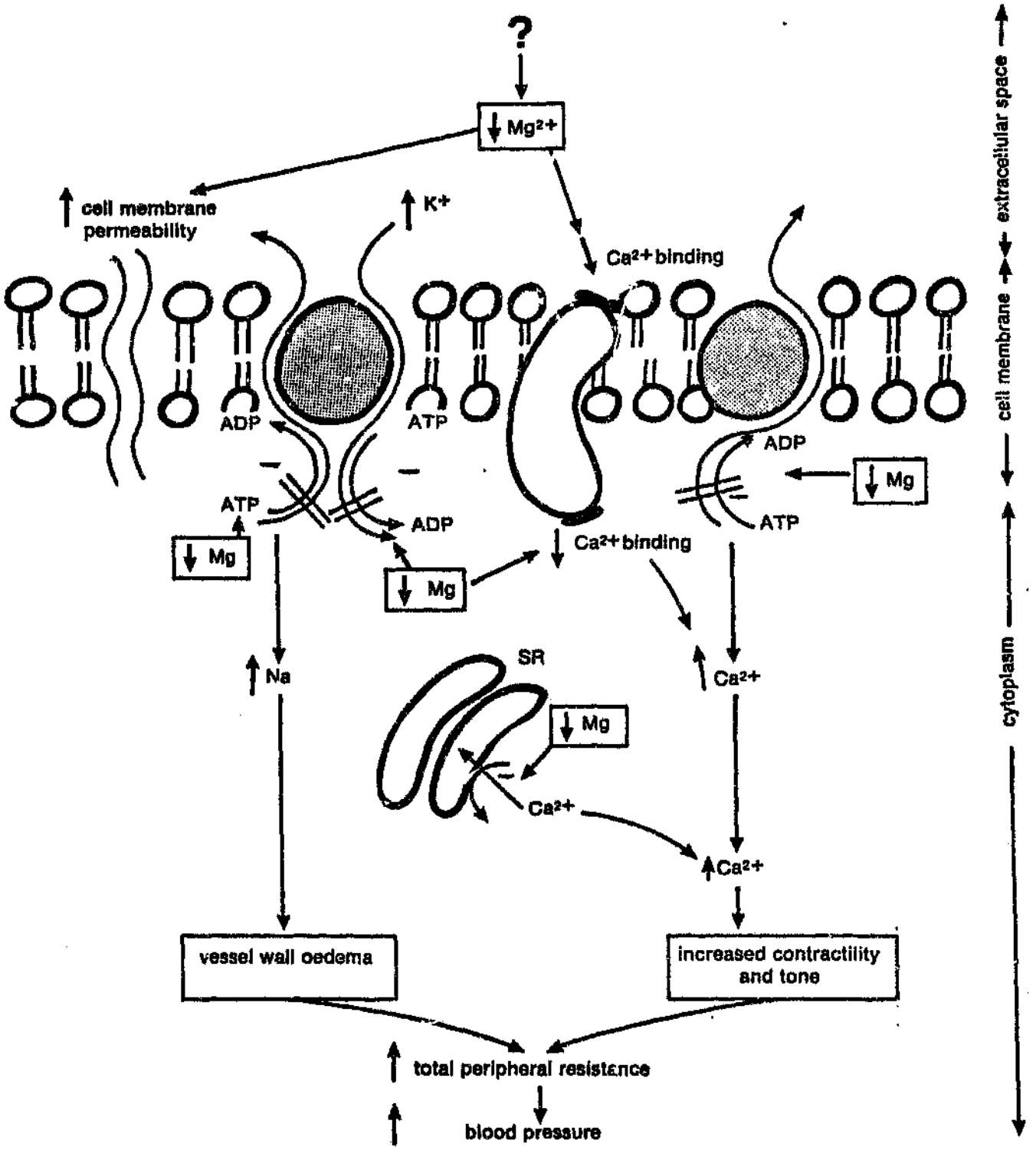


Figure 2.2 Magnesium defeciency. Magnesium deficiency results in increased intracellular Na^+ and Ca^{2+} concentrations. SR = sarcoplasmic reticulum; — = negative effect.

9. In normal states, magnesium enhances calcium binding, it stabilises the cell membrane and it activates the magnesium dependent pumps (Ca^{2+}-ATPase and Na^{+}-K^{+}-ATPase). The internal milieu is thus maintained (Figure 2.1).
10. In magnesium deficient states, cell membrane calcium binding is altered, cell membrane permeability is increased and CA^{2+}-ATPase and Na^{+}-K^{+}-ATPase activities are depressed. These abnormalities result in disrupted cellular cation homeostasis with consequent cytosolic sodium and calcium accumulation (Figure 2.2).

It is hypothesised that:-

a) magnesium deficiency and associated cellular abnormalities may play a role in the pathogenesis of essential hypertension.
b) the cellular defect is widespread occurring in different cells in all essential hypertensive patients and
c) the more severe the membrane lesion, the higher the blood pressure.

It will become evident in the following studies that the above hypotheses did not in fact hold true for all groups of hypertensive patients (see Chapter 14).

CHAPTER 3

AIMS OF THE STUDY

3. AIMS OF THE STUDY

This study is divided into two major sections. Section I deals with essential hypertension, and Section II with malignant hypertension. Each section comprises a human and animal experimental component.

3.1 Section I ESSENTIAL HYPERTENSION

3.1.1 Human study

The aims of this study were:-

1. To determine the relationship between extra- and intracellular monovalent and divalent cations and blood pressure in black and white patients with essential hypertension.
2. To assess cell membrane status in essential hypertension:-
   The paramaters studied included:-
   i) cell membrane adenosinetriphosphatase activity, and
   ii) calcium binding to cell membranes,
   in black and white patients with essential hypertension.
3. To determine the role that magnesium plays in cellular events in essential hypertension.

3.1.2 Experimental study

The aims of this study were to determine the effects of

dietary magnesium supplementation on blood pressure in spontaneously hypertensive rats and normotensive Wistar Kyoto rats.

3.2 Section II MALIGNANT HYPERTENSION

3.2.1 Human study

The aims of this study were:-

1. To assess the status of extra- and intracellular cations in patients with malignant hypertension.
2. To determine cell membrane adenosinetriphosphatase activity in patients with malignant hypertension.

3.2.2 Experimental study

The aims of this study were:-

1. To establish an experimental model of malignant hypertension.
2. To assess the cellular cation changes and cell membrane ion transport alterations that occur in the transition from benign to malignant hypertension.

CHAPTER 4

THE STUDY CELL MODELS

4 THE STUDY CELL MODELS

4.1 Platelets

The structures directly responsible for determining the peripheral resistance are the vascular smooth muscle cells. Ideally, cellular studies in essential hypertension should be performed on these cells. There are however a number of problems associated with the direct examination of vascular smooth muscle. These include the facts that:-

i) The vessel wall is complex comprising a variety of tissues including vascular smooth muscle.

ii) In vivo studies examining cellular cation metabolism using radioactive isotopes have been performed in large vessels such as the aorta and its branches. These vessels differ from the smaller resistance vessels and consequently may not be a good cell model for essential hypertension studies.

iii) The vascular wall undergoes structural changes when hypertension is present. It is thus difficult to differentiate whether alterations in the vascular smooth muscle cells are due to the increase in blood pressure or to intrinsic pathoaetiologic factors in hypertension.

iv) It is ethically and practically difficult to obtain samples of blood vessels from living normotensive and hypertensive individuals.

For these reasons, other cells have been studied. Blood is easily accessible and therefore most studies examining cellular events in hypertension have been performed on blood cells.

When studying cells other than those directly involved in the physiological mechanisms of vascular contraction a number of underlying assumptions need to be made:-

i) alterations in circulating blood cells are representative of a generalised phenomenon,

ii) if similar alterations occur in vascular smooth muscle cells, they can predispose or contribute to blood pressure elevation in susceptible subjects.

iii) in-vitro findings resemble in-vivo processes. (Swales, 1982; Aviv and Lasker, 1990).

Studies examining cellular cation regulation in essential hypertension have been performed on erythrocytes and leukocytes. These cells, and particularly mature erythrocytes are not representative of other cells. Platelets have recently been used to examine cellular changes in hypertension. Platelets exhibit several anatomical and functional characteristics similar to vascular smooth muscle cells and are therefore an ideal cell model to study in essential hypertension.

4.1.2 Similarities between platelets and vascular smooth muscle cells (VSM)

The main features which these two cell types share include:-

i) Both cell types possess an α_2-adrenoceptor system which, when inhibited, results in vasodilation of smooth muscle and inhibition of platelet activation (Erne et al, 1983; Bolli et al, 1983).

ii) Platelets and VSM cells have common receptors, including the 5-HT, prostaglandin and vasopressin receptors (Erne et al, 1985).

iii) Both cell types contract via active calcium-contractile protein coupling (Hinssen et al, 1978; Niederman and Pollard, 1975).

iv) There are similar calcium pools for regulation of cytosolic calcium - the dense tubular system in platelets and the sarcoplasmic reticulum in smooth muscle cells (Buhler and Resink, 1988).

v) Both platelets and vascular smooth muscle cells contain contractile proteins whose hormonal response is mediated through the 'second messenger' system of cyclic AMP and calcium and through calcium mobilisation by phosphoinositide hydrolysis (Bolli et al, 1983; Zavoico and Feinstein, 1984; Agranoff et al, 1983).

vi) The physiological alterations of platelets and VSM are closely related, e.g. release of thromboxane by platelets results in vasoconstriction, whereas release of prostacyclin by VSM inhibits platelet activation (Robertson et al, 1981; Moncada et al, 1976).

vii) Hypertensive states are associated with an increased risk for thromboembolic disorders (Kannel and Dawber, 1973).

Besides the similarity between these cell types, platelets have additional advantages over other tissues. They are clinically easily accessible and are a homogeneous cell line.

In this study, both platelets and erythrocytes were used as cell models. Platelets were studied because of their accessibility and resemblance to vascular smooth muscle.

4.2 Erythrocytes were studied as a baseline of comparison and also to determine whether alterations in hypertension are restricted to specific cell types.

SECTION I

ESSENTIAL HYPERTENSION

CHAPTER 5

EXPERIMENTAL DESIGN :

Studies on essential hypertension

5. EXPERIMENTAL DESIGN

5.1 Definitions

5.1.1 The population sample

Black and white normotensive and hypertensive adults of both sexes were studied. All subjects were resident in Johannesburg. The normotensive, hypertensive black and white subjects were matched for age, height and weight.

Definition of essential hypertension

The criteria for hypertension used in this study were based on data defined by the World Health Organisation (1978).

1. Systolic blood pressure (SBP) greater than 160 mmHg and/or
2. Diastolic blood pressure (DBP) greater than 95 mmHg and/or
   Mean arterial pressure (MAP) greater than 117 mmHg.

Mean arterial pressure was calculated from the standard formula:-

$$MAP = DBP + 1/3 (SBP - DBP).$$

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   $$MAP = DBP + 1/3 (SBP - DBP).$$

5.1.3 Subjects

5.1.3.1 Normotensive control subjects

5.1.3.1 (a) Black subjects

Male and female healthy normotensive volunteers from the nonmedical staff of the Johannesburg Hospital (a major academic hospital) were studied. All were older than 25 years and satisfied the following inclusion criteria:

Inclusion criteria

1. Systolic blood pressure below 140 mmHg.
2. Diastolic blood pressure below 90 mmHg.
3. No current or previous systemic illness.
4. No previous history of pregnancy associated hypertension or eclampsia.
5. No history of hospital admissions (except for obstetric reasons).
6. No antihypertensive medication.
7. No medication for the past six months.
8. Not malnourished.
9. Normal renal function.
10. Resident in Johannesburg for at least five years.

5.1.3.1 (b) White subjects

Healthy normotensive male and female white volunteers

entered into the study. They were obtained from the nonmedical staff of the University teaching hospitals. All were older than 25 years and all satisfied the same inclusion criteria indicated in Section 5.1.3.1 (a).

5.1.3.2 Essential hypertensive patients

5.1.3.2 (a) Black hypertensive patients

Black male and female patients with essential hypertension were studied. They were obtained from the Outpatient Departments of the Hillbrow and Johannesburg Hospitals (academic hospitals). The patients had all been referred to the hospitals by either private general practitioners, medical officers at municipal clinics or from the medical centre at the local labour bureau for full assessment and management of hypertension. On arrival at the Outpatient department, the subject's blood pressure was measured by the medical officer who was unaware of the study. Hypertension was diagnosed as systolic blood pressure above 160 mmHg and/or diastolic blood pressure greater than 95 mmHg on three separate occasions. (Reading 1 by private doctor; reading 2 by medical officer, reading 3 by the author). All patients who participated in the study satisfied the inclusion criteria stated in Section 5.1.3.1 (a) except that systolic blood pressure (SBP) had to be greater than 160 mmHg on three separate occasions, and/or diastolic blood pressure (DBP) greater than 95 mmHg on

three separate occasions.

5.1.3.2 (b) White hypertensive patients

The white hypertensive subjects included male and female patients from the Hypertension Clinic at the Johannesburg Hospital. As with the black hypertensive patients, hypertension was diagnosed if blood pressure was raised (according to the definitions in section 5.1.2) on three separate occasions. The inclusion criteria were the same as 5.1.3.1 (a).

5.1.3.2 (c) Diagnosis of essential hypertension

Only patients with diagnosed essential hypertension participated in the study. Secondary renal hypertension was excluded on the basis of

1. Normal general medical examination
2. Normal renal function (urinalysis and serum creatinine)
3. Normal renal sonar

5.2 Subject examination and collection of demographic data

5.2.1 History and questionnaire

The subjects were studied under standardised conditions by

the same medical doctor (RMT). Subject examination was performed every Monday and Tuesday morning (January 1988 to October 1989), between 08h30 and 14h00. A questionnaire which enquired about diet (24 hour recall), alcohol, smoking, occupation, education and medical history was completed by all the subjects.

No special dietary instructions were given, but all subjects were requested to refrain from smoking for at least two hours prior to blood pressure measurement.

5.2.2 Medical examination

The subjects all underwent a general medical examination. The following parameters were also measured on each individual:

1. Height: - without shoes, using a standardised centimetre marked tape measure.
2. Weight: - in underclothes only - using a standardised balanced scale.
3. Radial pulse - the right pulse was measured three times at five minute intervals. The mean value was recorded for analysis.
4. Urinalysis - prior to the medical examination, spot urine samples were collected for analysis. The urine was tested with the Combur-9-test sticks (Boehringer Mannheim). These strips determine for leukocytes, nitrite, pH, protein, glucose, ketones, urobilinogen,

bilirubin and blood in urine. Patients with abnormal dipsticks results were excluded from the study. Females who tested positive for blood due to menstruation were included.

5. Blood pressure.

5.2.3 Measurement of blood pressure

The same procedure was used to measure blood pressure in all the subjects. After a minimum of fifteen minutes rest, blood pressure was measured in the dominant arm with the subject in the seated position. A standard mercury sphygmomanometer which was regularly calibrated was used. Three readings, five minutes apart were taken. Systolic and phase V diastolic pressures corresponding respectively to the first and fifth phases of the Korotkoff sounds were measured. For subjects with an arm girth less than 35 cm, the standard size cuff was used. For subjects with arm girth greater than 35 cm, the large cuff was employed.

To minimise interoperator error, every tenth hypertensive reading for each operator was noted and compared: also the means $\pm$ SD of twenty two readings for each doctor was computed and compared using the Students-t-test. There were no significant differences between the mean values ($t = 0,06$; $p < 0,0001$), and the "tenth readings" did not vary between doctors.

5.3 Blood specimen collection

5.3.1 Patient preparation

Venous blood was obtained from the median cubital vein in the antecubital fossa, in the non dominant arm. The skin of the cubital fossa was cleaned with sterile isopropyl alcohol swabs (Webcol^{R}-Macmed, S.A.).

Various biological factors can affect the cation and protein concentrations of serum and plasma. These include posture of the patient, immobilisation, exercise, circadian variation, smoking and venous stasis at the time of blood sampling. Ionised calcium concentrations are lower in immobilised individuals compared to ambulatory subjects. (Robertson 1981). Exercise or muscular activity increases plasma protein and cation levels (Renoe, 1980). Stasis causes water efflux from the vascular compartment, increasing serum protein and cation concentrations. (Renoe, 1980). For these reasons, in this study, blood was collected in the recumbent position, without the use of a tourniquet.

5.3.2 Venepuncture

Fifty millilitres of blood was obtained using vacuated blood tubes and an 21-gauge needle. Sterile, lithium

heparin (0,2 mg/ml blood) coated glass tubes (Vac-U-test, Radem Laboratories Eqmt. Sandton, S.A.) were used. The vacuated tubes were employed because they are easy to manipulate and there is less likelihood of contamination of their outside with blood.

Forty millilitres of blood was immediately transferred to a 50 ml polyurethane tube containing 7 ml acid citrate anticoagulant (75 mm trisodium citrate, 42 mm citric acid, H_2O, 138 mm dextrose) (Dacie, 1984).

The citrated blood was used for all platelet studies and the heparinised blood for serum and erythrocyte analysis. The blood samples were mixed by gentle inversion and were centrifuged within 2 to 3 hours of collection. Details of anticoagulants used are described in Appendix B.

CHAPTER 6

GENERAL MATERIALS AND METHODS

6. GENERAL MATERIALS AND METHODS

In this study the following biochemical parameters were measured:-

1. Serum Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} concentrations

2. Platelet Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} concentrations

3. Erythrocyte Mg^{2+}, Ca^{2+}, Na^{+} and K^{+} concentrations

4. Platelet membrane activity of Na^{+}-K^{+}-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase

5. Erythrocyte membrane activity of Na^{+}-K^{+}-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase

6. Calcium binding to the outer cell membranes of platelets

7. Calcium binding to the outer cell membrane of erythrocytes

Magnesium and Ca^{2+} were determined by atomic absorption spectrophotometry (AAS) (Appendix G).

Sodium and K^{+} were determined by flame photometry (Appendix G).

Cell membrane ATPase activity was determined by measuring the amount of inorganic phosphate released when exposed to ATPase (Appendix L).

Cell membrane Ca^{2+} binding was determined by measuring the amount of Ca^{2+} removed from the outer cell membranes when exposed to various chelating agents (Appendix M).

Chemicals and reagents used in this study

The sources of chemicals and reagents used in this study are listed in Appendix C. All chemicals were of the highest purity grade unless otherwise stated.

CHAPTER 7

STATISTICAL ANALYSIS

7 STATISTICAL ANALYSIS

The following general statistical analyses were performed:-

1. Descriptive analysis.
2. Comparative studies.
3. Correlation studies.
4. Precision studies.

7.1 Descriptive analysis

The data are presented as means $\pm$ 1 standard deviation. The number of subjects is represented by 'n'.

7.2 Comparative analysis

To determine whether age or mean arterial pressure (MAP) should be considered as a covariate, it was tested whether the groups differed with respect to these two factors. Since MAP was significant, covariance analysis was applied for all the variables which correlated with MAP.

The second step in the procedure was to determine which variables were correlated with MAP and if so whether the slope of the regression lines were the same within the group.

A two-way analysis of variance model with interaction was used to compare changes in the variables among the groups.

For correlations with the same slope regression lines, MAP was used as a covariate corrected overall. For correlations with different slopes of regression lines, MAP was used as a covariate corrected separately within each group. Other confounding variables accounted for included QI, serum GGT, serum creatinine and serum albumin.

For intragroup comparisons the least square means were calculated and compared by means of the t-test to obtain probabilities. According to Bonferroni for any of these probabilities to be significant the p value must be smaller than 0,003 and anything between 0,05 and 0,003 can be considered a strong indication of a possible difference.

7.3 Correlation studies

Correlations between the variables within each group, and in combined groups were determined using the Pearsons correlation coefficient.

7.4 Precision studies for methods

The coefficient of variation was determined for repeated measurements on the same samples.

To determine the accuracy of the methods, measurements of the same sample were performed repeatedly on the same day and on different days and the coefficient of variation was

determined.

To determine the intraoperator accuracy, the same technician performed repeated measurements of the same sample, on separate occasions. The coefficient of variation was determined.

For interoperator accuracy, the coefficient of variation was determined for measurements of the same sample performed by different technicians.

Special statistical analyses are detailed in chapter 13.

CHAPTER 8

INTRA- AND EXTRACELLULAR MAGNESIUM, CALCIUM, SODIUM AND POTASSIUM STATUS IN BLACK AND WHITE ESSENTIAL HYPERTENSION

8 INTRA- AND EXTRACELLULAR MAGNESIUM, CALCIUM, SODIUM AND POTASSIUM STATUS IN BLACK AND WHITE ESSENTIAL HYPERTENSION

8.1 INTRODUCTION

8.1.1 Monovalent and divalent cations in essential hypertension

Epidemiological, clinical and experimental studies have suggested a relationship between cations and the development of hypertension. The four major body cations include sodium, potassium, magnesium and calcium. In relation to hypertension, sodium and potassium have been extensively investigated. Recent studies have concentrated on the role of calcium in hypertension. There is a void in the literature regarding the relationship between magnesium and blood pressure (Simpson, 1985a).

8.1.1.1 Sodium

8.1.1.1 (i) Epidemiology: sodium and blood pressure

Dahl first reported an interpopulation relationship between blood pressure and sodium in 1960. Since then many larger, well controlled studies have confirmed the original data (Fromert 1979; Simpson 1985b; Stamler et al, 1991; Elliott, 1991). In unacculturated societies, including rural

Africa, blood pressure does not increase with age and the prevalence of hypertension is low (Freis, 1976; Seedat, 1982). Recent data from large studies support these findings. Four remote populations had the lowest average blood pressure and dietary salt intake among 52 populations studied in INTERSALT, an international cooperative investigation of electrolytes and blood pressure (Carvalho et al, 1989; Kaplan, 1990). Subjects from primitive societies with low blood pressure have a very low sodium intake; however they also ingest large amounts of potassium and magnesium, are leaner and smaller and are more physically active than their westernised counterparts.

Results from intrapopulation studies have been less consistent than those from interpopulation studies. Most reports have failed to demonstrate a positive relationship between blood pressure and sodium intake. A Dutch study reported a significant negative correlation between sodium intake and blood pressure while significant positive associations have been described in studies from Belgium, Kashmir, California and Japan (Shibata and Hatano, 1979; Kesteloot and Joosens, 1988; Mir and Newcombe, 1988; Khaw and Barret-Connor, 1988). When the National Health and Examination Survey (NHANES) study was analysed using different analytical methods, no positive and negative correlations of sodium with blood pressure were described (Harlan et al, 1984; McCarron et al, 1984; Gruchow et al, 1985).

Reasons for the conflicting results in intrapopulation studies may be due to the fact that most studies have used urinary sodium excretion as a surrogate for dietary sodium intake with very few studies analysing sodium dietary intake directly (Watt and Foy, 1982; McCarron et al, 1984; Elliot, 1991). Also, the range of habitual sodium intake is relatively high in westernised societies compared to nacculturated societies. Furthermore, sodium intake in industrialised societies may be high enough to increase blood pressure only in genetically susceptible salt-sensitive subjects, who represent a minority of the population and therefore there will be no correlation between blood pressure and sodium intake in the entire population (Fujita et al, 1980; Luft and Weinberger, 1982). The failure to report a consistent sodium-blood pressure association within populations is often cited as evidence that sodium intake is not related to blood pressure in any clinically important way within the general population (Simpson, 1979).

Important methodological problems are inherent to both inter- and intra-population studies of sodium and blood pressure. Interpopulation studies are generally positive but rely on unstandardised data. The intrapopulation studies generally lack statistical power, but a recent pooling analysis that uses 24 hour urinary sodium excretion to quantify intake has demonstrated highly significant positive correlations between sodium and blood pressure

(Elliott, 1991).

8.1.1.1 (ii) The effects of sodium on blood pressure

Experimental and clinical studies have also suggested a link between sodium and hypertension. Certain forms of experimental hypertension may be induced by feeding animals a high salt diet (Koletsky, 1959; Dahl, 1972). The clinical implications of these studies are however uncertain, as the relative amount of salt required to induce hypertension in animals is greatly in excess of the usual content of human diets. Moreover, in some rat models, blood pressure increases rather than decreases with reduced salt intake (Seymour, 1980; Webb et al, 1987).

Most salt loading studies in humans have been short term. A recent study demonstrated that dietary salt supplementation significantly increased blood pressure in normotensive adults (Mascioli et al, 1991). Administration of daily 1500 mmol NaCl to hypertensive patients increases blood pressure in some individuals but not others (Luft et al, 1979a; Roos et al, 1985). A subgroup of salt-sensitive hypertensive individuals has been defined. These patients are older, black, have severe hypertension and haptoglobin 1-1 phenotype (Weinberger et al, 1987; Luft et al, 1991). Salt sensitive individuals also have increased forearm vascular resistance, decreased venous compliance, suppressed renin release and lower circulating aldosterone

concentration (Sullivan, 1991). Salt-sensitive hypertensive patients do not all have similar defects. At least six different mechanisms have been proposed to account for a substantial change in blood pressure when a patient's salt intake is modified. These include primary aldosteronism, bilateral renal artery stenosis, bilateral renal parenchymal disease, acromegaly, low renin hypertension and non-modulating essential hypertension (Williams and Hollenberg, 1991).

The blood pressure effects of salt restriction on individuals and populations have also been studied. A review of combined data concluded that the decrease in blood pressure with salt restriction is related to the initial blood pressure, i.e. the higher the pressure, the greater the reduction to be expected with salt restriction (Morgan and Nowson, 1986). There is also an age-related effect, where the advantages of sodium restriction are greater in older individuals than in younger subjects (Grobbee and Hofman, 1986a).

Results from individual studies have not been as positive as the combined analysis, with most reports showing only a small effect on blood pressure (Watt et al, 1983; Silman, 1989). Dietary sodium restriction may have a role as an adjunctive measure to drug therapy. The desired effect however is only achieved when sodium intake is less than 80 mmol/day (Weinberger et al, 1988). Almost 50% of

hypertensive patients will lower their systolic blood pressure by 5 mmHg or more when they decrease their daily dietary sodium from the usual 200 mmol to 80 mmol.

Despite the general enthusiasm for the putative role of a high salt intake in the genesis of essential hypertension, definitive conclusions have not yet been reached. Experimental studies examining cellular sodium metabolism in essential hypertension may provide some of the answers linking sodium and blood pressure.

8.1.1.1 (iii) Cellular sodium and hypertension

The relationship between cellular sodium and blood pressure was first reported in 1952 (Tobian and Binion, 1952). Since then increased arterial, myocardial and venous sodium levels in experimental and human hypertension have been reported (Pamnani and Overbeck, 1976). Whether this sodium is primarily intracellular or extracellular is unclear. Most studies examining the association between sodium and blood pressure have been performed on cells other than those from the vascular wall itself - particularly erythrocytes and leukocytes.

8.1.1.1 (iii a) Erythrocyte sodium content

The first direct evidence for increased intracellular sodium in hypertension was reported in 1960, where Losse

described an increase in intraerythrocyte sodium content in hypertensive patients. Subsequent studies on erythrocyte sodium and blood pressure have failed to consistently demonstrate elevated intracellular sodium in hypertension.

Parker and Berkowitz (1983) reviewed 21 published reports and found that erythrocyte sodium of hypertensive subjects was raised in 10 studies and unchanged in 11. Hilton (1986) analysed data pooled from 20 published reports and found that in about half the studies, erythrocyte sodium of hypertensive patients was increased and in the other half it was unchanged. These conflicting data have been attributed to methodological variations, mismatch of groups within the same study and non-comparable populations sampled. Intraerythrocyte sodium is higher in black normotensive and hypertensive subjects compared to whites (Tuck et al, 1984; Mbuyamba Kabangu et al, 1984). Also, the direct correlations of erythrocyte sodium with age and body weight and the effect of antihypertensive drugs on erythrocyte sodium transport may account for the above conflicting results (Bramley et al, 1986; Ringel et al, 1987).

In studies conducted under experimental conditions that resembled in vivo situations, erythrocyte sodium was reduced and not increased (Simon and Conklin, 1986; Simon and Engel, 1987). These findings have been confirmed (Engelhardt and Scholze, 1988).

8.1.1.1 (iii b) Leukocyte sodium content

In contrast to the conflicting data regarding intra-erythrocyte sodium there is agreement that the sodium content of leukocytes in general, and specifically lymphocytes, is increased in human and experimental hypertension (Edmonson et al, 1975; Furspan and Bohr, 1985; Hilton, 1986; Seon and Forrester, 1989; Ng et al, 1990). In large studies, increasing levels of diastolic blood pressure are associated with increasing levels of intraleukocyte sodium (Chien and Zhao, 1984; Hilton, 1985). A few studies have failed to report increased intraleukocyte sodium in hypertension. Most of these however are derived from the same laboratory (Heagerty et al, 1982; Bradlaugh et al, 1984; Bing et al, 1986a).

8.1.1.1 (iii c) Vascular muscle sodium content

Although it is assumed that erythrocytes and leukocytes are representative of vascular smooth muscle, this remains to be proven. Analysis of intracellular sodium content in intact arteries is more difficult than in erythrocytes or leukocytes. Several methods are available to measure intracellular sodium in blood vessels, but none of these is precise enough to detect small differences (Friedman, 1974). Studies on vascular muscle sodium content in hypertension have been conflicting (Abel et al, 1981; Simon et al, 1986; Simon, 1989).

In summary the data from erythrocyte studies suggests that intracellular sodium levels are increased in hypertensive patients. However, recent evidence indicates that when measurements are taken within seconds of blood withdrawal, erythrocyte sodium content is reduced, not increased in human hypertension. In contrast, there is widespread agreement that essential hypertension is associated with elevated levels of leukocyte sodium. Results on vascular smooth muscle sodium content are inconclusive. Although platelets are a better cell model for hypertension studies than erythrocytes or leukocytes, there is no data in the literature regarding platelet sodium content in hypertensive patients.

The exact mechanisms of how sodium retention produces hypertension are unknown. Peripherally, increased sodium may cause decreased peripheral systemic vascular resistance by 'vascular wall oedema', increased vascular reactivity, circulating natriuretic hormone and increased catecholamines (Haddy et al, 1978; Mendelowitz, 1982). The causes of increased body and cellular sodium content in essential hypertension are also unclear, but abnormal transmembrane ion transport systems have been implicated.

8.1.1.2 Potassium

8.1.1.2 (i) Epidemiology:- potassium and blood pressure

Potassium may have a protective effect against hypertension. Epidemiological studies have reported that in primitive communities where the prevalence of hypertension is low, the consumption of potassium rich diets is high (Oliver et al, 1975; Simpson, 1985a). Japanese, American and Swedish studies demonstrated a decreased incidence of hypertension in communities that consumed a high potassium diet compared to neighbouring populations that had the same sodium intake but a low potassium intake (Sasake, 1962; Watson, 1980; Ljungman et al, 1981). In community studies, the urinary sodium to potassium ratio is more closely related to blood pressure than is the sodium output alone (Yamori et al, 1981; Khaw et al, 1988). Serum and body potassium is inversely related to blood pressure in some population studies (Bulpitt et al, 1981; Beretta-Piccoli et al, 1982; Rinner et al, 1989). These findings have not been confirmed by other investigators (Berenson et al, 1979; Kesteloot and Joossens, 1988; Pan et al, 1990).

8.1.1.2 (ii) The effect of potassium on blood pressure

In experimental hypertensive models, especially Dahl-salt sensitive rats, increased potassium intake has a protective effect against hypertension (Dahl et al, 1972; Batterbee et al, 1979). The potassium loads in these rats were very large and the blood pressure responses small and variable. Some animal experiments have demonstrated that reduced

potassium intake lowers blood pressure while potassium repletion increases blood pressure (Freed et al, 1951). Furthermore, Tobian (1989) reported that a high potassium intake appears to protect hypertensive rats from vascular disease independent of any blood pressure-lowering effects.

In human studies, potassium supplementation was found to attenuate the hypertensive effect of massive sodium loading (Luft et al, 1979a). Although most of the controlled trials of potassium supplementation in hypertensive patients involve small numbers of subjects of short durations, a number of studies have reported significant decreases in blood pressure with dietary potassium loading in normotensive and hypertensive subjects (Richards et al, 1984; Svetskey et al, 1987; Siani et al, 1987; Barden et al, 1991). Others however have failed to confirm these results (Miller et al, 1987; Grimm et al, 1990).

Potassium excretion and intake in blacks is lower compared to whites (Grim et al, 1980; Veterans Administration Cooperative Study, 1987). Blood pressure of young black females is significantly related to the sodium:potassium urinary ratio (Langford and Watson, 1975). Furthermore most studies on the antihypertensive effects of potassium supplementation have been performed in black hypertensive subjects (Matlou et al, 1986; Obel, 1989). Although the role of potassium in hypertension is unresolved, black hypertensive patients, who have a diet low in potassium,

may benefit from potassium supplementation (Kaplan and Rom, 1990).

8.1.1.2 (iii) Cellular potassium and hypertension

Certain forms of hypertension (primary aldosteronism and primary hyperreninaemia) and certain antihypertensive agents are associated with altered cellular potassium homeostasis (Rabinowitz, 1989). The role of dietary potassium intake in the pathogenesis, maintenance and treatment of essential hypertension is controversial (Maxwell and Waks, 1987; Haddy, 1987). Homeostatic regulation of potassium excretion is via a negative feedback system. Potassium intake increases plasma potassium concentration, elevated plasma potassium stimulates aldosterone release and both aldosterone and increased plasma potassium independently stimulate renal potassium excretion (Young, 1985). Non-homeostatic control of potassium excretion involves the rate of distal tubular flow and sodium delivery, tubular fluid anion concentration and acid-base disturbances (Giebish et al, 1986).

The intracellular:extracellular potassium ratio is determined by the balance of the Na^+-K^+-ATPase pump-driven potassium influx and potassium fluxes through other pathways (Rabinowitz, 1989). Potassium fluxes are influenced by intra- and extracellular factors. Extracellular factors include adrenaline, insulin and

aldosterone (Bia and De Fronzo, 1981; De Fronzo and Bia, 1983). These hormones with other humoral and neural mechanisms, regulate the intracellular:extracellular potassium ratio and may set the plasma potassium concentration independently of changes in total body potassium content (De Fronzo and Bia , 1983). The intracellular factors affecting cellular potassium homeostasis include intracellular volume, pH, cell metabolism and other ions (Rabinowitz, 1989).

Recent research has suggested that the central nervous system may also be involved in cellular potassium regulation and renal potassium excretion (Aizman et al, 1985; Rabinowitz, 1988). It has been speculated that analogous but separate systems exist for sodium and for potassium each involving the brain and each acting through specific humoral factors (Yoshimatsu et al, 1986; Katafuchi et al, 1987; Rabinowitz, 1989). For sodium a hypothalamic derived ouabain-like agent may be involved in renal sodium handling and cellular sodium homeostasis (Anderseon et al, 1969). Both of these mechanisms play a role in blood pressure regulation. Under pathological conditions, for example hypertension, where the ouabain-like substance is increased, renal sodium excretion decreases and intracellular sodium increases. A similar system may exist for potassium, and if so, may explain the role of potassium in blood pressure regulation and hypertension.

8.1.1.3 Calcium

8.1.1.3 (i) Epidemiologic perspectives

The first report of a calcium blood pressure association was in 1973 when Langford and Watson described a low calcium intake and a low urinary ratio of sodium to calcium in black schoolgirls with high systolic blood pressure levels. Since reports appeared on the relationship between drinking water hardness and cardiovascular mortality and morbidity, epidemiologists have studied the relationship of divalent cations, particularly calcium and magnesium to blood pressure (Schroeder, 1960; Stitt et al, 1973; Neri and Johnson, 1978).

Epidemiologic studies examining various populations have reported both negative and positive relationships between calcium intake and blood pressure. Cuttler and Brittain (1990) have recently reviewed 25 studies conducted in 17 populations. These studies did not include subsets and were matched methodologically as closely as possible. They concluded that although there are inconsistencies, the majority of epidemiologic studies demonstrate a significant negative correlation between calcium intake and blood pressure. The largest population studies (both carried out in America) included the NHANES and Nurses Health Study. Analysis of the NHANES I revealed an inverse relation between dietary intake of calcium and blood pressure

(McCarron et al, 1984). Reanalysis of this data (as for potassium) demonstrated that the relationship did not hold for all subgroups and that the relationship was only significant in black populations (Harlan et al, 1984; Gruchow et al, 1985). The only purely prospective study of dietary factors, including calcium and magnesium in relation to blood pressure is the Nurses' Health Study (Witteman et al, 1989). This survey, which examined more than 58 000 nurses, supported the results of many of the earlier cross sectional studies. Over a four year period the relative risk of self reported hypertension for a calcium intake greater than the recommended daily allowance (800 mg/day) was significant when compared with an intake of less than 400 mg/day (Witteman et al, 1989).

Population studies that have failed to demonstrate an inverse correlation between blood pressure and calcium intake may be due to small sample sizes, uncontrolled confounding variables, such as obesity and alcohol intake, and to variations in methodology (Cuttler and Brittain, 1990). Other possible effect modifications include population subgroups, vitamin D and dietary sodium intake (Sowers et al, 1985; Gruchow et al, 1988). Taking all these factors into consideration and with the growing number of large epidemiological studies, blood pressure and dietary calcium intake appear to be inversely related.

8.1.1.3 (ii) Effects of calcium on blood pressure

Calcium supplements have been found partly to inhibit the development of hypertension in hypertensive rats and to lower blood pressure in humans (Ayyani, 1979; Belizan et al, 1983a; McCarron et al 1989; Hattori et al, 1991). This blood pressure lowering effect of calcium has been observed in pregnant females, normal young males, elderly subjects and low renin hypertensive patients (Belizan et al, 1983b; Lyle et al, 1987; Grobee and Hofman, 1986b; Luft et al, 1986; Resnick and Laragh, 1983). Analysis of 19 studies on calcium supplementation revealed that increased oral calcium intake, whether supplemental or dietary, lowers blood pressure (Cuttler and Brittain, 1990). Cappuccio et al (1989) reviewed 15 studies and concluded that the overall effect of oral calcium on blood pressure is very small and it is therefore inappropriate to recommend oral calcium supplementation for the treatment of hypertension. Intravenous calcium infusion increases blood pressure in normotensive and hypertensive subjects (Suzuki and Aoki, 1988). Some studies have not been able to demonstrate a hypotensive effect of calcium (Nowson and Morgan, 1986; Cappuccio et al, 1987; Siani et al, 1988). The variable blood pressure responses to calcium supplementation appears to be due to differences in the backgrounds of the subjects and/or the design and size of the trials (Mikani et al, 1990).

The mechanisms by which oral calcium supplementation may reduce blood pressure are unknown (McCarron and Morris, 1986). Altered transmembrane calcium transport, calcium-sodium interactions and calcium regulating hormones may play a role (Doris, 1985; Kang et al, 1990; Brickman et al, 1990).

8.1.1.3 (iii) Calcium levels in blood

Serum, plasma and ionised calcium levels have been evaluated in normal and hypertensive subjects. A number of large studies, investigating more than 10 000 individuals, demonstrated a significant increase in blood pressure with increasing levels of total serum calcium (Bulpitt et al, 1976; Robinson et al, 1982; Kesteloot et al, 1984a; 1984b; Kesteloot and Joosens, 1988). A few reports have failed to demonstrate a relationship between serum calcium and blood pressure (Strazzulo et al, 1983; Kaplan and Meese, 1988). McCarron (1982) reported that hypertensive patients have lower mean serum-ionised calcium levels than normotensive controls. This was confirmed by Resnick et al (1983) but only in low renin hypertensive patients. Hunt et al (1991) recently reported a relationship between ionised calcium and renin status in normotensive subjects. Folson et al (1986) demonstrated a small decrease in ionised calcium levels of hypertensive males but not females. Recent reports have documented a significant inverse relationship between plasma ionised calcium and blood pressure,

independent of platelet calcium levels (Hvarfner et al, 1987; Hvarfner et al, 1988). Several relationships between serum ionised calcium and blood pressure (Buckley et al, 1987; Cooper and Shamsi, 1987). Hunt et al (1984) demonstrated that normotensive individuals with a family history of hypertension had a significantly higher plasma ionised calcium than individuals without a family history of hypertension.

Kaplan and Meese (1986) summarised 17 large studies examining the relationship between blood calcium levels and blood pressure. They reported that the majority of the studies showed a direct correlation. Many of these studies were conducted in diverse populations, using both cross sectional and case control designs. The inconsistencies of deficient calcium intake and raised blood calcium levels in hypertension are difficult to explain, but may be related to the complexity of calcium metabolism and regulation.

8.1.1.3 (iv) Cellular calcium and hypertension

Cellular calcium metabolism may be altered in essential hypertension at both the whole body and cellular levels (Buhler et al, 1986; Young et al, 1988). This may be related to cell membrane Ca^{2+}-ATPase, an intrinsic membrane-binding protein, a cell membrane calcium channel, parathyroid hypertensive factor or perhaps some other process (Kowarski et al, 1986; Roullet et al, 1987;

Postnov, 1990; Benishan et al, 1990).

Increased basal and stimulated platelet free calcium levels in hypertensive patients have been repeatedly demonstrated (Erne et al, 1984; Lindner et al, 1987; Le Quan et al, 1985; Zemel et al, 1990). Also, basal calcium levels are elevated in cultured cells from spontaneously hypertensive rats (Sugiyama et al, 1990; Papageorgiou and Morgan, 1990). Some studies however have failed to demonstrate alterations in intracellular calcium levels in both human and experimental hypertension (Nabika et al, 1985; Lew et al, 1985). Pritchard et al (1989) demonstrated a significant correlation of blood pressure with platelet but not lymphocyte intracellular free calcium concentrations. These conflicting results suggest that if a defect in calcium handling by cell membranes does exist in hypertension, it does not have the effect of increasing intracellular calcium in all cell types.

Since platelets resemble vascular smooth muscle cells, the biochemical findings in platelets may be extrapolated to vascular smooth muscle. If vascular smooth muscle calcium levels are elevated, the underlying cause for enhanced contractility and raised peripheral resistance in essential hypertension may be explained (see Section 1.3).

8.1.1.4 Magnesium

8.1.1.4 (i) Epidemiology: magnesium and blood pressure

Interest in the role of magnesium in human cardiovascular disease was stimulated by studies that demonstrated an inverse relationship between water hardness and cardiovascular death rates (Crawford et al, 1968; Masironi, 1970). Magnesium is a major contributor to water hardness. Dawson et al, 1978 demonstrated an inverse association between drinking-water-magnesium levels and hypertension in 24 West Texas communities. A study of 489 British males showed significantly lower diastolic blood pressures in men living in hard-water towns compared to those living in soft-water areas (Stitt et al, 1973). The relation of dietary magnesium with hypertension has been investigated in only a few studies. The strongest evidence for an association between dietary magnesium and blood pressures comes from the Honolulu Heart Study, where a low magnesium intake was found to be the dietary factor most strongly associated with blood pressure (Joffres et al, 1987). These results were confirmed by the recent Nurses Health Study, where the relation of various nutritional factors with hypertension was examined in over 58 000 females (Witteman et al, 1990). In this study, dietary magnesium (and calcium) had independent and significant inverse associations with hypertension.

Data from the Belgian interuniversity research on Nutrition and Health Study (8058 subjects) reported a significant negative correlation between dietary magnesium intake and systolic blood pressure in females (Kesteloot and Joossens, 1988).

Few studies have examined the within-person relation between magnesium intake and blood pressure. In an American hospital-based study, white normotensive individuals had higher intakes of dietary magnesium than white hypertensive and black normotensive and hypertensive subjects (Zemel et al, 1988). McCarron et al (1983) also reported that magnesium intake was lower in hypertensive patients than in normotensive controls. Thulin et al (1980) did not find an association between magnesium intake and blood pressure in Swedish females.

8.1.1.4 (ii) The effects of magnesium on blood pressure

The hypotensive effect of magnesium was documented in the 1930s when parenteral magnesium sulfate was used to treat preeclampsia and nephritis (Blackfan and McKhann, 1931; Lazard, 1933). Studies examining the effect of orally administered magnesium on blood pressure have produced conflicting results. Some studies have demonstrated a significant blood pressure lowering effect with oral magnesium, while others have failed to show a change in blood pressure (Dyckner and Wester, 1983; Cappuccio et al,

1985; Motoyama et al, 1989; Daly et al, 1990: Patki et al, 1990). Karppanen et al (1984) who conducted the largest and the longest clinical trial on magnesium supplementation reported a significant reduction in systolic blood pressure. Resnick et al (1983) demonstrated that only those patients with high renin activity benefited from magnesium supplementation. A recent study has indicated that diuretic-induced magnesium loss can aggravate hypertension and increase the drug requirement among these patients (Singh et al, 1989). Magnesium supplementation, in patients receiving long-term thiazide diuretics significantly reduces blood pressure (Saito et al, 1988; Dyckner et al, 1988). Oral and parenteral magnesium have also been used in patients with resistant essential hypertension (Singh et al, 1989).

Although not conclusive, results of the above studies favour the proposal that magnesium supplementation may lower blood pressure, but carefully performed randomised clinical trials are needed before definitive conclusions can be made. Magnesium supplementation may be a potentially useful nonpharmacologic form of treating hypertension.

8.1.1.4 (iii) Serum and urine levels of magnesium

Urinary magnesium levels, in the steady state, reflects dietary intake of magnesium (Whelton and Klag, 1989). Data

from the study of 24 West Texas communities indicated an inverse correlation between mean urinary magnesium levels for the community and hypertension mortality (Dawson et al, 1978). In a community-based cross-sectional study of elderly white Americans, urinary magnesium was negatively associated to systolic blood pressure in males, but not females (Whelton and Klag, 1989). In a Belgian study of over 4500 subjects, 24-hour magnesium was related to diastolic but not systolic blood pressure (Kesteloot, 1984b). Lai et al (1989) reported that of the number of urinary electrolytes studied, only the calcium-magnesium ratio was a predictor of systolic blood pressure.

Other studies examining Chinese, Korean, Belgian and Zairean populations did not demonstrate a relationship between urinary magnesium levels and blood pressure (Staessen et al, 1983; Mbuyamba Kabangu et al, 1986; Kesteloot et al, 1987).

Studies investigating the relationship between serum magnesium levels and blood pressure have also produced conflicting results. An early clinical study reported higher serum magnesium levels in patients with severe hypertension compared to normotensive controls (Walker and Walker, 1936). In 1958, Albert reported the opposite - that hypertensive patients had decreased serum magnesium concentrations. More recent studies examining elderly Danish white, black South African males and American adults

have demonstrated lower serum magnesium levels in hypertensive subjects compared to normotensive controls (Peterson et al, 1977; Sempos et al, 1983; Touyz et al, 1987). In contrast to these results, data from community-based cross-sectional American and Dutch studies and results from the NHANES study did not reveal an association between serum magnesium levels and blood pressure (Harlan et al, 1984; Whelton and Klag, 1989; Rinner et al, 1989).

Hvarfner et al (1987) reported a positive association between serum magnesium and blood pressure in a Swedish population. Buemi et al (1988) demonstrated that plasma magnesium levels decreased in normotensive subjects after the cold pressor test, whereas there were no changes in magnesium in hypertensive patients. A number of cases of severe hypermagnesaemia with refractory hypotension have been documented (Mordes et al, 1975; Ferdinandus et al, 1981). These studies further support the thesis that magnesium disturbances may be associated with hypertension. Although the data are conflicting, there is a need in clinical medicine to measure serum magnesium concentrations in all patients with hypertension as those who are hypomagnesaemic may benefit from magnesium supplementation.

8.1.1.4 (iv) Cellular magnesium and hypertension

Since magnesium is essentially an intracellular cation, cytoplasmic magnesium levels may be of more importance than

urinary or serum levels (Reinhart, 1988). Resnick et al (1984) demonstrated a strong inverse relation between intraerythrocyte levels of free magnesium, measured using a nuclear magnetic resonance technique, and diastolic blood pressure. Intracellular levels of magnesium were lower in untreated hypertensive subjects than in either treated hypertensives or normotensive controls (Resnick et al, 1984). In a cross-sectional study of 296 black South African males, hypertensive patients had significantly lower erythrocyte total magnesium levels than normotensive subjects (Touyz et al, 1989).

A study in junior [illegible] school students demonstrated that subjects with a positive family history of hypertension had higher systolic blood pressures with significantly lower erythrocyte magnesium concentrations compared to subjects with a negative family history (Shibutani et al, 1988). In pre-eclamptic patients intra-erythrocyte magnesium levels were significantly depressed compared to healthy, normotensive pregnant females (Kisters et al, 1990). Many studies have shown that in diuretic treated hypertensive patients, intracellular magnesium is significantly decreased (Dorup et al, 1988a; 1988b).

Some studies have failed to demonstrate intracellular magnesium depletion in hypertension. Seller et al (1965) reported decreased serum magnesium and increased erythrocyte magnesium levels in hypertensive patients.

Kjeldsen et al (1990) recently demonstrated that in untreated essential hypertensive white males, erythrocyte magnesium was significantly raised compared to normotensive controls. Gunther et al (1990) found no differences in erythrocyte magnesium concentrations between normotensive and hypertensive adults.

Most clinical studies examining intracellular magnesium in hypertension have been performed using erythrocytes. Several studies suggest that the mononuclear blood cell magnesium may be a better predictor of intracellular magnesium and total body magnesium status than the concentration of magnesium in plasma or erythrocytes (Elin, 1988; Yang et al, 1989a). Ryan et al (1981) reported intraleukocyte deficiency in essential hypertensive and diuretic treated hypertensive patients. Of the circulating blood cells, platelets resemble vascular smooth muscle the closest and therefore should be used in cellular studies on hypertension. A recent publication has confirmed that platelet magnesium is a better predictor of body magnesium status than erythrocyte magnesium concentrations (Touyz and Milne, 1991). There is no data in the literature on platelet magnesium status and hypertension.

Mechanisms of how intracellular magnesium may be related to blood pressure are discussed in section 1.2.5. Alterations of intracellular or extracellular magnesium concentrations may affect cell function through their effects on calcium

handling. Magnesium may bind competitively to the same sites as calcium, producing the appropriate physiologic response; it may bind competitively with calcium but not exert an effect; or it may affect calcium distribution by altering the flux of calcium across the cell membrane or by displacing it from the intracellular binding sites resulting in increased cyctoplasmic calcium concentrations (Cholst et al, 1984). In vascular smooth muscle increased intracellular calcium stimulates contraction with a consequent rise in peripheral resistance and tone (Robinson, 1984). The calcium-magnesium interactions may be important in hypertension. Luft et al (1988) and Evans et al (1990) recently demonstrated that the hypotensive effect of calcium may be mediated by magnesium depletion.

Resnick et al (1986) demonstrated that independent of dietary calcium intake, the intracellular free magnesium levels directly participates in the final common pathway of events regulating blood pressure and vasoconstrictor tone.

In addition to the calcium-magnesium effects, magnesium deficiency may be associated with blood pressure via other mechanisms. These include:- the attenuation of beta-adrenergic and prostaglandin responses, inhibition of ATPase transport systems, enhanced platelet aggregation and atherogenesis, enhanced intracellular potassium deficiency, effects on the renin-antiogensin system and effects on endothelium derived relaxing factor (Singh et al, 1989;

Gold et al, 1990; Atarashi et al, 1990).

Although there is no definitive evidence to support the use of oral magnesium supplements in the prophylaxis and treatment of hypertension, a magnesium rich diet or supplements, may be beneficial in subgroups of hypertensive patients, specifically those who are magnesium deficient (elderly, blacks, diuretic treated). Theoretically, magnesium should have a hypotensive effect.

8.1.2 Summary: cations and hypertension

The available data that too little or too much dietary sodium, potassium, calcium or magnesium is responsible for the pathogenesis of essential hypertension, or that alterations in the intake of these cations will consistently lower blood pressure is incomplete. In certain patients or in specific subgroups, defects in cellular cation metabolism may be important. Although the mean blood pressure change with altered cation intake in most studies is small or absent, individual changes are often significant.

8.2 AIMS

The aims of this study were:-

8.2.1 To determine the extracellular (serum) and intracellular (platelet and erythrocyte) concentrations of total magnesium, calcium, sodium and potassium in white and black patients with essential hypertension

8.2.2 To examine the relationships between the major cations in essential hypertension

8.2.3 To assess whether there are racial differences in the intracellular cations in essential hypertension

8.3 SUBJECTS AND METHODS

8.3.1 Subjects

One hundred and fifty four subjects entered into the study. Fifty two black healthy normotensive volunteers (23 females; 29 males), 52 black patients with newly diagnosed essential hypertension (30 females; 22 males); 26 white normotensive volunteers (13 females; 13 males) and 24 white hypertensive patients (14 females; 10 males) were studied. Inclusion criteria and the procedure for the medical examination are described in Section 5.1.3.

Venous blood was obtained according to procedures described in Section 5.3.2.

8.3.2 Materials and Methods

8.3.2.1 Preparation of blood - handling and centrifugation

Within 2 to 3 hours of venesection, the blood was transported at 20°C to the laboratory where it was centrifuged. A Clements G5100 centrifuge was used fo all spinning of samples except where otherwise specified.

8.3.2.1 (i) Preparation of platelets

8.3.2.1 (a) Platelet rich plasma (PRP)

In order to prevent platelet activation, all platelet preparations were performed using polyethylene or polypropylene plastic ware and siliconised glassware (Dacie, 1984). The citrated blood was divided into two equal aliquots - one for Na^+ and K^+ determination and the other for Mg^{2+} and Ca^{2+} analysis.

PRP can be prepared by two methods:-

i) the erythrocytes are allowed to settle under the influence of gravity and the PRP can be transferred to a fresh, polyethylene or siliconised glass tube.

ii) the whole blood in citrate, dextrose or heparin can be centrifuged. Ideal spinning speed is not consistent in the literature (Mustard et al 1972; Hallem and Rink 1985; Zemel et al, 1990) although most

researchers use low speed centrifugation to obtain PRP.

In this study, the second method was employed for isolating PRP. To determine optimal centrifugation conditions, preliminary studies were performed on six citrated blood samples (Appendix D).

8.3.2.1 (b) Methods for separating platelets from plasma

A variety of methods have been described for platelet isolation from plasma. The three most common procedures used are by chromatography (gel filtration) by density gradient centrifugation and by repeated centrifugation and washing in physiological solutions. (See Appendix E for details).

Platelet isolation in this study

The PRP was centrifuged at 600 x g for 10 minutes at room temperature producing a platelet pellet and platelet poor plasma. The platelet pellet for Na^+ and K^+ determination was suspended in a buffer consisting of 100 mM $MgCl_2$, 1 mM $MgHPO_4.3H_2O$ 5 mM glucose and 20 mM Hepes (pH 7,4) and for Mg^{2+} and Ca^{2+} determination it was suspended in a buffer comprising 145 mM NaCl, 1 mM NaH_2PO_4, 5 mM glucose and 20 mM Hepes (pH 7,4). The platelet pellet suspended in

buffer was then centrifuged at 600 x g for 15 minutes and the pellet washed twice more in the washing buffer. The final supernatant Na^+ and K^+ wash was retained for Mg^{2+} and Ca^{2+} analysis and the final supernatant Mg^{2+} and Ca^{2+} wash was retained for Na^+ and K^+ analysis. The ions analysed were not detected in the final supernatant washes. After the last wash, the platelet pellet, suspended in 1 ml of buffer was mixed on a Coulter mixer for 5 minutes. Finally, platelet counts were determined in the washed platelet suspension.

Centrifugation and cellular contamination

The centrifugation procedure described should ensure that the maximum number of platelets is retained without any (or absolute minimal) erythrocyte or leukocyte contamination using an automated technique. A full blood count was determined on the intact washed platelets suspended in buffer. These counts were performed at the South African Institute for Medical Research using an automated method (Technicon HI system. Technicon diagnostics, Belgium). The platelet count varied between 300 and 500 x 10^9/l. The erythrocyte and leukocyte counts varied between 0 and 0,02 x 10^{12}/l and 0 and 0,9 x 10^9/l respectively.

The platelet count was adjusted to 1 x 10^8 cells/ml with buffer. Counts were adjusted using the formula

$$\frac{\text{platelet count obtained x volume (y)}}{\text{required platelet count (1 x } 10^8\text{)}} = x$$

x - y = volume that must be added to y to obtain required platelet count.

Throughout this study, the platelet count was standardised at 1 x 10^8 cells/ml.

8.3.2.1 (ii) Preparation of erythrocytes

The method used to separate erythrocytes from plasma was similar to that described for platelets of repeated washings and centrifugation.

The heparinised blood was centrifuged at 450 x g for 10 minutes. The plasma was aspirated and transferred to plastic test tubes for analysis. The buffy coat (containing leukocytes and platelets) was aspirated and discarded. The remaining erythrocyte sediment (about 4,5 - 5,0 ml) was divided into two equal aliquots, one for measuring Na^+ and K^+, and the other for measuring Mg^{2+} and Ca^{2+}. Washing of the erythrocytes was performed three times by suspension in an iso-osmolar NaCl (0,9%) solution for Mg^{2+} and Ca^{2+} determination and in iso-osmolar $MgCl_2$ (112 mmol/l) for Na^+ and K^+ analysis. The cells were washed by centrifugation, at 450 x g for 10 minutes at room

temperature, and aspiration of the supernatant. The final supernatants were retained for estimation of the ions. There was no detectable Na^+ and K^+ in the $MgCl_2$ wash from the cells prepared for Na^+ and K^+ analysis and there was no detectable Mg^{2+} and Ca^{2+} in the NaCl wash from the cells prepared for Mg^{2+} and Ca^{2+} analysis.

Correction for trapped plasma

The washed intact erythrocytes were gently inverted for 5 minutes on a Coulter mixer (Coulter electronics). A microhaematocrit capillary sample was taken for packed cell volume (PCV) determination using a microcentrifuge (Heraeus Christ GmbH). The PCV of the erythrocyte samples varied between 85% and 92%.

Validations of techniques for isolating platelets and erythrocytes are presented in Appendix F.

8.3.2.2 Cation analysis

8.3.2.2.1 Serum cations

8.3.2.2.1 (i) Preparation of serum

The cation content of serum was measured. The plasma obtained from the heparinised blood samples was allowed to stand at room temperature for one hour. The fibrin clots

were removed leaving the clear serum. This serum was used for the determination of the ion concentration.

8.3.2.2.1 (ii) Methods

8.3.2.2.1 (ii a) Sodium and potassium

Sodium and K^+ were measured undiluted by digital flame photometry (543 Flame photometer, Instrumentation Laboratories). The serum samples were aspirated directly into the flame photometer and Na^+ and K^+ were measured simultaneously. See Appendix G (a and c) for details regarding the principles and methods of photometry.

8.3.2.2.1 (ii b) Magnesium and calcium

Magnesium and Ca^{2+} were determined by Atomic absorption spectroscopy (Varian Techtron AA 175) (Dawson and Heaton, 1961). See Appendix G (b and d) regarding the principles and methods of AAS. For Mg^{2+} and Ca^{2+} analysis, 0,1 ml of serum was diluted to 5 ml in 0,2% lanthanum oxide for Mg^{2+} and in 0,1% lanthanum oxide for Ca^{2+}. The standard electrolyte solutions were diluted in the same manner and used to calibrate the AAS. The standard concentrations for Mg^{2+} were 1,0 - 4,0 mmol/l and for Ca^{2+} 1,0 - 3,0 mmol/l. The ions were determined individually, and the concentration obtained directly from the digital printout.

8.3.2.2.2 Platelet cations

8.3.2.2.2 (i) Preparation of platelet lysates

Sodium and K^+ were measured by flame photometry in the platelet preparation for Na^+ and K^+ and Mg^{2+} and Ca^{2+} were measured by AAS in the platelet preparation for Mg^{2+} and Ca^{2+}. None of the cations were detectable under these conditions.

The washed, intact platelets at concentrations of 1×10^8 cells/ml were then lysed by adding 10 µl of a 20% Saponin solution to each platelet suspension. The lysed suspension was mixed well for 5 minutes on a Coulter mixer.

Sodium, K^+, Mg^{2+} and Ca^{2+} were measured in the lysate. Since the ions were not detected in the prelysed suspension, the concentration of cations measured in the lysate was that derived from the lysed platelets.

8.3.2.2.2 (ii) Methods

8.3.2.2.2 (ii a) Magnesium and calcium

Magnesium and Ca^{2+} were analysed by AAS, the technique of which is described in Appendix G.b. The samples were prepared as for serum (Dawson and Heaton, 1961). Low standard concentrations (1,0 to 3,0 µmol/ml) were diluted

in the same way as the platelet samples. The instrument was zeroed on distilled, deionised water and calibrated with the standards. Results were read directly off the digital printout.

8.3.2.2.2. (ii b) Sodium and Potassium

Sodium and K^+ were measured by flame photometry. See Appendix G.a. for methods describing the use of flame photometry. The samples were prepared as for serum except that the instrument was calibrated with different standards - Na^+ - 5,0 /umol/ml; K^+ - 100 /umol/ml.

The sensitivity of the machine was increased by expanding the Scale 10 fold for Na^+ analysis and by using the 0-200 /umol/ml (0-200 mmol/l) scale for K^+ measurement.

The final content of platelet cations was expressed as

/umol/ml/l x 10^8 cells/ml

⟶ /umol/l x 10^8 cells

8.3.2.2.2 (iii) Platelet cations - unit of measurement

An important property of a result is the units of measurement. The units determine the ease of comparison among measurements; within a study and between investigators. Biochemical results are usually expressed as a ratio of two measurements. The numerator for fluid

and tissue analysis is often mass or molecular units which are easily interconverted. The denominator unit for fluid is volume. For cellular analysis, the denominator unit is a potential problem where several units may be chosen depending on the technique e.g. cell count, DNA analysis and protein concentration (Elin, 1988; Ladefoged and Hagen, 1988; Ralston et al, 1989). Since these units can not readily be converted from one to another, comparisons of results between studies are difficult. The differences between "concentration" and "content" are defined by the denominator. The definition of concentration is "the quantity of a substance per unit volume or weight" and the definition of content is "that which is contained within the thing" (Stedmans medical dictionary, 1982). When expressing "content", the number of cells is used as the denominator whereas in that for expressing "concentration", the denominator relates to the volume or mass of the cell.

In this study the unit of expression for platelet cations was umol/cell number - consequently, according to the above definition, the platelet cation content was determined. One of the problems with this measurement is that the unit (the cell) is dependent on the volume or size of the unit. Since the platelet volumes were within normal limits for all the platelet specimens, inter sample variability was minimised.

8.3.2.2.3 Erythrocyte cations

8.3.2.2.3 (i) Preparation of erythrocyte lysates

The ions were measured in the final supernatant wash. Magnesium and Ca^{2+} were not detected in the erythrocyte preparation for Mg^{2+} and Ca^{2+} and Na^{+} and K^{+} were not detected in the preparation for Na^{+} and K^{+}.

The method for determining erythrocyte cation concentrations was based on that described by Fortes Mayer and Starkey (1977). The washed erythrocyte suspensions were lysed by the addition of 20 µl of a 20% Saponin solution. The erythrocyte lysate was mixed well on a Coulter mixer.

8.3.2.2.3 (ii) Methods

8.3.2.2.3 (ii a) Sodium and potassium

For Na^{+} and K^{+} analysis 0,3 ml of the well-mixed lysate was added to 10 ml of lithium nitrate diluent (15 mmol/l) with an automatic pipette (Oxford Laboratories Ltd, U.K.). Because the lysate was very viscous (PCV 85-92%, pre-lysing) a "washout" pipetting technique was employed. The standard cation solutions were diluted in the same manner as the erythrocyte samples. The standard concentrations used to calibrate the instrument were:-

Na^+ : 5,0 mmol/l

K^+ : 100 mmol/l

To increase the sensitivity of the flame photometer the scale for Na^+ was adjusted ten times and the range for K^+ was changed to the 0 - 200 mmol/l scale. Since manual dilutions were made, the automatic diluter of the flame photometer was not used. The diluted lysed samples were aspirated directly into the instrument. The cation concentrations were read off the digital printout. (Appendix Ga).

In order to correct for the trapped extracellular fluid, the packed cell volume (PCV) was taken into account when calculating the final concentrations. The final erythrocyte Na^+ and K^+ concentrations were determined from the formulae:-

- Erythrocyte Na^+ (mmol/l) = recorded Na^+ value x $\frac{100}{PCV}$

- Erythrocyte K^+ (mmol/l) = recorded K^+ value x $\frac{100}{PCV}$

8.3.2.2.3 (ii b) Magnesium and calcium

Erythrocyte Mg^{2+} and Ca^{2+} were measured by AAS. (See Appendix G.b). For Mg^{2+} analysis, 0,1 ml of well mixed erythrocyte lysate was diluted to 5,0 ml with 0,2% lanthanum oxide. The Mg^{2+} standards varied between 1,5 and 4,0 mmol/l and were diluted in 0,2% lanthanum oxide as for

the lanthanum oxide samples. For Ca^{2+} determination, 0,1 ml of erythrocyte lysate was mixed with 4,9 ml 0,1% lanthanum oxide. The standards (1,0 - 10,0 µmol/l) were diluted in the same way. For Mg^{2+} determination the AAS was set at a wavelength of 285.2 nm and for Ca^{2+} at a wavelength of 422.7 nm. The instrument was zeroed with distilled, deionised water and calibrated with the standards. The samples were aspirated and the concentration read off the digital printout. The ions were measured separately.

To correct for the trapped extracellular fluid, the PCV was taken into account in the final calculations. Final erythrocyte Mg^{2+} and Ca^{2+} concentrations were calculated from the formulae:

- erythrocyte Mg^{2+} (mmol/l) = recorded Mg^{2+} value x $\frac{100}{PCV}$

- erythrocyte Ca^{2+} (umol/l) = recorded Ca^{2+} value x $\frac{100}{PCV}$

Validations of the techniques for preparing cells for cation determination are presented in Appendix F. iii. For all determinations duplicate samples were prepared. Three measurements were performed on each sample. Thus for each subject, six readings were obtained for each parameter studied. The mean value was taken for analysis. After processing five samples, the instruments (Flame photometer and AAS) were recalibrated with the blanks and standards.

8.3.2.4 Serum gamma glutamyl transferase, creatinine and albumin

Serum gamma glutamyl transferase (GGT) albumin and creatinine concentrations were determined by automated methods at the South African Institute for Medical Research (S.A.I.M.R). The samples were processed blind and the technician performing the tests was unaware from which group the blood samples were obtained. Details of the methods are described in Appendix H.

The accuracy of techniques is detailed in Appendix I.

8.4 RESULTS

One hundred and fifty four subjects entered into the study - 104 blacks and 50 whites. In the black group 52 subjects had essential hypertension and 52 had normal blood pressure. In the white group, 26 subjects were normotensive and 24 were hypertensive. There were 29 males and 23 females in the black normotensive group and 22 males and 30 females in the black hypertensive group. In the white group, there were 13 males and 13 females in the normotensive group and 10 males and 14 females in the hypertensive group.

8.4.1 Clinical characteristics of the groups

8.4.1.1 Blood pressure

8.4.1.1 (i) Black group (Table 8.1)

The mean SBP and DBP in the normotensive group was 123 ± mmHg and 76 ± 8 mmHg respectively. In the hypertensive group, the mean SBP was 167 ± 22 mmHg and the mean DBP was 105 ± 11 mmHg. The mean MAP in the normotensive and hypertensive groups were 92 ± 8 mmHg and 126 ± 12 mmHg respectively. Systolic blood pressure, DBP and MAP were significantly higher in the hypertensive group compared to the normotensive group ($P < 0,0001$).

8.4.1.1 (ii) White group (Table 8.2)

In the normotensive group the mean SBP was 122 ± 9 mmHg and the mean DBP was 71 ± 6 mmHg. The mean SBP and DBP in the hypertensive group was 176 ± 19 mmHg and 107 ± 17 respectively. The mean MAP in the normotensive group was 88 ± 5 mmHg and 130 ± 15 mmHg in the hypertensive group. Systolic blood pressure, DBP and MAP were significantly increased in the hypertensive subjects compared to their normotensive counterparts ($P < 0,0001$).

Table 8.1: Clinical characteristics of the black group

	Black Group	
	Normotensive	Hypertensive
Total n	52	52
Female n	23	30
Male n	29	22
Variable	Mean ± SD	Mean ± SD
Age (years)	43 ± 9	47 ± 11
Height (m)	1,69 ± 0,08	1,63 ± 0,09
Weight (Kg)	71 ± 12	70 ± 11
QI (Kg/m^2)	25 ± 5	26 ± 4
SBP (mmHg)	123 ± 11	167 ± 22*
DBP (mmHg)	76 ± 8	105 ± 11*
MAP (mmHg)	92 ± 8	126 ± 12*
HR (beats/min)	67 ± 8	71 ± 10

SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate; QI = quetelet index
* $P < 0,0001$ hypertensive versus normotensive

Table 8.2: Clinical characteristics of the white group

	White Group	
	Normotensive	**Hypertensive**
Total n	26	24
Female n	13	14
Male n	13	10
Variable	Mean ± SD	Mean ± SD
Age (years)	44 ± 10	47 ± 12
Height (m)	1,72 ± 0,07	1,70 ± 0,07
Weight (Kg)	.. ± 15	75 ± 16
QI (Kg/m^2)	24 ± 4	27 ± 5
SBP (mmHg)	122 ± 9	176 ± 19*
DBP (mmHg)	71 ± 6	107 ± 17*
MAP (mmHg)	88 ± 5	130 ± 15*
HR (beats/min)	69 ± 10	78 ± 12

SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate; QI = quetelet index.
* $P < 0,0001$ hypertensive versus normotensive

8.4.1.1 (iii) Groups combined (black and white)
(Table 8.3)

The mean SBP, DBP and MAP in the normotensive group was 123 ± 10; 75 ± 8; 91 ± 7 mmHg respectively. In the hypertensive group, mean SBP was 170 ± 21 mmHg; mean DBP 106 ± 13 mmHg and mean MAP 127 ± 13 mmHg.

Comparing the groups, SBP, DBP and MAP were significantly higher in the hypertensive subjects compared to the normotensive subjects ($P < 0,0001$).

8.4.1.2 Age (Tables 8.1-8.3)

The mean age of the black normotensive group was 43 ± 9 years and 47 ± 11 years in the black hypertensive group. In the white subjects, the mean age in the normotensive group was 44 ± 10 years and 47 ± 12 years in the hypertensive group. In the combined black and white groups, the mean age in the normotensive group was 44 ± 10 years and 47 ± 12 years in the hypertensive group.

The normotensive and hypertensive groups (black, white and combined) were age matched, with no significant difference in age between the two groups.

Table 8.3: Clinical characteristics of the combined black and white groups.

	Combined black and white groups	
	Normotensives	Hypertensives
	BNT + WNT	BHT + WHT
	(n = 78)	(n = 76)
Variable		
Age (years)	44 ± 10	47 ± 12
Height (m)	1,70 ± 0,07	1,65 ± 0,08
Weight (Kg)	72 ± 13	72 ± 12
QI (Kg/m^2)	25 ± 5	26 ± 5
SBP (mmHg)	123 ± 10	170 ± 21*
DBP (mmHg)	75 ± 8	106 ± 13*
MAP (mmHg)	91 ± 7	127 ± 13*
HR (beats/min)	67 ± 9	73 ± 11
GGT (U/l)	29 ± 28	25 ± 31
Albumin (g/l)	43 ± 4	44 ± 5
Creatinine (µmol/l)	90 ± 13	89 ± 16

SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; HR = heart rate; QI = quetelet index.

* P < 0,0001 hypertensive versus normotensive

8.4.1.3 Height, weight, Quetelet Index (QI)
(Tables 8.1-8.3)

There were no significant differences in height, weight and QI between the normotensive and hypertensive groups. (black, white and combined groups). The white normotensive males were heavier than the white normotensive females (Table 8.6).

8.4.1.4 Heart rate (HR) (Tables 8.1-8.3)

Although HR was higher in the hypertensive groups compared to the normotensive groups, there was no significant difference in HR between the groups.

8.4.1.5 Serum albumin (Table 8.4)

The mean serum albumin level in the black normotensive group was 42 ± 4 g/l and 42 ± 4 g/l in the black hypertensive group. In the white subjects, the mean serum albumin level was 45 ± 4 g/l in the normotensive and 46 ± 5 g/l in the hypertensive group. There were no significant differences in serum albumin concentration between the normotensive and hypertensive groups.

8.4.1.6 Serum creatinine (Table 8.4)

In the black group, the normotensive subjects had a mean

serum creatinine level of 99 ± 13 umol/l and the hypertensive subjects a level of 88 ± 16 umol/l. The mean serum creatinine concentrations in the white normotensive and hypertensive groups were 82 ± 14 umol/l and 90 ± 16 mmol/l respectively. Serum creatinine levels did not differ significantly between the normotensive and hypertensive groups.

8.4.1.7 Serum GGT (Table 8.4)

The mean serum GGT levels in the black normotensive and hypertensive groups were 37,4 ± 31 U/l and 30,8 ± 35 U/l respectively. In the white normotensive group, the serum GGT concentration was 12,6 ± 8 U/l and 11,8 ± 8 U/l in the white hypertensive group. Serum GGT levels did not differ significantly between the normotensive and hypertensive groups. When comparing the black and white groups, (normotensive and hypertensive groups), serum GGT was significantly elevated in the black subjects ($P < 0,05$) (Table 8.5).

Tables 8.6-8.11 present the clinical characteristics in the male and female, normotensive and hypertensive, black and white groups.

Table 8.4: Serum albumin, creatinine and gammaglutamyl transferase (GGT) levels in the different groups

	Albumin (g/l)		Creatinine (µmol/l)		GGT (U/l)	
Group	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
BNT (total)	42 ± 4	37 - 46	99 ± 13	89 - 113	37,4 ± 31	6 - 120
female	41 ± 4	37 - 45	100 ± 5	102 - 112		
male	43 ± 3	39 - 46	101 ± 12	89 - 113		
BHT (total)	42 ± 4	37 - 46	88 ± 16	72 - 104	30,8 ± 35	5 - 200
female	42 ± 4	37 - 46	80 ± 16	59 - 100		
male	42 ± 3	39 - 43	96 ± 8	88 - 104		
WNT (total)	45 ± 4	41 - 49	82 ± 14	68 - 96	12,6 ± 8	3 - 36
female	41 ± 4	37 - 45	77 ± 4	72 - 81		
male	48 ± 4	41 - 52	86 ± 14	66 - 97		
WHT (total)	46 ± 5	41 - 51	90 ± 16	84 - 106	11,8 ± 8	1 - 32
female	42 ± 6	36 - 48	80 ± 17	63 - 97		
male	49 ± 3	46 - 52	103 ± 9	94 - 112		

BNT = black normotensive; BHT = black hypertensive;
WNT = white normotensive; WHT = white hypertensive

Table 8.5: Pairwise-t-test comparisons for serum gammaglutamyl transferase between the black and white groups. Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
BNT	NS	-		
WHT	0,003	0,0004	-	
WNT	0,003	0,0004	NS	-

NS = not significant

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

Table 8.6: Clinical characteristics in the black normotensive and hypertensive, male and female groups

	Normotensive Group		Hypertensive Group	
	Females	Males	Females	Males
Variable				
Age (years)	43 ± 8	42 ± 9	47 ± 11	48 ± 14
Height (m)	1,62 ± 0,03	1,76 ± 0,05	1,57 ± 0,05	1,72 ± 0,0
Weight (Kg)	74 ± 16,4	76 ± 11	70 ± 13	72 ± 9
QI (Kg/m^2)	28 ± 2	25 ± 4	28 ± 4	24 ± 3
SBP (mmHg)	117 ± 10	128 ± 11	167 ± 24*	167 ± 10*
DBP (mmHg)	75 ± 8	77 ± 7	105 ± 11*	104 ± 12*
MAP (mmHg)	89 ± 8	94 ± 7	126 ± 13*	125 ± 11*
HR (beats/min)	67 ± 7	66 ± 8	69 ± 8	74 ± 12

* $p < 0,0001$ hypertensive versus normotensive

Table 8.7: Clinical characteristics in the white normotensive an hypertensive, male and female groups

	Normotensive Group		Hypertensive Group	
	Females	Males	Females	Males
Variable				
Age (years)	42 ± 11	47 ± 10	51 ± 12	42 ± 12
Height (m)	1,66 ± 0,05	1,79 ± 0,02	1,63 ± 0,06	1,72 ± 0,0
Weight (Kg)	64 ± 16	81 ± 9*	70 ± 18	81 ± 10
QI (Kg/m^2)	23 ± 5	25 ± 2,7	26 ± 7	27 ± 3
SBP (mmHg)	119 ± 9	126 ± 7	174 ± 20*	181 ± 17*
DBP (mmHg)	72 ± 6	70 ± 6	106 ± 14*	107 ± 21*
MAP (mmHg)	88 ± 6	89 ± 5	129 ± 13*	132 ± 18*
HR (beats/min)	74 ± 7	64 ± 10	77 ± 13	80 ± 9*

* $p < 0,0001$ hypertensive versus normotensive

Table 8.8: Students-t-test comparison of MAP between the male and female, black and white, normotensive and hypertensive groups. Significant P values are presented.

Groups	1	2	3	4	5	6	7	8
1	-							
2	NS	-						
3	0,0001	0,0001	-					
4	0,0001	0,0001	NS	-				
5	NS	NS	0,0001	0,0001	-			
6	NS	NS	0,0001	0,0001	NS	-		
7	0,0001	0,0001	NS	0,09	0,0001	0,0001	-	
8	0,0001	0,0001	NS	NS	0,0001	0,0001	NS	-

Group
1 = black (B) hypertensive (HT) female
2 = BHT male
3 = B normotensive (NT) female
4 = BNT male
5 = white (W) HT female
6 = WHT male
7 = WNT female
8 = WNT male

NS = not significant

Table 8.9: Students-t-test comparison of DBP between the male an female, black and white, normotensive and hypertensiv groups. Significant P values are presented.

Groups	1	2	3	4	5	6	7
1	-						
2	NS	-					
3	0,0001	0,0001	-				
4	0,0001	0,0001	NS	-			
5	NS	NS	0,0001	0,0001	-		
6	NS	NS	0,0001	0,0001	NS	-	
7	0,0001	0,0001	NS	NS	0,0001	0,0001	-
8	0,0001	0,0001	NS	NS	0,0001	0,0001	NS

Group
1 = black (B) hypertensive (HT) female
2 = BHT male
3 = B normotensive (NT) female
4 = BNT male
5 = white (W) HT female
6 = WHT male
7 = WNT female
8 = WNT male

NS = not significant

Table 8.10: Students-t-test comparison of SBP between the male and female, black and white, normotensive and hypertensive groups. Significant P values are presented.

Groups	1	2	3	4	5	6	7	8
1	-							
2	NS	-						
3	0,0001	0,0001	-					
4	0,0001	0,0001	0,02	-				
5	NS	NS	0,0001	0,0001	-			
6	0,03	0,03	0,0001	0,0001	NS	-		
7	0,0001	0,0001	NS	NS	0,0001	0,0001	-	
8	0,0001	0,0001	NS	NS	0,0001	0,0001	NS	-

Group
1 = black (B) hypertensive (HT) female
2 = BHT male
3 = B normotensive (NT) female
4 = BNT male
5 = white (W) HT female
6 = WHT male
7 = WNT female
8 = WNT male

NS = not significant

Table 8.11: Students-t-test comparison for Age between the male and female, black and white, normotensive and hypertensive groups. Significant P values are presented.

Groups	1	2	3	4	5	6	7	8
1	-							
2	NS	-						
3	NS	NS	-					
4	NS	NS	NS	-				
5	NS	NS	0,02	0,008	-			
6	NS	NS	NS	NS	0,03	-		
7	0,05	0,05	NS	NS	0,009	NS	-	
8	NS	NS	NS	NS	NS	NS	NS	-

Group
1 = black (B) hypertensive (HT) female
2 = BHT male
3 = B normotensive (NT) female
4 = BNT male
5 = white (W) HT female
6 = WHT male
7 = WNT female
8 = WNT male

NS = not significant

8.4.2 Correlation studies between the clinical variables and blood pressure (Tables 8.12; 8.13)

In the black group, the only significant correlation with blood pressure was height in the normotensive subjects (P = 0,009). There were no significant correlations between the variables of age, QI, HR, serum albumin, serum creatinine, serum GGT and MAP in any of the groups.

When the black and white, normotensive and hypertensive groups were combined, age was significantly related to MAP (r = 0,23; P = 0,004).

8.4.3 Biochemical Data

8.4.3.1 Serum ions (Tables 8.14-8.17; Figures 8.1-8.4)

8.4.3.1 (i) Black group

Serum K^+ and serum Mg^{2+} concentrations were significantly lower in the hypertensive group compared to the normotensive group (P < 0,01). There were no significant differences in serum Na^+ and serum Ca^{2+} between the groups. Serum ion concentrations were not significantly different between males and females (Tables 8.14; 8.15).

Table 8.12: Pearsons correlation coefficients (r) and probabilities (P) for correlations between MAP and the clinical variables in the black group.

	Black Group			
	Normotensive		Hypertensive	
	r	P	r	P
Age (years)	0,23	0,09	0,08	0,53
Height (m)	0,35	0,009*	0,04	0,75
Weight (Kg)	0,02	0,85	0,04	0,76
QI (Kg/m^2)	0,05	0,71	0,11	0,42
SBP (mmHg)	0,76	0,0001*	0,86	0.0001*
DBP (mmHg)	0,83	0,0001*	0,83	0,0001*
HR (beats/min)	0,16	0,23	0,08	0,54

* significant correlation

SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; QI = quetelet index.

Table 8.13: Pearsons correlation coefficients for correlations between MAP and the clinical variables in the white group.

	White Group			
	Normotensive		Hypertensive	
	r	p	r	p
Age (years)	0,05	0,78	0,04	0,84
Height (m)	0,31	0,10	0,10	0,63
Weight (Kg)	0,37	0,06	0,23	0,26
QI (Kg/m^2)	0,30	0,10	0,28	0,16
SBP (mmHg)	0,67	0,0002*	0,74	0.0001*
DBP (mmHg)	0,81	0,0001*	0,91	0,0001*
HR (beats/min)	-0,22	0,28	0,33	0,11

SBP = systolic blood pressure; DBP = diastolic blood pressure;
HR = heart rate; QI = quetelet index.
* = significant correlation

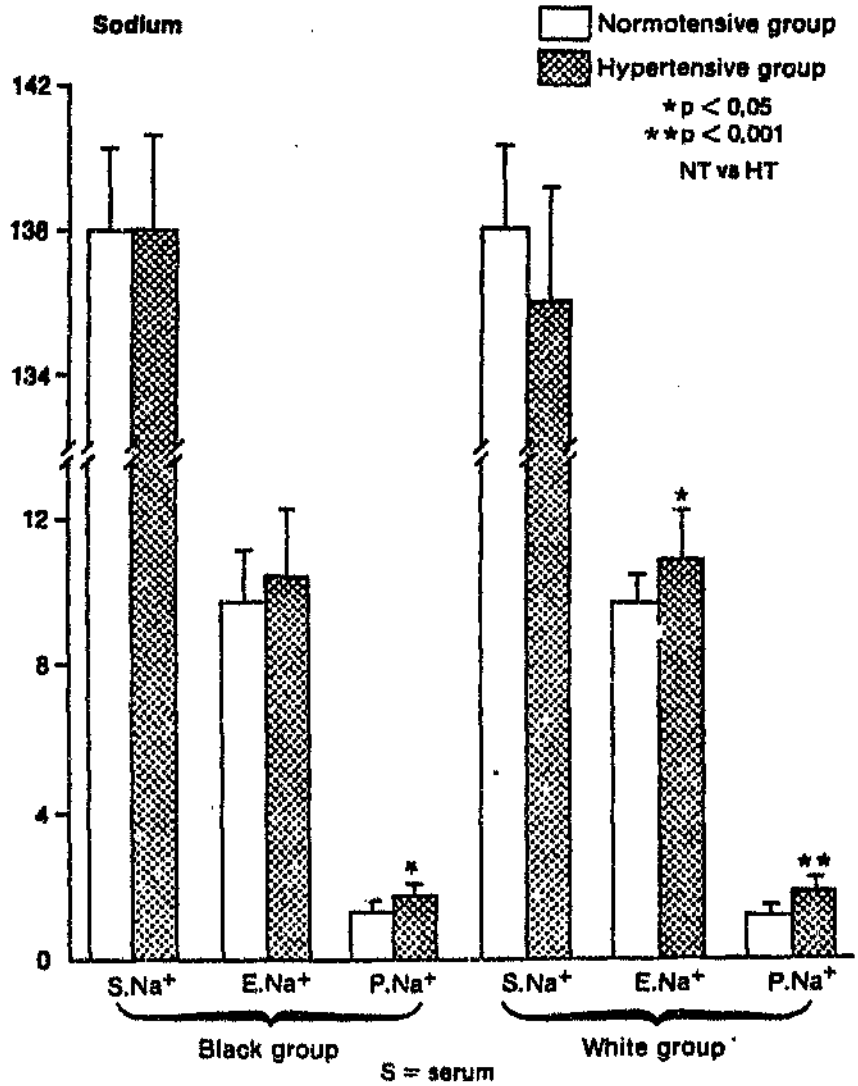


Figure 8.1. Serum, platelet and erythrocyte sodium concentrations in the black and white normotensive and hypertensive groups.

Serum and erythrocyte values expressed as mmol/l, platelet values expressed as $\mu mol/l \times 10^6$

Table 8.14: Serum, erythrocyte and platelet cations levels in the black normotensive and hypertensive groups.

Variable	Black Group: Normotensive	Black Group: Hypertensive	P value: Normotensive vs Hypertensive
Serum Na^+	138 ± 2,6	138 ± 3,1	0,11
Erythrocyte Na^+	9,9 ± 1,7	10,7 ± 2,7	0,08
Platelet Na^+	1,44 ± 0,33	1,64 ± 0,28	0,003*
Serum K^+	4,01 ± 0,34	3,79 ± 0,34	0,0009*
Erythrocyte K^+	83 ± 7	86 ± 10	0,07
Platelet K^+	4,49 ± 1,34	4,61 ± 1,34	0,70
Serum Ca^{2+}	1,99 ± 0,13	2,08 ± 0,32	0,07
Erythrocyte Ca^{2+}	4,64 ± 1,15	6,77 ± 1,12	0,0001*
Platelet Ca^{2+}	1,48 ± 0,35	1,62 ± 0,41	0,05*
Serum Mg^{2+}	0,86 ± 0,12	0,71 ± 0,10	0,0001*
Erythrocyte Mg^{2+}	2,53 ± 0,37	2,18 ± 0,41	0,0001*
Platelet Mg^{2+}	2,09 ± 0,40	1,54 ± 0,41	0,0001*

Serum values are expressed as mmol/l, erythrocyte values as mmol/l except erythrocyte Ca^{2+} which is expressed as /umol/l, and platelet values as /umol/l x 10^8 cells.

* = significant difference

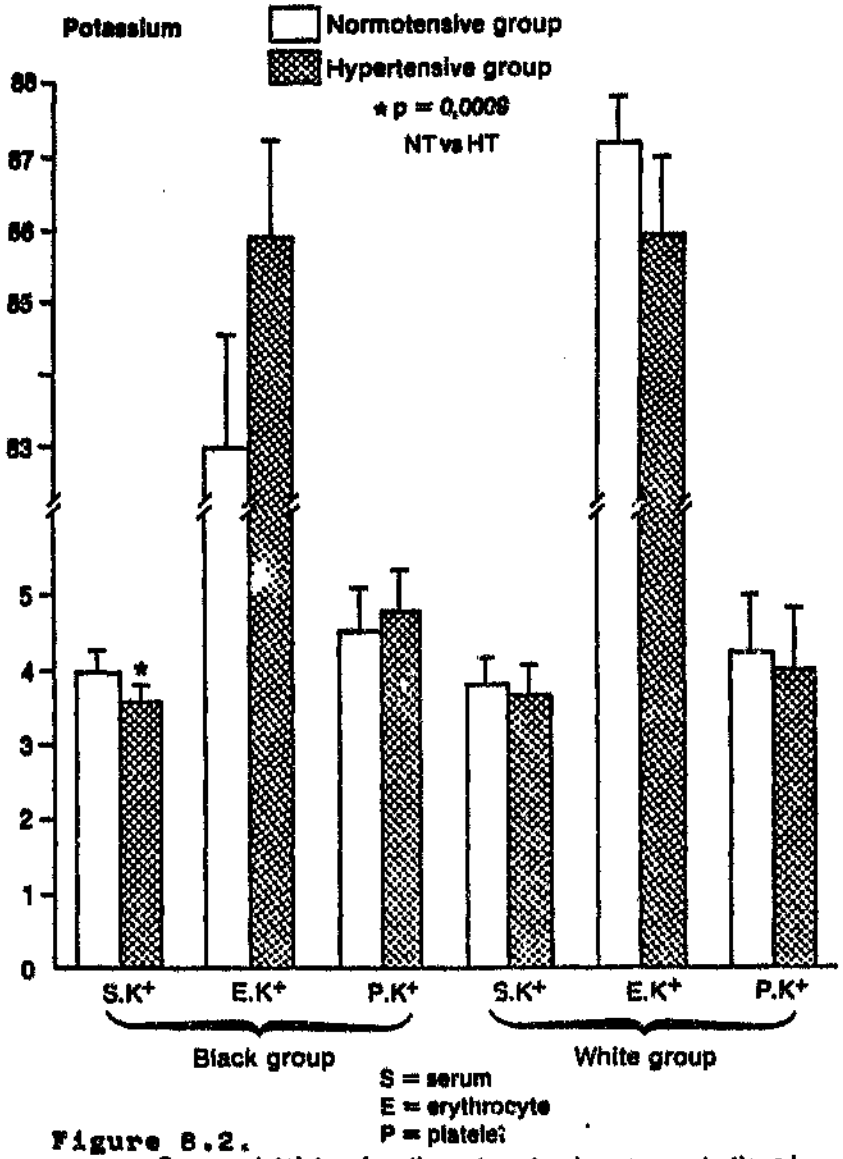


Figure 8.2. Serum, platelet and erythrocyte potassium concentrations in the the black and white normotensive and hypertensive groups

Serum and erythrocyte values expressed as mmol/l, platelet values expressed as 1×10^8

Table 8.15: Serum, erythrocyte and platelet cation concentrations in the female and male normotensive and hypertensive black groups.

	Black Group			
	Normotensive		Hypertensive	
Variable	Female	Male	Female	Male
Serum Na^+	138 ± 2,0	139 ± 2,9	138 ± 3,2	138 ± 2,1
Erythrocyte Na^+	9,7 ± 1,30	10,2 ± 1,92	11,2 ± 3,2	10,1 ± 2,0
Platelet Na^+	1,42 ± 0,33	1,45 ± 0,33	1,56 ± 0,20	1,69 ± 0,29*
Serum K^+	4,1 ± 0,37	3,89 ± 0,25	3,71 ± 0,35	3,83 ± 0,32
Erythrocyte K^+	80 ± 7,4	83 ± 7,3	87 ± 10,7	86 ± 8,5
Platelet K^+	4,8 ± 1,30	4,3 ± 1,33	4,6 ± 1,40	4,6 ± 1,41
Serum Ca^{2+}	1,98 ± 0,18	2,10 ± 0,09	2,10 ± 0,33	2,09 ± 0,31
Erythrocyte Ca^{2+}	4,3 ± 1,11	4,9 ± 1,10	6,8 ± 1,30	6,7 ± 0,01
Platelet Ca^{2+}	1,51 ± 0,42	1,46 ± 0,29	1,60 ± 0,51	1,62 ± 0,32
Serum Mg^{2+}	0,90 ± 0,13	0,83 ± 0,09	0,70 ± 0,11	0,73 ± 0,09
Erythrocyte Mg^{2+}	2,48 ± 0,26	2,57 ± 0,44	2,24 ± 0,40	2,11 ± 0,42
Platelet Mg^{2+}	2,1 ± 0,50	2,0 ± 0,30	1,52 ± 0,40	1,55 ± 0,37

Serum values are expressed as mmol/l, erythrocyte values as mmol/l except erythrocyte Ca^{2+} which is expressed as /umol/l, and platelet values as /umol/l x 10^8 cells.

* $p < 0,01$ males versus females

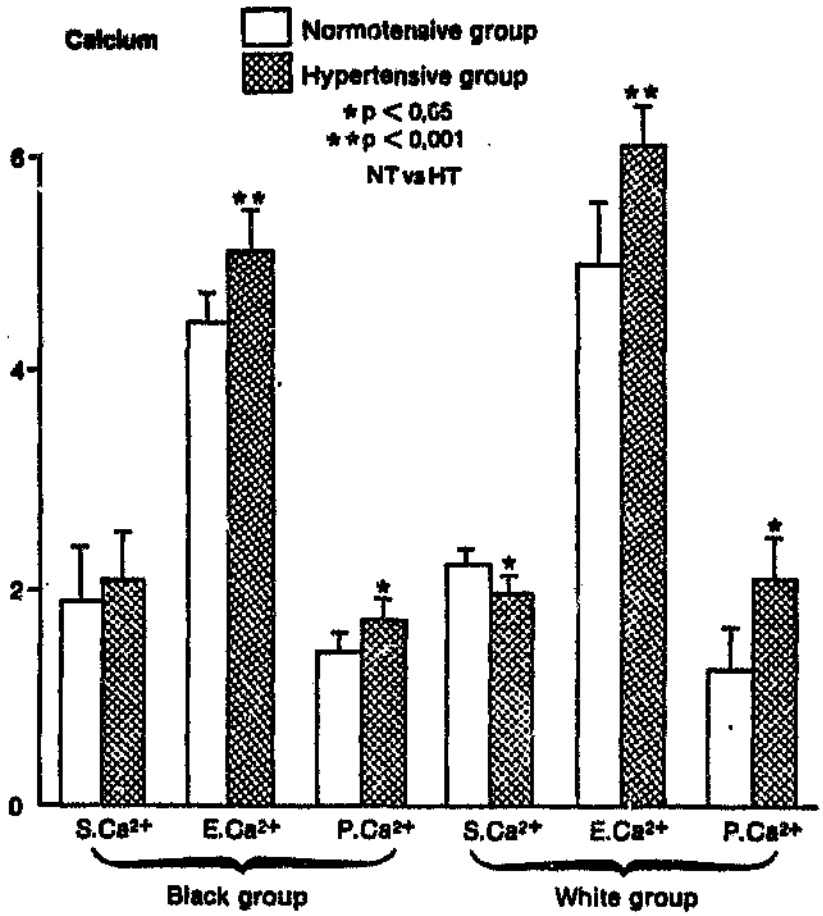


Figure 8.3.

Serum, platelet and erythrocyte calcium concentrations in the black and white normotensive and hypertensive groups.

Serum and erythrocyte values expressed as mmol/l, erythrocyte calcium expressed as µmol/l and platelet values as µmol/1x10⁸ cells

Table 8.16: Serum, erythrocyte and platelet cations levels in the white normotensive and hypertensive groups.

	White Group		
	Normotensive	Hypertensive	
Variable			P value Normotensive vs Hypertensive
Serum Na^+	138 ± 2,9	136 ± 3,2	0,06
Erythrocyte Na^+	9,8 ± 2,31	10,7 ± 2,93	0,005*
Platelet Na^+	1,45 ± 0,42	1,76 ± 0,30	0,001*
Serum K^+	3,91 ± 0,36	3,73 ± 0,41	0,16
Erythrocyte K^+	88 ± 9,1	86 ± 14,3	0,37
Platelet K^+	4,19 ± 1,04	3,99 ± 1,11	0,66
Serum Ca^{2+}	2,18 ± 0,27	2,02 ± 0,35	0,02*
Erythrocyte Ca^{2+}	5,17 ± 2,18	6,18 ± 1,67	0,009*
Platelet Ca^{2+}	1,45 ± 0,29	1,65 ± 0,44	0,05*
Serum Mg^{2+}	0,85 ± 0,05	0,79 ± 0,17	0,08
Erythrocyte Mg^{2+}	2,51 ± 0,45	2,31 ± 0,52	0,06
Platelet Mg^{2+}	2,11 ± 0,32	1,53 ± 0,51	0,02*

Serum values are expressed as mmol/l, erythrocyte values as mmol/l except erythrocyte Ca^{2+} which is expressed as /umol/l, and platelet values as /umol/l x 10^8 cells.
* = significant difference

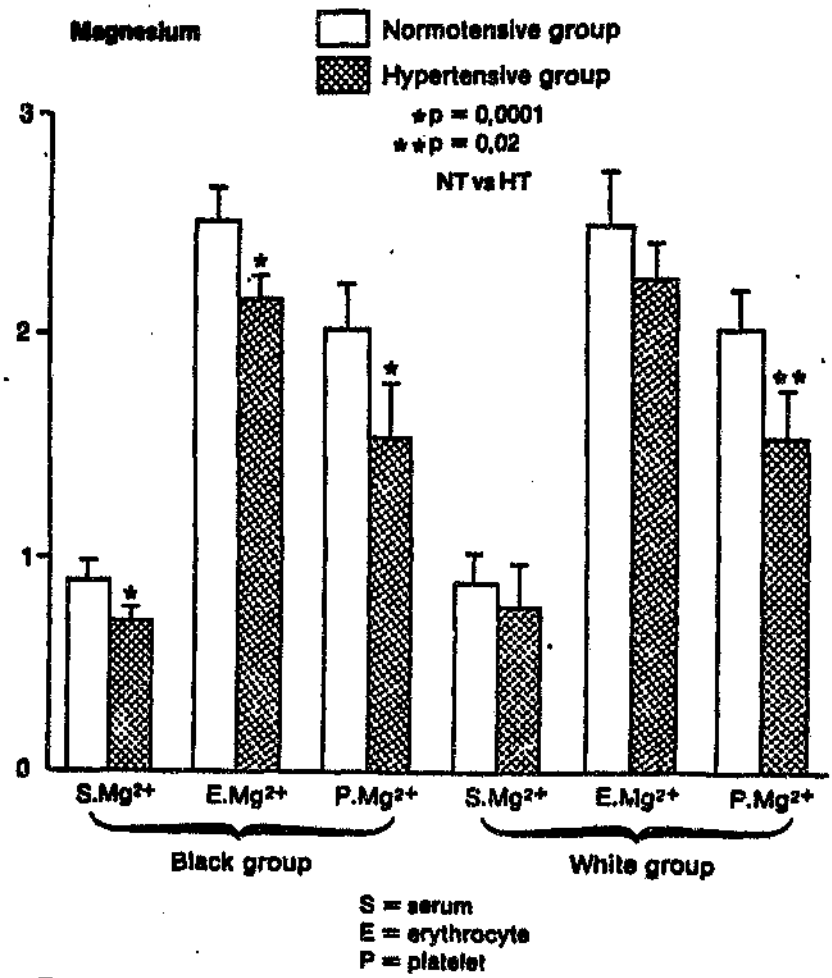


Figure 8.4.

Serum, platelet and erythrocyte magnesium concentrations in the black and white normotensive and hypertensive groups.

Serum and erythrocyte values expressed as mmol/l, platelet values expressed as $\mu mol/1 \times 10^8$ cells

Table 8.17: Serum, erythrocyte and platelet cation concentrations in the female and male normotensive and hypertensive white groups.

	White Group			
	Normotensive		Hypertensive	
Variable	Female	Male	Female	Male
Serum Na^+	137 ± 3	139 ± 2	135 ± 3	138 ± 3
Erythrocyte Na^+	9,8 ± 2,91	9,8 ± 1,72	9,6 ± 2,20	12,2 ± 0,27
Platelet Na^+	1,43 ± 0,58	1,45 ± 0,17	1,71 ± 0,32	1,82 ± 0,27
Serum K^+	3,7 ± 0,43	4,0 ± 0,21*	3,6 ± 0,29	3,9 ± 0,48
Erythrocyte K^+	90 ± 6	85 ± 11	90 ± 13	81 ± 14
Platelet K^+	4,5 ± 0,99	3,9 ± 1,06	3,8 ± 1,30	4,2 ± 0,73
Serum Ca^{2+}	2,22 ± 0,32	2,15 ± 0,21	2,10 ± 0,42	1,99 ± 0,22
Erythrocyte Ca^{2+}	6,5 ± 1,98	5,9 ± 1,31	5,6 ± 2,50	4,6 ± 1,49
Platelet Ca^{2+}	1,52 ± 0,32	1,39 ± 0,24	1,55 ± 0,47	1,79 ± 0,39
Serum Mg^{2+}	0,83 ± 0,05	0,87 ± 0,04	0,83 ± 0,15	0,74 ± 0,20*
Erythrocyte Mg^{2+}	2,3 ± 0,26	2,7 ± 0,53*	2,4 ± 0,48	2,2 ± 0,57
Platelet Mg^{2+}	2,1 ± 0,32	2,2 ± 0,33	1,50 ± 0,60	1,61 ± 0,36

Serum values are expressed as mmol/l, erythrocyte values as mmol/l except erythrocyte Ca^{2+} which is expressed /umol/l, and platelet values as /umol/l x 10^8 cells.

* $p < 0,01$ males versus females

8.4.3.1 (ii) White group

Serum Ca^{2+} levels were significantly lower in the hypertensive subjects compared to their normotensive counterparts ($P = 0,02$). There were no significant differences in serum Na^{+}, K^{+} and Mg^{2+} between the groups. In the normotensive group serum K^{+} was significantly lower in the females then the males ($P < 0,05$). There were no other significant differences in serum ions between the sexes. (Tables 8.16; 8.17).

8.4.3.1 (iii) Combined black and white groups

In the combined black and white group, serum K^{+} and serum Mg^{2+} concentrations were significantly lower in the hypertensive group ($P < 0,01$) (Figure 8.5; Table 8.18).

8.4.3.2 Intracellular cations (Tables 8.14-8.17; Figures 8.2-8.5)

8.4.3.2.1 Erythrocyte cations

8.4.3.2.1 (i) Black group

Erythrocyte Mg^{2+} concentration was significantly lower and erythrocyte Ca^{2+} significantly higher in the hypertensive patients compared to the normotensive subjects ($P = 0,0001$). There were no significant differences in

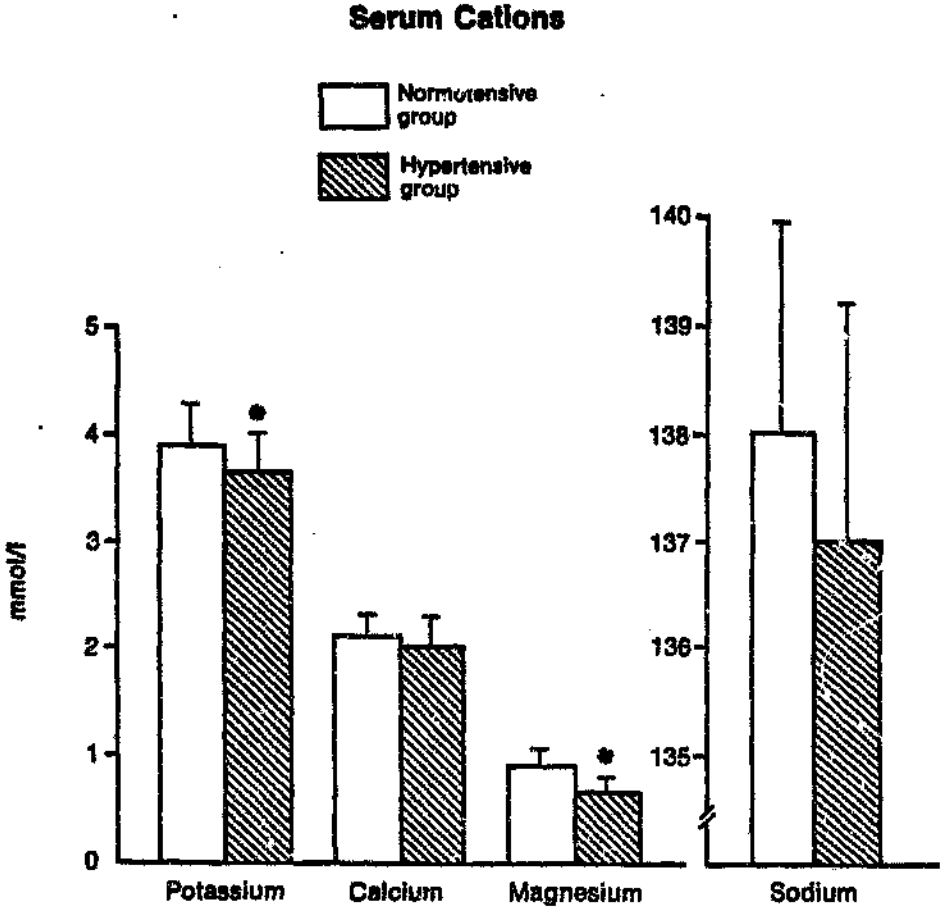


Figure 8.5. Combined groups.

Histogram of serum potassium, calcium, magnesium and sodium levels in the normotensive and hypertensive groups

* $p < 0.05$. Normotensive vs hypertensive.

Table 8.18: Serum, erythrocyte and platelet cation levels in the combined black and white groups.

	Normotensive Group Black and White (n = 78)	Hypertensive Group Black and White (n = 76)
Serum Na^+	138 ± 3	137 ± 3
Erythrocyte Na^+	9,9 ± 1,93	10,7 ± 2,7
Platelet Na^+	1,44 ± 0,36	1,67 ± 0,29*
Serum K^+	3,98 ± 0,35	3,78 ± 0,36*
Erythrocyte K^+	84 ± 8	86 ± 11
Platelet K^+	4,39 ± 1,24	4,41 ± 1,39
Serum Ca^{2+}	2,05 ± 0,21	2,06 ± 0,33
Erythrocyte Ca^{2+}	5,15 ± 1,52	6,26 ± 1,69*
Platelet Ca^{2+}	1,47 ± 0,33	1,63 ± 0,42*
Serum Mg^{2+}	0,86 ± 0,11	0,74 ± 0,13*
Erythrocyte Mg^{2+}	2,52 ± 0,39	2,22 ± 0,45
Platelet Mg^{2+}	2,10 ± 0,37	1,53 ± 0,44*

Serum values are expressed as mmol/l, erythrocyte values as mmol/l except erythrocyte Ca^{2+} which is expressed as /umol/l, and platelet values as /umol/l x 10^8 cells.

* $P < 0,05$

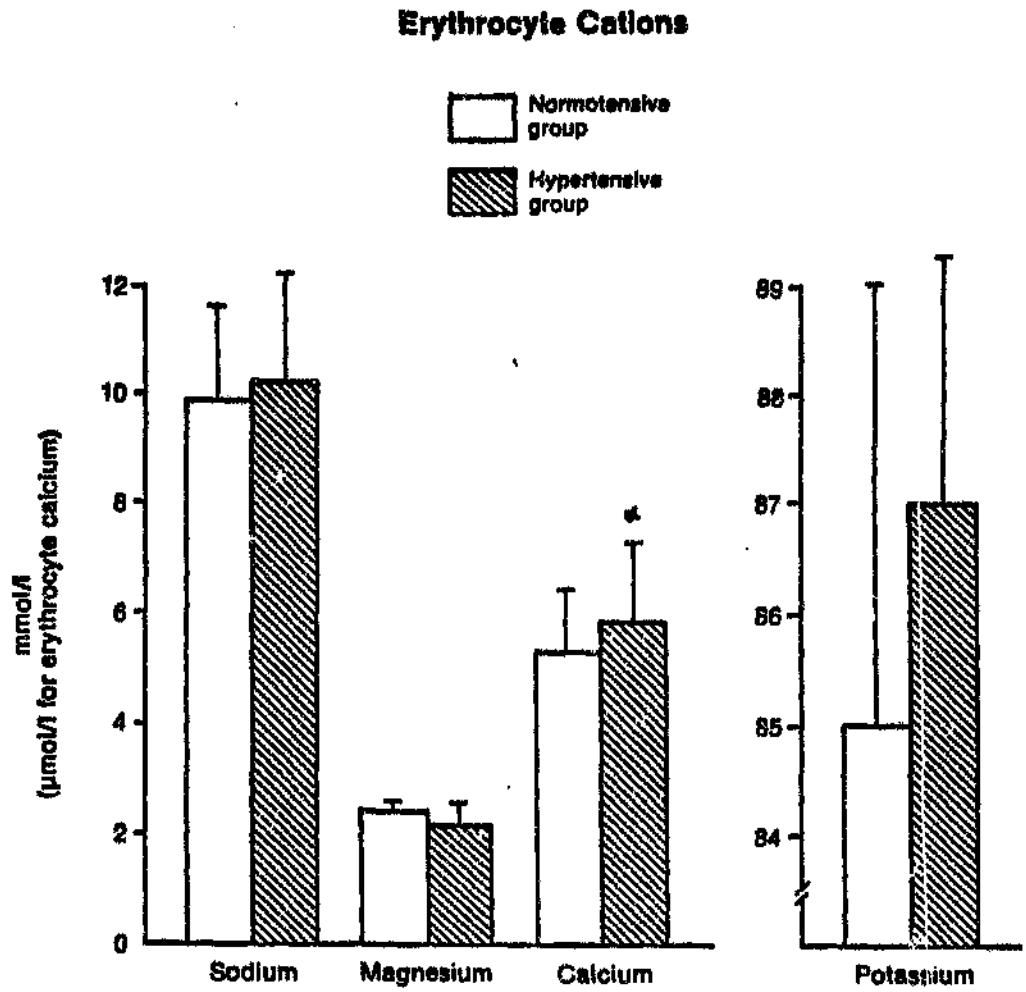


Figure 8.6. Combined groups.
Histogram of erythrocyte sodium, magnesium, calcium and potassium levels in the normotensive and hypertensive groups. Units for erythrocyte calcium are expressed as umol/l

* $p < 0,05$ Normotensive vs hypertensive

erythrocyte Na^+ and K^+ between the groups. Erythrocyte Mg^{2+} and K^+ was significantly lower in the normotensive females compared to the normotensive males. There were no other sexual differences for erythrocyte ions in the black group (Tables 8.14; 8.15).

8.4.3.2.1 (ii) White group

In the hypertensive group, erythrocyte Na+ and Ca^{2+} concentrations were significantly increased compared to the normotensive group ($P < 0,01$). Erythrocyte Mg^{2+} and K^+ levels were similar in the normotensive and hypertensive subjects. In the normotensive group, erythrocyte Mg^{2+} concentration was significantly lower in the females compared to the males ($P = 0,04$). In the hypertensive group, erythrocyte Na^+ was significantly raised in the males ($P = 0,02$) (Tables 8.16; 8.17).

8.4.3.2.1 (iii) Combined black and white group

In the combined group, the only significant difference between the normotensive and hypertensive subjects was erythrocyte Ca^{2+}. The hypertensive patients had a significantly elevated erythrocyte Ca^{2+} concentration compared to the control subjects ($P = 0,02$) (Table 8.18; Figure 8.6).

8.4.3.2.2 Platelet cations (Tables 8.14-8.17; Figures 8.2-8.5).

8.4.3.2.2 (i) Black group

Platelet Na^+ and Ca^{2+} levels were significantly higher in the hypertensive patients compared to the normotensive subjects ($P < 0,05$). In the hypertensive group, platelet Mg^{2+} was significantly decreased ($P = 0,0001$) compared to the normotensive group. In the hypertensive subjects, the females had lower levels of platelet Na^+ compared to the males ($P = 0,05$) (Tables 8.5.14, 8.5.15).

8.4.3.2.2 (ii) White group

Platelet Na^+ and Ca^{2+} concentrations were significantly elevated in the hypertensive group compared to the normotensive group ($P < 0,05$). Platelet Mg^{2+} was significantly lower in the hypertensive compared to the normotensive subjects ($P = 0,02$). There were no significant differences in platelet cations between males and females in both the normotensive and hypertensive groups (Tables 8.5.16, 8.5.17).

8.4.3.2.2 (iii) Combined black and white group

Platelet Mg^{2+} was significantly decreased in the hypertensive group compared to the normotensive group ($P =$

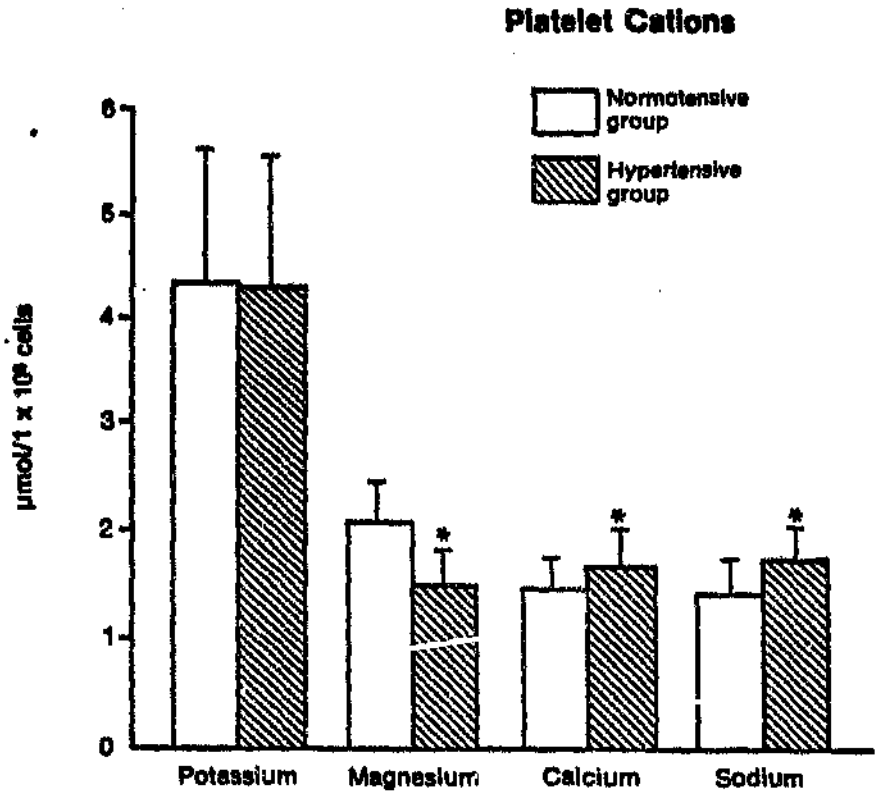


Figure 8.7. Combined groups

. Histogram of platelet potassium, magnesium, calcium and sodium levels in the normotensive and hypertensive groups

* $p < 0,01$ Normotensive group vs hypertensive group

0,001). In the hypertensive black and white combined group, platelet Na^+ and Ca^{2+} concentrations were significantly higher compared to the levels in the normotensive group ($P < 0,01$). There was no significant difference in the platelet K^+ concentration between the normotensive and hypertensive groups (Table 8.18; Figure 8.7).

8.4.4 Comparisons of variables between black and white hypertensive groups

Erythrocyte calcium was significantly higher in the black hypertensive group compared to the white hypertensive group. This was true for males and females. Serum magnesium was significantly lower in the black hypertensive subjects compared to the white hypertensive group. There were significant differences between the hypertensive black males, black females and white females. There were no significant differences for sodium and potassium between the black and white hypertensive groups (Tables 8.19-8.26).

8.4.5 Correlation Studies

8.4.5.1 Correlations of biochemical variables with MAP

8.4.5.1 (i) Black group (Table 8.27)

In the normotensive group, there was a significant inverse

Table 8.19: Pairwise-t-test comparisons for sodium between the black and white groups. Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
	NS			
BNT	NS	-		
	0,003			
	NS	NS		
WHT	NS	NS	-	
	NS	0,0002		
	NS	NS	NS	
WNT	NS	NS	0,005	-
	0,01	NS	0,001	

Top value represents serum Na^+, middle value erythrocyte Na^+ and bottom value platelet Na^+.

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

Table 8.20: Pairwise-t-test comparisons for sodium between the male and female, black and white, normotensive and hypertensive groups. Significant P values are presented.

Group	1	2	3	4	5	6	7	8
1	-							
2	NS NS NS	-						
3	NS NS NS	NS NS 0,01	-					
4	NS NS NS	NS NS 0,01	NS NS NS	-				
5	0,004 NS NS	0,009 NS NS	0,001 NS 0,01	0,001 NS 0,02	-			
6	NS 0,006 NS	NS 0,004 NS	NS 0,01 0,003	NS 0,01 0,004	0,03 0,003 NS	-		
7	NS 0,04 NS	NS NS 0,03	NS NS NS	0,03 0,05 NS	NS 0,04 0,03	NS 0,001 0,009	-	
8	NS 0,04 NS	NS NS 0,05	NS NS NS	NS 0,05 NS	0,0007 0,04 0,05	NS 0,001 0,01	NS NS NS	-

Top value represents serum Na^+, middle value for erythrocyte Na^+ and bottom value for platelet Na^+.

Group

1 = BHT female
2 = BHT male
3 = BNT female
4 = BNT male

Group

5 = WHT female
6 = WHT male
7 = WNT female
8 = WNT male
NS = not significant

Table 8.21: Pairwise-t-test comparisons for potassium between the black and white groups. Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
	0,0009			
BNT	NS	-		
	NS			
	NS	0,002		
WHT	NS	NS	-	
	NS	NS		
	NS	NS	NS	
WNT	NS	NS	NS	-
	NS	NS	NS	

Top value represents serum K^+, middle value erythrocyte K^+ and bottom value platelet K^+.

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

Table 8.22: Pairwise-t-test comparisons for potassium between the male and female, black and white, normotensive and hypertensive groups. Significant P values are presented.

Group	1	2	3	4	5	6	7	8
1	-							
2	NS NS NS	-						
3	0,0001 0,001 NS	0,001 0,04 NS	-					
4	NS 0,06 NS	NS NS NS	0,005 NS NS	-				
5	NS NS 0,07	NS NS 0,01	0,0001 0,002 0,03	0,01 0,02 NS	-			
6	NS 0,05 NS	NS NS NS	0,07 NS NS	NS NS NS	0,02 0,01 NS	-		
7	NS NS NS	NS NS NS	0,001 0,002 NS	NS 0,01 NS	NS NS NS	NS 0,01 NS	-	
8	0,01 NS NS	0,06 NS NS	NS NS NS	NS NS NS	0,001 NS NS	NS NS NS	0,03 NS NS	-

Top value represents serum K^+, middle value for erythrocyte K^+ and bottom value for platelet Na^+.

Group			Group		
1	=	BHT female	5	=	WHT female
2	=	BHT male	6	=	WHT male
3	=	BNT female	7	=	WNT female
4	=	BNT male	8	=	WNT male
			NS	=	not significant

Table 8.23: Pairwise-t-test comparisons for calcium between the black and white groups. Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
	NS			
BNT	0,0001	-		
	0,05			
	NS	0,003		
WHT	0,0001	NS	-	
	NS	NS		
	NS	NS	0,02	
WNT	NS	0,0001	0,009	-
	NS	NS	0,05	

Top value represents serum Ca^{2+}, middle value erythrocyte Ca^{2+} and bottom value platelet Ca^{2+}.

Group: BHT = black hypertensive group
NT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

Table 8.24: Pairwise-t-test comparisons for calcium between the male and female, black and white, normotensive and hypertensive groups. Significant P values are presented.

Group	1	2	3	4	5	6	7	8
1	-							
2	NS NS NS	-						
3	NS 0,0001 NS	NS 0,0001 NS	-					
4	NS 0,0001 NS	NS 0,0001 NS	NS NS NS	-				
5	NS 0,012 NS	NS 0,02 NS	NS 0,009 NS	NS NS NS	-			
6	NS 0,0001 NS	NS 0,0002 NS	NS NS 0,07	NS NS 0,02	NS NS NS	-		
7	NS NS NS	NS NS NS	0,01 0,0001 NS	0,02 0,0001 NS	NS NS NS	0,01 0,002 NS	-	
8	NS 0,07 0,07	NS NS 0,08	0,06 0,001 NS	NS 0,03 NS	NS NS NS	0,05 0,03 0,01	NS NS NS	-

Top value is for serum Ca^+, middle value for erythrocyte Ca^+ and bottom value for platelet Ca^+.

Group

1 = BHT female
2 = BHT male
3 = BNT female
4 = BNT male

Group

5 = WHT female
6 = WHT male
7 = WNT female
8 = WNT male
NS = not significant

Table 8.25: Pairwise-t-test comparisons for magnesium between the black and white groups.
Significant p values are presented.

Group	BHT	BNT	WHT	WNT
BHT	-			
	0,0001			
BNT	0,0001	-		
	0,0001			
	0,04	0,02		
WHT	NS	0,02	-	
	NS	0,04		
	0,0002	NS	NS	
WNT	0,001	NS	NS	-
	0,02	NS	0,02	

Top value represents serum Mg^{2+}, middle value erythrocyte Mg^{2+} and bottom value platelet Mg^{2+}.

Group: BHT = black hypertensive group
BNT = black normotensive group
WHT = white hypertensive group
WNT = white normotensive group

NS = not significant

Table 8.26: Pairwise-t-test comparisons for magnesium between the male and female, black and white, normotensive and hypertensive groups.
Significant P values are presented.

Group	1	2	3	4	5	6	7	8
1	-							
2	NS NS NS	-						
3	0,0001 0,04 0,0001	0,001 0,003 0,0001	-					
4	0,0004 0,003 0,0001	0,01 0,0002 0,0001	0,06 NS NS	-				
5	0,003 NS NS	0,03 0,04 NS	NS NS 0,0001	NS NS 0,0001	-			
6	NS NS NS	NS NS NS	0,002 0,06 0,0004	0,06 0,01 0,002	NS NS NS	-		
7	0,003 NS 0,0001	0,03 NS 0,0004	NS NS NS	NS NS NS	NS NS 0,0004	NS NS 0,005	-	
8	0,0003 0,001 0,0001	0,004 0,0001 0,0001	NS NS NS	NS NS NS	NS 0,06 0,0001	0,02 0,004 0,0009	NS 0,03 NS	-

Top value is for serum Mg^{2+}, middle value for erythrocyte Mg^{2+} and bottom value for platelet Mg^{2+}.

Group

1 = BHT female
2 = BHT male
3 = BNT female
4 = BNT male

Group

5 = WHT female
6 = WHT male
7 = WNT female
8 = WNT male
NS = not significant

Table 8.27: Pearsons correlation coefficients for correlations between cations and MAP in the black group.

		Black Normotensive		Black Hypertensive	
		r	p	r	p
Serum	Na^+	0,18	0,77	-0,05	0,70
Erythrocyte	Na^+	-0,06	0,66	0,24	0,08
Platelet	Na^+	0,13	0,37	0,09	0,49
Serum	K^+	-0,20	0,14	-0,03	0,79
Erythrocyte	K^+	0,14	0,29	0,05	0,70
Platelet	K^+	-0,23	0,08	0,16	0,24
Serum	Ca^{2+}	0,06	0,66	-0,36	0,0009*
Erythrocyte	Ca^{2+}	0,36	0,009*	0,39	0,003*
Platelet	Ca^{2+}	0,02	0,87	0,37	0,007*
Serum	Mg^{2+}	0,10	0,44	-0,28	0,05*
Erythrocyte	Mg^{2+}	0,02	0,90	0,02	0,82
Platelet	Mg^{2+}	-0,32	0,02*	-0,28	0,05*

Table 8.28: Pearsons correlation coefficients for correlations between cations and MAP in the white group.

		White Normotensive		White Hypertensive	
		r	p	r	p
Serum	Na^+	-0,14	0,48	-0,04	0,84
Erythrocyte	Na^+	-0,29	0,14	0,21	0,31
Platelet	Na^+	0,27	0,17	0,42	0,04*
Serum	K^+	-0,06	0,74	0,12	0,55
Erythrocyte	K^+	-0,07	0,71	-0,10	0,60
Platelet	K^+	0,01	0,96	0,35	0,09
Serum	Ca^{2+}	-0,14	0,50	-0,18	0,38
Erythrocyte	Ca^{2+}	0,02	0,91	-0,09	0,64
Platelet	Ca^{2+}	0,01	0,95	-0,02	0,90
Serum	Mg^{2+}	-0,06	0,80	-0,30	0,08
Erythrocyte	Mg^{2+}	-0,32	0,11	-0,13	0,56
Platelet	Mg^{2+}	-0,12	0,5	-0,27	0,19

correlation between platelet Mg^{2+} and MAP (r = -0,32; P = 0,02) and a significant direct correlation between erythrocyte Ca^{2+} and MAP (r = 0,36; P = 0,009). In the hypertensive subjects, MAP was inversely related to serum Mg^{2+} (r = -0,25; P = 0,05) platelet Mg^{2+} (r = -0,28; P = 0,05) and serum Ca^{2+} (r = -0,36; P = 0,01). Erythrocyte Ca^{2+} and platelet Ca^{2+} were positively correlated to MAP in the hypertensive group; r = 0,36; P = 0,003 and r = 0,37; P = 0,007 respectively.

8.4.5.1 (ii) White group (Table 8.28)

In the normotensive group, there were no significant correlations between the biochemical variables and MAP. In the hypertensive group, there was a significant positive correlation between platelet Na^{+} and MAP (r = 0,42; P = 0,04).

8.4.5.2 Correlations between the cations

8.4.5.2 (i) Black group (Tables 8.29)

In the normotensive group, there were significant positive correlations between:

- serum Na^{+} and erythrocyte Na^{+} (r = 0,36; P = 0,009)
- serum K^{+} and platelet K^{+} (r = 0,32; P = 0,02)
- platelet Mg^{2+} and platelet Ca^{2+} (r = 0,33; P = 0,01)

Significant inverse correlations in the normotensive group included:-

platelet K^+ and platelet Na^+ ($r = -0,32$; $P = 0,05$)
platelet K^+ and serum Ca^{2+} ($r = -0,41$; $P = 0,002$)
serum Ca^{2+} and erythrocyte Na^+ ($r = -0,33$; $P = 0,01$)
platelet Mg^{2+} and platelet Na^+ ($r = -0,32$; $P = 0,05$)

In the hypertensive group, significant direct correlations were found between:-

- serum Na^+ and platelet K^+ ($r = 0,32$; $P = 0,03$)
- serum Na^+ and erythrocyte K^+ ($r = 0,31$; $P = 0,02$)
- serum Na^+ and serum Mg^{2+} ($r = 0,46$; $P = 0,0005$)
- platelet Ca^{2+} and platelet K^+ ($r = 0,43$; $P = 0,001$)

In the hypertensive group, platelet Na^+ was negatively correlated to platelet Mg^{2+} ($r = -0,41$; $P = 0,002$) and serum K^+ was inversely related to erythrocyte Mg^{2+} ($r = -0,54$; $P = 0,0001$).

Pearsons correlation coefficients for the cations in the separate sexes are presented in Tables 8.30 and 8.31.

8.4.5.2 (ii) White group (Tables 8.32)

In the normotensive subjects, there were significant positive correlations between

- serum Na^+ and erythrocyte Na^+ ($r = 0,47$; $P = 0,01$)
- serum Na^+ and serum K^+ ($r = 0,46$; $P = 0,01$)
- platelet K^+ and erythrocyte K^+ ($r = 0,41$; $P = 0,03$)
- platelet Mg^{2+} and serum K^+ ($r = 0,42$; $P = 0,03$)
- erythrocyte Ca^{2+} and erythrocyte K^+ ($r = 0,53$; $P = 0,005$)

Significant inverse correlations in the normotensive subjects included:-

- erythrocyte Ca^{2+} and serum Na^+ ($r = -0,46$; $P = 0,02$)
- erythrocyte Ca^{2+} and serum K^+ ($r = -0,46$; $P = 0,02$)

In the hypertensive patients, there were significant direct correlations between:-

- serum Na^+ and serum Mg^{2+} ($r = 0,41$; $P = 0,05$)
- serum Mg^{2+} and erythrocyte K^+ ($r = 0,45$; $P = 0,03$)
- serum Mg^{2+} and erythrocyte Mg^{2+} ($r = 0,38$; $P = 0,05$)
- platelet Ca^{2+} and serum K^+ ($r = 0,51$; $P = 0,01$)
- serum Na^+ and platelet Na^+ ($r = 0,40$; $P = 0,05$)

Significant inverse correlations in the hypertensive group included:-

- serum Mg^{2+} and erythrocyte Na^+ ($r = -0,44$; $P = 0,03$)
- erythrocyte Ca^{2+} and erythrocyte Na^+ ($r = -0,47$; $P = 0,02$)
- serum GGT and serum K^+ ($r = -0,48$; $P = 0,01$)

Table 8.29: Pearsons correlation coefficients for correlations between the cations in the black normotensive and hypertensive groups.

	SNa^+	ENa^+	PNa^+	SK^+	EK^+	PK^+	SMg^{2+}	EMg^{2+}	PMg^{2+}	SCa^{2+}	ECa^{2+}	PCa^{2+}	GGT
SNa^+													
ENa^+	0,36**	-											
	0,14												
PNa^+	0,17	0,12	-										
	0,26	0,24											
SK^+	-0,12	0,07	0,08	-									
	0,06	0,09	0,29										
EK^+	0,23	0,18	0,18	-0,16	-								
	0,31*	0,30	0,11	-0,12									
PK^+	0,22	0,25	-0,32*	0,32**	0,05								
	0,32*	0,19	0,01	0,11	0,11								
SMg^{2+}	-0,13	-0,17	-0,09	-0,02	0,01	-0,13	-						
	0,46**	0,09	0,12	0,13	0,20	0,02							
EMg^{2+}	-0,03	-0,03	0,06	0,01	-0,11	-0,02	0,06	-					
	0,20	0,13	-0,30	-0,54**	0,17	0,01	0,02						
PMg^{2+}	-0,19	-0,11	-0,31**	0,11	0,11	0,06	-0,01	0,11	-				
	0,19	0,06	-0,41**	0,15	0,01	0,01	0,22	0,07					
SCa^{2+}	-0,22	-0,33**	0,08	-0,14	-0,09	-0,41**	-0,04	-0,08	-0,10	-			
	0,14	-0,13	0,06	0,18	0,04	0,09	0,04	^ 09	0,01				
ECa^{2+}	-0,03	0,08	0,21	-0,19	0,08	-0,30	0,0?	:4	0,03	0,10	-		
	0,01	0,01	0,01	0,12	0,05	0,01	0,1	,02	-0,22	0,17			
PCa^{2+}	0,05	0,19	0,08	0,01	0,15	0,17	0,12	0,07	0,33**	0,19	0,14	-	
	0,06	0,16	-0,14	0,06	0,32	0,43**	0,11	0,17	0,07	0,20	0,05		
GGT	0,24	0,24	0,02	0,21	0,16	0,17	0,01	0,17	0,11	0,17	0,16	0,14	-
	0,13	0,19	0,21	0,08	0,21	0,16	0,01	0,22	0,06	0,05	0,04	0,01	

* $p < 0,05$; ** $p < 0,01$; S = serum; E = erythrocyte, p = platelet

Top value refers to normotensive group;

Bottom value refers to hypertensive group.

Table 8.30: Pearsons correlation coefficients for correlations between the cations in the male and female black normotensive groups.

	SNa^+	ENa^+	PNa^+	SK^+	EK^+	PK^+	SMg^{2+}	EMg^{2+}	PMg^{2+}	SCa^{2+}	ECa^{2+}	PCa^{2+}
SNa^+												
ENa^+	0,17 0,42*	-										
PNa^+	0,32 0,07	0,01 0,19	-									
SK^+	-0,31 0,11	0,04 0,26	0,09 0,04	-								
EK^+	0,06 0,31	0,01 0,26	0,04 0,24	0,23 0,05	-							
PK^+	0,02 0,39*	0,44* 0,34*	0,09 0,38*	0,37 0,16	0,07 0,02	-						
SMg^{2+}	0,08 0,20	0,06 0,32	0,28 0,13	0,27 0,01	0,01 0,13	0,06 0,50**	-					
EMg^{2+}	0,06 0,04	0,20 0,01	0,22 0,01	0,25 0,04	0,01 0,21	0,01 0,01	0,17 0,05	-				
PMg^{2+}	-0,19 -0,21	0,22 0,01	-0,16 0,49**	0,19 0,16	0,22 0,03	0,03 0,08	0,03 0,05	0,18 0,13	-			
SCa^{2+}	-0,29 -0,23	-0,53** -0,24	0,10 0,06	0,12 0,07	0,06 0,22	-0,50** -0,31	0,08 0,14	0,08 0,15	0,01 -0,31	-		
ECa^{2+}	-0,04 -0,09	-0,04 0,09	0,27 0,17	-0,31 0,14	-0,19 0,22	0,01 -0,44**	0,18 0,46**	0,02 0,15	0,25 0,15	0,26 0,18	-	
PCa^{2+}	-0,05 0,17	0,01 0,39*	0,06 0,13	0,09 0,29	0,28 0,01	0,28 0,04	0,08 0,24	0,05 0,09	0,55** 0,04	0,26 0,02	0,31 0,02	

* $p < 0,05$; ** $p < 0,01$; S = serum; E = erythrocyte; p = platelet

Top value represents the females and the bottom value represents the males

Table 8.31: Pearsons correlation coefficients for correlations between the cations in the male and female black hypertensive group.

	SNa^+	ENa^+	PNa^+	SK^+	EK^+	PK^+	SMg^{2+}	EMg^{2+}	PMg^{2+}	SCa^{2+}	ECa^{2+}	PCa^{2+}
SNa^+	-											
ENa^+	0,18 0,06											
PNa^+	0,32 0,19	0,23 0,17	-									
SK^+	0,04 0,09	0,01 0,29	-0,43** -0,22	-								
EK^+	0,43** 0,11	0,38* 0,25	0,11 0,15	0,13 0,09	-							
PK^+	0,11 -0,68**	0,31 0,01	0,22 0,24	0,05 -0,35*	0,24 0,08	-						
SMg^{2+}	0,41** 0,58	0,09 0,24	0,16 0,01	0,13 0,18	0,31 0,03	0,18 -0,35*	-					
EMg^{2+}	0,19 0,19	0,08 0,34	-0,51** 0,21	0,43** 0,77**	0,19 0,19	0,07 0,06	0,21 0,19	-				
PMg^{2+}	0,27 0,06	0,05 0,31	0,36 0,50	0,12 0,21	0,16 0,27	0,02 0,04	0,18 0,26	0,24 0,18	-			
SCa^{2+}	0,28 0,07	0,04 0,28	0,15 -0,36*	0,09 0,30	0,05 0,02	0,05 0,16	0,04 0,16	0,05 0,31	0,01 0,01	-		
ECa^{2+}	0,08 0,16	0,06 0,16	0,01 0,08	0,07 0,21	0,02 0,14	0,04 0,03	0,14 0,12	0,07 0,08	0,19 0,26	0,19 0,15	-	
PCa^{2+}	0,03 0,25	0,20 0,01*	-0,36** 0,28	0,01 0,22	0,39* 0,13	0,63** 0,05	0,09 0,12	0,09 0,38*	0,10 0,10	0,24 0,14	0,04 0,09	

* $p < 0,05$; ** $p < 0,01$; S = serum; E = erythrocyte; p = platelet

Top value represents the females and the bottom value represents the males

Table 8.32: Pearsons correlation coefficients for correlations between the cations in the white normotensive and hypertensive groups.

	SNa^+	ENa^+	PNa^+	SK^+	EK^+	PK^+	SMg^{2+}	EMg^{2+}	PMg^{2+}	SCa^{2+}	ECa^{2+}	PCa^{2+}	GGT
SNa^+													
ENa^+	0,47**	-											
	0,22												
PNa^+	0,07	0,09	-										
	0,40*	0,16											
SK^+	0,46**	0,26	0,02	-									
	0,06	0,01	0,06										
EK^+	-0,31	0,03	0,07	-0,29	-								
	0,06	0,02	0,006	-0,34									
PK^+	-0,17	0,11	0,08	0,24	0,41*	-							
	0,08	0,14	0,24	0,04	-0,34								
SMg^{2+}	0,36	0,07	0,36	0,19	0,12	0,07	-						
	0,41*	-0,44*	0,28	0,13	0,45*	0,06							
EMg^{2+}	0,08	0,22	0,01	0,09	0,16	0,15	0,22	-					
	0,05	0,23	0,05	0,24	0,02	0,26	0,38*						
PMg^{2+}	0,34	0,01	-0,31	0,42*	-0,39	0,11	0,11	0,03	-				
	0,22	0,30	0,12	0,07	0,01	0,25	0,06	0,02					
SCa^{2+}	0,16	0,05	0,21	-0,25	-0,27	0,14	0,33	0,12	0,22	-			
	0,01	0,01	-0,34	-0,22	0,07	0,16	0,14	0,05	0,09				
ECa^{2+}	-0,46**	0,06	0,04	-0,46**	0,53**	0,21	0,29	0,08	0,30	0,06	-		
	0,21	-0,47*	0,21	0,21	0,09	0,23	0,12	0,31	0,14	-0,24			
PCa^{2+}	0,26	0,07	0,35	0,10	0,10	0,22	0,01	0,21	0,22	0,35	0,18	-	
	0,22	0,02	0,06	0,51**	0,10	0,34	0,14	0,14	0,34	0,21	0,18		
GGT	0,05	0,08	0,08	0,18	-0,41*	0,07	0,15	0,16	0,02	0,31	0,28	0,09	-
	0,17	0,02	0,06	-0,48*	0,15	-0,28	0,19	0,12	0,15	0,05	0,16	0,23	

* $p < 0,05$; ** $p < 0,01$; S = serum; E = erythrocyte; p = platelet

Top value refers to normotensive group;

Bottom value refers to hypertensive group.

Table 8.33: Pearsons correlation coefficients for correlations between the cations in the white normotensive males and females.

	SNa^+	ENa^+	PNa^+	SK^+	EK^+	PK^+	SMg^{2+}	EMg^{2+}	PMg^{2+}	SCa^{2+}	ECa^{2+}	PCa^{2+}
SNa^+	-											
ENa^+	0,58* 0,33											
PNa^+	0,08 0,02	0,07 0,20	-									
SK^+	0,47 0,07	0,46 0,26	0,04 -0,51*	-								
EK^+	0,01 0,34	0,28 0,31	0,06 0,42	-0,44 0,04	-							
PK^+	0,19 0,05	0,09 -0,45	0,11 0,81**	-0,04 -0,39	0,01 0,51*	-						
SMg^{2+}	0,33 0,12	0,06 0,39	0,44 0,23	0,01 0,24	0,21 0,19	0,19 0,25	-					
EMg^{2+}	0,26 0,33	0,16 0,41	-0,31 0,38	-0,36 0,13	0,39 0,32	0,17 0,36	0,01 0,17	-				
PMg^{2+}	0,46 0,19	0,06 0,09	-0,31 -0,55*	0,43 0,40	-0,51* 0,35	0,11 0,26	0,01 0,17	0,06 0,21	-			
SCa^{2+}	0,07 0,54*	0,25 0,48	0,36 -0,46	0,31 0,01	0,23 0,82**	0,01 0,44	0,50* 0,20	0,21 -0,34	0,18 0,34	-		
ECa^{2+}	0,36 0,56*	0,02 0,14	0,14 0,43	-0,42 0,43	0,61* 0,57*	0,11 0,31	-0,16 0,44	0,26 0,14	0,24 0,48	0,40 -0,77**	-	
PCa^{2+}	0,33 0,51*	0,05 0,36	0,49 0,02	0,44 0,33	-0,29 0,16	0,22 0,13	0,24 0,22	0,09 0,19	0,36 0,16	0,29 0,08		-

* $p < 0,05$; ** $p < 0,01$; S = serum; E = erythrocyte; p = platelet
Top value represents the females and the bottom value represents the males;

Table 8.34 Pearsons correlation coefficients for correlations between the cations in the white hyporte: sive males and females.

	SNa^+	ENa^+	PNa^+	SK^+	EK^+	PK^+	SMg^{2+}	EMg^{2+}	PMg^{2+}	SCa^{2+}	ECa^{2+}	PCa^{2+}
SNa^+	-											
ENa^+	0,24 0,61*											
PNa^+	0,61* -0,06	0,18 0,01	-									
SK^+	0,27 -0,49	0,40 -0,58*	0,02 0,03	-								
EK^+	-0,33 0,74*	0,12 0,53	0,06 0,08	0,39 -0,81**	-							
PK^+	0,19 0,51	0,08 0,07	0,29 0,01	0,36 0,16	0,21 -0,60*	-						
SMg^{2+}	0,41 0,81**	0,44 0,81**	0,47 0,19	0,57* 0,42	0,16 0,62*	0,01 0,10	-					
EMg^{2+}	0,10 0,22	0,04 0,74**	0,33 -0,51*	0,10 0,22	0,18 0,02	0,26 0,46	0,25 0,41	-				
PMg^{2+}	0,05 0,63*	0,21 0,50	0,17 0,06	0,23 0,02	0,03 0,19	0,38 0,36	0,14 0,54*	0,11 0,37	-			
SCa^{2+}	0,03 0,39	0,41 0,07	0,39 0,12	0,04 0,42	0,19 0,52*	0,01 0,78**	0,28 0,03	0,01 0,34	0,13 0,07	-		
ECa^{2+}	0,09 0,25	-0,42 -0,53*	0,27 0,07	0,06 0,84**	0,23 -0,49	0,18 0,27	0,21 0,28	-0,39 -0,41	0,15 0,19	0,38 0,04	-	
PCa^{2+}	0,27 0,13	0,03 0,21	0,19 0,32	0,16 0,80**	0,24 -0,45	0,57* 0,02	0,10 0,09	-0,22 0,08	0,37 0,29	0,15 0,17	0,08 0,81**	-

* $p < 0,05$; ** $p < 0,01$; S = serum; E = erythrocyte; p = platelet

Top value represents the females and the bottom value represents the males

Correlation coefficients for the separate sexes are shown in Tables 8.33-8.34.

8.4.6 Summary of results:-

1. The normotensive and hypertensive groups were matched for age, height and weight.
2. In the black group, the hypertensive patients had significantly:
   (i) increased platelet Na^+
   (ii) decreased serum K^+
   (iii) increased erythrocyte Ca^{2+}
   (iv) increased platelet Ca^{2+}
   (v) decreased serum Mg^{2+}
   (vi) decreased erythrocyte Mg^{2+}
   (vii) decreased platelet Mg^{2+}
3. In the white group, the hypertensive patients had significantly:
   (i) increased platelet Na^+
   (ii) increased erythrocyte Na^+
   (iii) decreased serum Ca^{2+}
   (iv) increased erythrocyte Ca^{2+}
   (v) increased platelet Ca^{2+}
   (iv) decreased platelet Mg^{2+}
4. In the combined black and white groups, the hypertensive patients had significantly:
   (i) increased platelet Na^+
   (ii) decreased serum K^+

(iii) increased platelet Ca^{2+}

(iv) increased erythrocyte Ca^{2+}

(v) decreased serum Mg^{2+}

(vi) decreased platelet Mg^{2+}

5. Serum Mg^{2+} was significantly lower and erythrocyte Ca^{2+} significantly higher in the black hypertensive group compared to the white hypertensive group.

6. Correlations with MAP

a) In the black group

Normotensive subjects; MAP was -

- directly correlated to erythrocyte Ca^{2+}
- inversely correlated to platelet Mg^{2+}

Hypertensive subjects; MAP was -

- directly correlated to - erythrocyte Ca^{2+}
  - platelet Ca^{2+}
- inversely correlated to - serum Mg^{2+}
  - platelet Mg^{2+}
  - erum Ca^{2+}

b) In the white group:-

Normotensive subjects - no significant correlations with MAP.

Hypertensive subjects - MAP was - positively correlated to platelet Na^{+}

7. Correlations between cations:

a) Black group

In the normotensive subjects there were significant positive correlations between:-

- extracellular Na^+ and intracellular Na^+
- extracellular K^+ and intracellular K^+
- intracellular Mg^{2+} and intracellular Ca^{2+}

Significant inverse correlations included -

- extracellular Ca^{2+} and intracellular Na^+ and K^+

Hypertensive subjects - significant positive correlations included -

- extracellular Na^+ and intracellular K^+
- extracellular Na^+ and extracellular Mg^{2+}
- intracellular Ca^{2+} and intracellular K^+

Significant inverse correlations included -

- intracellular Na^+ and intracellular Mg^{2+}

b) White group

Normotensive subjects:-

Significant positive correlations included -

- extracellular Na^+ and intracellular Na^+ and K^+
- intracellular Mg^{2+} and extracellular K^+

- intracellular Ca^{2+} and intracellular K^+

Significant inverse correlations included -
- intracellular Mg^{2+} and intracellular K^+
- intracellular Ca^{2+} and extracellular Na^+ and K^+

In the Hypertensive subjects:-

Significant positive correlations included -
- extracellular Na^+ and intracellular K^+ and Mg^{2+}
- intracellular Ca^{2+} and extracellular K^+

Significant inverse correlations included -
- extracellular Mg^{2+} and intracellular Na^+
- intracellular Na^+ and intracellular Ca^{2+}

8.5 DISCUSSION

The results presented provide evidence that intracellular cations are altered in essential hypertension. Platelets were chosen for the study because they are easily accessible and have many features in common with vascular smooth muscle, including a calcium-dependent contractile system and an -2-adrenoceptor cyclase system (Hinssen et al, 1978; Erne et al, 1983) (Section 4.1.2). Little is known of platelet total content of magnesium, sodium, potassium and calcium. Erythrocytes in essential hypertension were also studied as a baseline cell of

comparison. The free cation level, which is the major determinant of vascular smooth muscle contractility, constitutes a small proportion of the intracellular concentration and therefore does not accurately reflect total cell cation content. For this reason, the total concentration of intracellular cations was measured in this study. The intracellular total calcium concentration may be a marker of the cytosolic free calcium concentration (see Appendix J).

Platelet sodium, unlike erythrocyte sodium, was significantly elevated in the hypertensive patients in the black and white group and in the combined group. Reported data on erythrocyte sodium in hypertension have been conflicting, although increased leucocyte sodium has been repeatedly reported in hypertensive patients (Edmonson et al, 1975; Boon et al, 1985). Platelet sodium has not been previously studied in essential hypertension and the consistently raised levels in the hypertensive group may be representative of vascular smooth muscle status in hypertension. In the white hypertensive females, platelet sodium was positively correlated with blood pressure whereas in the black hypertensive females erythrocyte sodium correlated with blood pressure. These results confirm others which report increased white blood cell sodium levels with direct associations between intracellular sodium and blood pressure (Ambrosioni et al, 1981). Mechanisms of how intracellular sodium retention

produces hypertension are unclear. Factors that have been implicated include increased total peripheral resistance and the interaction of intracellular sodium with the other cations and particularly calcium (Kurtz and Morris, 1990). Since there is a Na^{+}-Ca^{2+} exchange system, concentration changes in one ion will affect the other (Blaustein, 1977). The sodium-calcium interaction may be important in black hypertensive patients. Ernst et al (1990) recently demonstrated that calcium attenuates cardiovascular reactivity to sodium and stress in blacks. Dietary calcium may thus have a prophylactic blood pressure lowering effect in this group of patients. Zemel et al (1986) also demonstrated that in hypertensive black adults, dietary sodium causes an increased intracellular calcium which is prevented by calcium supplementation.

Much evidence has recently focused on calcium in hypertension (Cuttler and Brittain, 1990; Sugiyama et al, 1990). Results from this study showed that in the combined groups there were no significant differences for serum calcium between groups and no significant correlations between serum calcium and blood pressure. In the white hypertensive patients, serum calcium was significantly lower compared to the normotensive controls. In the combined analysis, and in the separate groups, intracellular calcium (platelet and erythrocyte) was significantly elevated in the hypertensive patients. In the black hypertensive group, intracellular calcium

(platelet and erythrocyte) was positively associated with mean arterial pressure. Platelet levels were correlated with blood pressure in both males and females, but direct correlations between erythrocyte levels and mean arterial pressure were only found in females. These results confirm others which have documented increased platelet free calcium in essential hypertension with strong positive correlations between the free calcium concentration and height of blood pressure (Le Quan Song et al, 1985; Cooper et al, 1987). The intracellular calcium concentration determines the development of tension in vascular smooth muscle and hence arteriolar resistance (Karaki, 1990). Raised intracellular calcium results in increased contractility, tone and resistance and ultimately elevated blood pressure. Cytosolic calcium accumulation may result from enhanced calcium influx, increased intracellular mobilisation and decreased efflux (Bolton et al, 1986). Defects in these mechanisms have been described in hypertension (Buhler et al, 1986; Robinson et al, 1984).

Platelet magnesium was consistently decreased in the hypertensive group, both in the combined analysis and in the black and white groups separately. Serum and erythrocyte magnesium were significantly lower in the hypertensive black patients compared to their normotensive counterparts. There were no significant differences for serum and erythrocyte magnesium in the white hypertensive and normotensive subjects. In the combined analysis, serum

and platelet magnesium were inversely correlated with mean arterial pressure. In the black hypertensive group, serum, platelet and erythrocyte magnesium levels were negatively associated with blood pressure. There were no correlations between magnesium (serum, platelet or erythrocyte) in the white group. The data relating to the black group support other studies which have reported hypomagnesaemia and erythrocyte Mg^{2+} depletion in hypertensive patients (Peterson et al, 1977; Touyz et al, 1989).

Resnick et al (1984) have repeatedly demonstrated that erythrocyte free magnesium is significantly lower in hypertensive patients compared to normotensive controls, with significant inverse correlations between magnesium levels and systolic and diastolic pressures. Other studies have failed to document any difference in serum magnesium between hypertensive and normotensive subjects, and have not found any correlation between magnesium and mean arterial pressure (Harlan et al, 1984; Wehling, 1988; Rinner et al, 1989). These data are in agreement with the results obtained for the white group studied here. A recent study reported increased erythrocyte magnesium levels in hypertensive patients (Kjeldsen et al, 1990). The conflicting reports may be explained on the basis of sample size and population group studied. The magnesium abnormalities and relation to hypertension may be restricted to a specific subgroup of hypertensive patients based on race, sex and renin status.

Reasons for the differences in magnesium status between hypertensive and normotensive subjects and between blacks and whites may be associated with dietary intake. Blacks have a lower potassium diet than whites, and a low potassium diet is also a low magnesium diet (Grim et al, 1980; Steyn et al, 1986). This is supported by the fact that in the black hypertensive patients studied, both serum potassium and serum magnesium were significantly decreased. Although there is conflicting evidence supporting a high potassium (and magnesium) diet as non-drug therapy in hypertension, the protective effect may be beneficial only in certain populations, such as the urban blacks studied here. Unlike other reports, there were no significant correlations between potassium (serum, platelet or erythrocytes) with blood pressure in this study.

Besides dietary intake there are other environmental factors that may influence cation status in hypertensive patients including excessive alcohol intake. An exact alcohol history was unobtainable, and serum gamma glutamyl transferase was taken as an indirect marker of alcohol intake (Ramsay, 1977). Since the levels were similar in the normotensive and hypertensive groups, and there were no significant correlations between GGT and the cations, alcohol was probably not a major cause of the cation alterations in the hypertensive patients. When comparing the black and white groups, serum GGT levels were significantly higher in the black group compared to the

white group. This may play a role in the differences in magnesium status between the blacks and whites.

Cell membrane defects may be associated with cation abnormalities in essential hypertension (Postnov, 1985). The racial differences that were observed in this study may be attributed to differences in ion regulation. Several differences in sodium and potassium transport between blacks and whites have been documented (Aviv and Gardner, 1990). Whether there are racial differences in cellular transport processes involving the other ions remains unclear.

The results of this study suggest that in essential hypertension there is platelet sodium and calcium overload with magnesium depletion. Unlike whites, black hypertensive patients have consistently lower serum magnesium and potassium levels with erythrocyte magnesium deficiency compared to normotensive subjects. The cation alterations are more widespread in the black hypertensives. It is proposed that cellular magnesium defects may be more important in black hypertension than in white hypertension.

CHAPTER 9

CELL MEMBRANE Na^+-K^+-ATPase, Ca^{2+}-ATPase AND Mg^{2+}-ATPase ACTIVITIES IN ESSENTIAL HYPERTENSION

9. CELL MEMBRANE ADENOSINE TRIPHOSPHATASE (ATPase) ACTIVITY IN ESSENTIAL HYPERTENSION

9.1 INTRODUCTION

9.1.1 The ATPase enzymes

Many cell membrane transport systems control the intracellular ionic environment. Abnormal ion transport in vascular smooth muscle cell membranes may lead directly to increased resistance by activating contraction and/or stimulating hypertrophy and hyperplasia (Blaustein, 1977; Folkow, 1978). Also, altered cation transport in cells other than those from the vasculature can influence peripheral resistance indirectly. For example through the cells and hormones that regulate fluid volume and by effects mediated through the autonomic system (Tosteson et al, 1981). Hence, defects in one or more of the cation transport systems may play a role in the pathogenesis of hypertension. The ion pumps are quantitatively the most important cation transport systems. The two major ATPase pumps are:- i) Na^+-K^+-ATPase and ii) Ca^{2+}-ATPase.

9.1.1.1 Na^+-K^+-ATPase

Na^+-K^+-ATPase, the electrogenic pump for sodium and potassium is localised in the cell membrane and is activated by sodium and potassium in the presence of

magnesium and adenosine triphosphate (ATP) (Stone and Xie, 1988). Na^+-K^+-ATPase has also recently been localised to intracellular membrane compartments (Caplan et al, 1986). The activity of the Na^+-pump in isolated membranes is inhibited by ouabain and by the removal of potassium and magnesium; similar conditions inhibit the electrogenic transport of sodium and potassium in intact cells (Thomas, 1972). In the vascular wall, the Na^+-pump contributes to the resting membrane potential by causing a net excess of positive charge outside the cell, thus controlling the degree of activity of the vascular smooth muscle cells (Thomas, 1972; Friedman, 1979). (For further detail see Section 1.3.9.1).

9.1.1.1 (i) Cell membrane Na^+-K^+-ATPase activity in hypertension

Failure of the Na^+-pump results in progressive sodium accumulation in the cell, cell volume expansion and decreased cell membrane potential. Observations of increased intracellular sodium in cells from hypertensive patients, stimulated researchers to investigate cell membrane Na^+-K^+-ATPase activity in essential hypertension. The Na^+-K^+ pump however has been inadequately examined, usually by indirect methods in heterogeneous populations often receiving treatment, and with conflicting results. Some studies have failed to demonstrate differences in erythrocyte ATPase activity in treated hypertensive and

normotensive subjects (Swartz et al, 1981; Lijnen et al, 1988). Other reports have documented increased erythrocyte ATPase activity in human and experimental hypertension (Hannaert et al, 1988; Hajem et al, 1990; Zicha and Duhm, 1990; Syme et al, 1990). Most studies however have demonstrated that cell membrane Na^+-pump activity is decreased in essential hypertensive patients and SHR (Postnov et al, 1977; Aderounmu et al, 1979; Poston et al, 1982; Lin et al, 1985; Chen et al, 1986; Ringel et al, 1987). One of the largest studies examining the relationship between Na^+-pump activity and blood pressure, demonstrated that systolic, diastolic and mean blood pressure levels were negatively correlated to erythrocyte Na^+-K^+-ATPase activity (Rygielski et al, 1987).

Studies that have determined the ouabain-sensitive sodium efflux component in hypertensive subjects have also produced conflicting results. Many studies reported a decreased ouabain-sensitive sodium efflux, whereas others reported higher or unchanged values in hypertensive subjects compared to normotensive subjects (Cole, 1985; Saito et al, 1984; Bramley et al, 1985).

The discrepancies between results may be due to confounding variables that were not accounted for in the various studies. Race, sex, pregnancy and antihypertensive drugs may affect sodium transport. Pregnancy and contraceptive agents increase rubidium 86 uptake by the cells and also

increase the number of ouabain-binding sites in erythrocytes (Lowenstein, 1962; Smith et al, 1982). Dorup et al, (1988b) demonstrated that Na^+-K^+ pump number is decreased in skeletal muscle during treatment with diuretics. Sexual and racial differences for ouabain-sensitive sodium efflux have also been described (Smith et al, 1988). Most studies show that blacks have reduced V_{max} for erythrocyte Na^+-K^+-ATPase, activity, decreased sodium pump density and increased intracellular sodium concentrations (Mbuyamba Kabangu, 1984; Lasker et al, 1985; Hopp et al, 1986). Rahman et al (1986) conducted a tightly controlled study where a homogenous group of black males, on no treatment was investigated. They reported significantly lower erythrocy Na^+-K^+-ATPase activity in the patients with essential hypertension compared to the normotensive controls. The difference w s entirely a result of the ouabain-sensitive moiety. Other studies have failed to demonstrate Na^+-pump activity differences between races (Tuck et al, 1987).

The increased ATPase activity in certain hypertensive patients may be a manifestation of a compensatory or adaptive mechanism. Deiz et al (1987) described a group of hypertensive subjects whose erythrocytes exhibit low affinity of the Na^+-pump to internal sodium. These and other cells with increased Na^+ leak have augmented, maximal Na^+-pump activity when loaded with sodium (Garay and Nazaret, 1985; Diez et al, 1987). The higher maximal

activity of Na^+-K^+-ATPase in these cells probably reflects an adaptive process. In this process, the cells increase their Na^+-pump density to compensate for the lower affinity to internal sodium or for the increased sodium entry.

The current data suggests that hypertensives represent specific and distinct subgroups with a spectrum of alterations in the Na^+-pump, some of which may be manifestations of adaptive processes.

Whether there is a cause-effect relationship between inhibition of ATPase activity and essential hypertension remains unclear. Millar and Bramley (1986) tested whether abnormal sodium transport in hypertension is a direct consequence of the increased arterial pressure. They found that acute changes in pressure had no effect on erythrocyte sodium efflux and concluded that abnormal sodium transport in hypertension was not a consequence of raised blood pressure. Reduced ATPase activity in peripheral blood cells may reflect similar inhibition in the Na^+-K^+ pump in vascular smooth muscle cells. Strong evidence suggests that ATPase inhibition in the vascular walls results in vasoconstriction (Hendrickx and Casteels, 1974). These data support the causative role that depressed Na^+-pump activity may have in the pathogenesis of essential hypertension.

9.1.1.1. (ii) Factors that inhibit Na^+-K^+-ATPase

The importance of reduced Na^+-K^+-ATPase activity to the development of high blood pressure is the relationship between sodium pump and the intracellular calcium content. Inhibition of the pump causes a rise in cytoplasmic calcium and in the vasculature results in increased smooth muscle tone. A variety of Na^+-K^+-ATPase inhibitors have been described (de Wardener, 1982; Hasegawa et al, 1987; Borghi et al, 1990). Most of these appear to be naturally occurring compounds and have been termed endogenous circulating sodium transport inhibitors (Poston, 1987).

The first suggestion that a humoral sodium pump inhibitor existed was in 1961, when de Wardener postulated that a sequence of events occurs whereby the initiating event in essential hypertension would be a renal defect in salt excretion, leading to volume expansion and to release of a circulating natriuretic substance with vasoconstrictor properties and inhibition of ATPase activity. The resulting natriuresis would lead to a new steady state, without volume expansion, but with higher blood pressure (de Wardener and MacGregor, 1982). The natriuretic effects of plasma and urine extracts from animals and man undergoing volume expansion have been extensively reported (review by de Wardener and Clarkson, 1985). Kelly et al (1985) isolated three fractions in normal plasma which inhibit the Na^+-pump and which cross-react with digoxin

antibodies. In 1984 Balzan et al demonstrated endogenous digitalis-like activity (EDLA) in deproteinised normal serum and in urine. A sodium transport inhibitor, suggested to be a glycosteroid, has been extracted from normal urine of hypertensive patients (Cloix et al, 1986). Fagoo and Godfraind (1985) isolated a Na^+-K^+ pump inhibitor, probably a lignan, which they described as 'cardiodigin'. These lignans have several structural similarities to cardiac glycosides.

The cardiac glycosides (digitalis) have constrictor effects on vascular smooth muscle. These effects are attributed to the interrelationships between Na^+-pump activity, membrane potential and to intracellular sodium and calcium concentrations (Blaustein, 1977; Mulvany, 1985). Digitalis is an ouabain-like factor that selectively inhibits the Na^+-K^+ pump. Endogenous compounds that have digitalis like actions have been termed 'digitalis-like factors'. Mammalian plasma contains abundant digitalis-like activity in assays for Na^+-K^+-ATPase activity, ouabain binding and digoxin immunoreactivity (Buckalew, 1988). These actions are elevated in some hypertensive subjects (Buckalew, 1988).

9.1.1.1 (ii.a) Digitalis-like factor (DLF)

Plasma digitalis-like factor (DLF) constitutes a complex array of factors. The major components of these include

lipids, steroids and unidentified chemicals (Buckalew and Haddy, 1990). Many different lipids may inhibit Na^+-K^+-ATPase, but the most important of these are free fatty acids (FFA) and lysophospholipids (Tamura et al, 1985; Buckalew and Haddy, 1990). Lipids do not inhibit the Na^+-pump in an ouabain like fashion. They probably alter membrane lipids in a way that inhibits the enzyme's activity (Kelly, 1987). The steroids which have been identified as contributing to plasma digitalis-like activity include dehydroepiandrosterone sulfate, hydrocortisone, 19-norhydroxyandrostenedione and 19-hydroxyandrostenedione (LaBella et al, 1979; Schreiber and Stephan, 1979). Several reports have suggested an endogenous substance which is neither lipid nor steroid (Haupert, 1987; Rauch and Buckalew, 1988). This factor has a low molecular weight, is stable in boiling hydrochloric acid and occurs in the hypothalamus. Whether the hypothalamic Na^+-pump inhibitor is also found in plasma remains unclear. Some reports have demonstrated increased plasma levels of this factor in subjects on high-salt diets and in hypertensive individuals (de Wardener et al, 1987; Rauch and Buckalew, 1988).

Some of the DLFs have natriuretic effects and may play a role in volume regulation (Buckalew and Haddy, 1990). The lysophospholipids (LPC) have been identified as putative natriuretic hormones (Blankley and Kaplan, 1984). Unlike the FFA, the LPC have a blood pressure lowering effect, and

their role in the pathophysiology of hypertension is unclear (Blankley and Kaplan, 1984).

9.1.1.1 (ii.b) Atrial natriuretic factor (ANF)

Atrial natriuretic peptide has potent natriuretic properties and is related to salt balance. It was thus suggested that this may be the 'natriuretic hormone' and also the sodium transport inhibitor of essential hypertension. Some of the natriuresis observed in the early volume expansion studies may have been related to ANF but this peptide has no effect on Na^+-K^+-ATPase and could therefore not explain the sodium pump inhibitory effects of extracts from urine or plasma in the volume expanded state (de Wardener and Clarkson, 1985). Also, ANF is a vasodilator and not a vasoconstrictor (Sonnenberg, 1985).

9.1.1.1 (iii) Summary

There seems little doubt that sodium transport is abnormal in essential hypertension and that this abnormality is related to a circulating sodium pump inhibitor. Whether this inhibitor plays a role in the aetiology of hypertension remains to be proven. Also, the relevance of the endogenous Na^+-pump inhibitors in normal subjects is unclear.

9.1.1.2 Ca^{2+}-ATPase

Ca^{2+}-ATPase pumps calcium out of the cell (Schatzmann, 1982). This enzyme is present in all eucaryotic plasma membranes (Carafoli et al, 1990). The pump is a high affinity enzyme, which interacts with calcium with a K_m significantly less than 1 μm (Schatzmann, 1982). It continuously exports calcium from the cell at a rate of 0,5 nmoles per milligram of membrane protein per second (Carafoli et al, 1990).

The Ca^{2+}-ATPase pump belongs to the P class of ATPases, that is, it forms an aspartyl-phosphate during the reaction cycle (Pedersen and Carafoli, 1987). It is a polypeptide of 140 kDa and contains 1220 amino acids (Carafoli and Zurini, 1982). A hydrophillic portion protrudes from the cytoplasmic surface of the membrane, and a hydrophobic core is inserted into the membrane bilayer (Carafoli et al, 1990) (Figure 1.10; Section 1.2.4.3).

The Ca^{2+}-ATPase enzyme catalyses the overall reaction:

$$ATP + Ca^{2+}_{in} \xrightarrow{Mg^{2+}} ADP + P_i + Ca^{2+}_{out}$$

The plasma membrane pump is a target of calmodulin stimulation (Gopinath and Vincenzi, 1977). Unlike other enzyme targets where calmodulin acts by promoting phosphorylation, the activation here is due to the direct interaction of calcium with the pump (James et al, 1988).

Also activation of a cAMP-dependent protein kinase leads to stimulation of Ca^{2+}-ATPase, thus potentiating the efflux of calcium from cells (Neyses et al, 1985; James et al, 1988).

Two intracellular compartments - the sarcoplasmic reticulum and the mitochondria, have the ability of sequestrating calcium by means of the Ca^{2+} - pumps. Ca^{2+}-ATPase is the major protein component of the sarcoplasmic reticulum (MacLennan, 1970). The sarcoplasmic reticulum accumulates 2 moles of calcium per mole of ATP hydrolysed (Hasselbach, 1964). Mitochondria isolated from almost all sources, can actively transport Ca^{2+} across the inner membrane into their matrix (Nicholls and Akerman, 1982). The driving force for this active transport is the electrical potential generated across the inner membrane during oxidation of metabolic substrates by the respiratory chain or during hydrolysis of ATP by the coupling ATPase (Carafoli et al, 1990). In the sarcoplasmic reticulum and mitochondria, the overall reaction catalysed by the pump is:-

$$ATP + Ca^{2+}_{out} \longrightarrow ADP + P_i + Ca^{2+}_{in}$$

9.1.1.2 (i) Cell membrane Ca^{2+}-ATPase activity in hypertension

The plasma membrane Ca^{2+}-ATPase is of major interest in hypertension because of its critical role in maintaining calcium homeostasis (Carafoli and Zurini, 1982). The

regulation of Ca^{2+}-ATPase is complex and in addition to calcium, magnesium and calmodulin other factors can modulate calcium efflux activity (Robinson, 1984; Postnov and Orlov, 1985; Buhler and Resink, 1988).

Postnov (1990) described the major pathological lesion in essential hypertension as being a widespread membranopathy. This 'membranopathy' constitutes increased calcium efflux, altered calcium binding by the inner surface of the plasma membrane and the alteration of Ca^{2+}-ATPase activity. The findings with respect to the Ca^{2+}-pump in hypertension are varied with no agreement concerning the nature of the Ca^{2+}-ATPase abnormality, whether Ca^{2+}-ATPase activity is decreased or increased; even the involvement of calmodulin-dependent or independent fractions is a matter of controversy (de la Sierra et al, 1990).

It has been postulated that decreased plasma membrane pump activity is responsible for cellular calcium overload in hypertension (Postnov et al, 1984). Decreased activity of the calmodulin-stimulated, Mg^{2+} dependent Ca^{2+}-pump has been demonstrated in erythrocytes and platelets of hypertensive patients, and in erythrocytes, synaptosomes and cardiomyocytes of SHR (Postnov et al, 1984; Orlov et al, 1988). These studies were followed up by the finding of increased cytoplasmic free calcium concentrations in platelets of essential hypertensive patients (Erne et al, 1984; Le Quan Song et al, 1985). In SHR, intracellular

calcium was increased in erythrocytes, platelets, synaptosomes, lymphocytes, vascular smooth muscle cells and skeletal muscle cells (Ochima et al, 1986; Orlov et al, 1988; Sugiyama et al, 1990; Touyz et al, 1991).

Resink et al (1986) showed that calmodulin stimulation of Ca^{2+}-ATPase in platelets from hypertensive patients was increased. This elevation in Ca^{2+}-ATPase activity in hypertensive subjects might indicate a primed calcium transport system which functions to protect against cellular calcium overload and to compensate for the inefficient degree of stimulation of the calcium extrusion system by calmodulin (Resink et al, 1986).

Most studies have examined the biochemical and pharmacological properties of the Ca^{2+}-pump in broken cell membranes or inside-out vesicles which cannot be directly extrapolated to the Ca^{2+}-pump under physiological conditions in intact cells. Dagher et al (1988) examined calcium transport in intact erythrocytes. They measured $^{45}Ca^{2+}$ extrusion from Ca^{2+} loaded cells and found that the erythrocyte Ca^{2+}-pump was not altered in essential hypertension. De la Sierra et al (1990) also conducted studies using intact erythrocytes. They demonstrated that essential hypertensive patients are heterogeneous with respect to calcium pump function. About 75% of the hypertensive patients studied had normal calcium pump fluxes, while 25% had a low apparent affinity for internal

calcium (De la Sierra et al, 1990).

Factors that may be associated with defective Ca^{2+}-ATPase activity in essential hypertension include hydrophobicity, acid phospholipids, polyphosphoinositides and proteolysis (Buhler and Resink, 1988). Takaya et al (1989) and De la Sierra et al (1990) demonstrated that defective platelet Ca^{2+}-pump function is associated with low-renin hypertension.

Although some studies have suggested that decreased Ca^{2+}-ATPase activity is a widespread phenomenon in essential hypertension, others disagree with this. De la Sierra (1990) may be correct in his conclusion that in a subgroup of hypertensive patients, there is defective Ca^{2+}-pump activity. This subgroup of hypertensive subjects has yet to be identified. It is still unknown whether Ca^{2+}-ATPase activity differs between sexes, ages and races.

9.1.1.3 Mg^{2+}-ATPase

Cell membrane Mg^{2+}-ATPase is a magnesium dependent, calcium independent pump. It is different to the magnesium dependent Ca^{2+}-ATPase transporter (Lin, 1985). The exact function of this enzyme is unknown, but it may be involved in transmembrane magnesium transport (Gunther and Vormann, 1986a; Flatman, 1988). It may also be a non-specific marker of all magnesium dependent ATPase pumps.

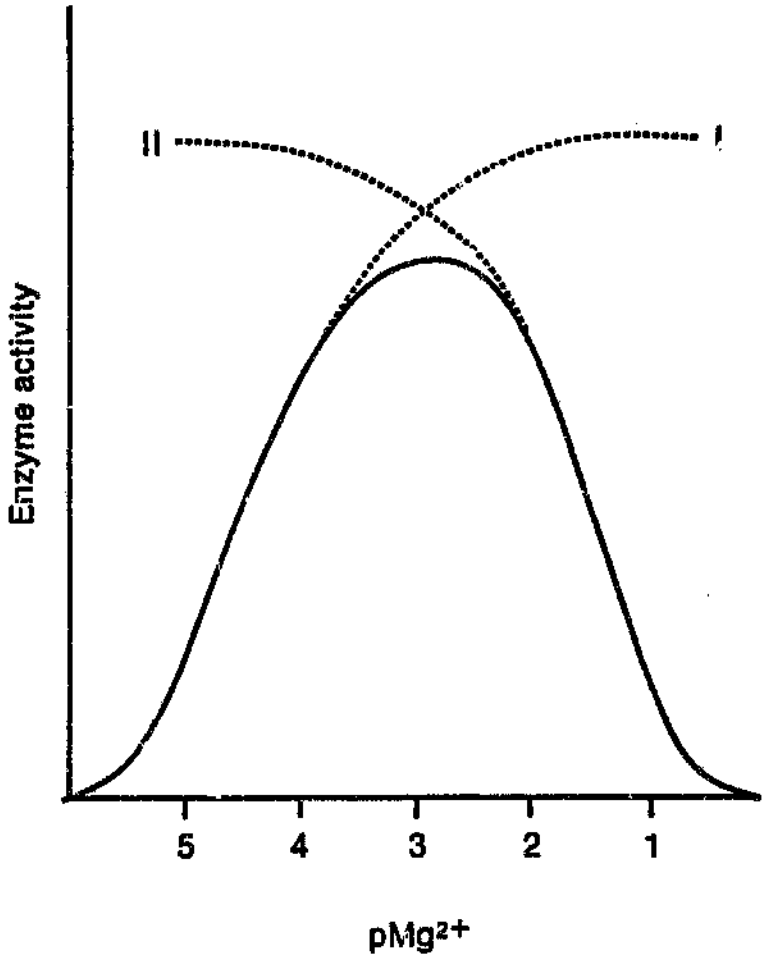


Fig. 9.1 Enzyme activity of a magnesium dependent enzyme (ATPase) as a function of pMg^{2+}
I = activation or II = inhibition of the enzyme depending on pMg^{2+} (Gunther, 1981)

9.1.2 ATPase activity and magnesium

Magnesium activates over 300 enzymes including all ATP-dependent enzyme reactions (Aikawa, 1981; Banting et al, 1983). Their catalytic activity shows a bell-shaped pMg^{2+} dependency (pMg^{2+} = -log $[Mg^{2+}]$ (Gunther, 1981). This means that:-

1. the catalytic activity increases with the logarithm of the concentration (ion activity) of magnesium;
2. there is a pMg^{2+} optimum in the range of pMg^{2+} 3;
3. at the pMg^{2+} optimum there is no change of the catalytic activity.

The bell-shaped pMg^{2+} dependency consists of two sigmoid curves:-

i) activation as a function of pMg^{2+} and

ii) inhibition as a function of pMg^{2+} (Figure 9.i)

The inhibition (e.g. the ATPase of myosin) is caused by unspecific binding of magnesium to the enzyme at higher magnesium concentrations. The activation of ATPase enzymes by magnesium results in the following reaction pattern:

$Mg^{2+} + S \longrightarrow Mg^{2+}\text{-}S$

$Mg^{2+} - S + E \longrightarrow Mg^{2+}\text{-}SE \longrightarrow E + Mg^{2+} + products$

where S = substrate

E = enzyme

Magnesium plays a key role in the coordination of metabolic

pathways where the limiting steps are represented by phosphorylation reactions (Vidair and Rubin, 1982; Gunther et al, 1984a). This cation is found in high levels in all cellular and subcellular membranes, in mitochondria, microsomes and the nucleus. It remains in an equilibrium between bound and free forms that is optimal for its effects (Gunther, 1986).

9.1.2.1 Magnesium and Na^+-K^+-ATPase

Magnesium has been established as an absolute requirement for the Na^+-K^+-ATPase and Ca^{2+}-ATPase pumps (Banting et al, 1983; Yang et al, 1988a). Flatman and Lew (1979) and Huang et al (1985) have shown that in intact red blood cells, magnesium at concentrations in the physiological range (10^{-4}-10^{-3} M) influences ion transport. It interacts reversibly with the Na^+-pump at the inner surface of the membrane (Flatman and Lew, 1979). Ellory et al (1983) have demonstrated that erythrocyte transport of sodium and potassium is affected by altering magnesium concentrations. Magnesium also influences the bumetanide-sensitive (Na^+-K^+-cotransport) and residual (ouabain and bumetanide insensitive) routes of sodium/potassium transport in erythrocytes (Ellory et al, 1983).

9.1.2.2. Magnesium and Ca^{2+}-ATPase

Although the magnesium dependence of the Ca^{2+}-ATPase pump

is undisputed, the direct role of magnesium in the ATP-driven calcium pump is unclear (Asselbach et al, 1981; Altura and Altura, 1985a; Morsy and Shamoo, 1985). Neither the stoichiometry nor the transport of magnesium during the calcium transport cycle is known. It has been suggested that magnesium is countertransported during active Ca^{2+}-transport (Kanazawa et al, 1971; Broderick and Somlyo, 1987). More recent studies however have failed to demonstrate this (Chiu and Haynes, 1980; Salama and Scarpa, 1983). Morsy and Shamoo (1988) reported that magnesium inside the sarcoplasmic reticulum vesicles stimulates the calcium transport into the sarcoplasmic reticular vesicles by up to 50%. They also failed to demonstrate magnesium influx during active calcium transport.

9.1.2.3 The cellular and subcellular stabilising effects of magnesium

Magnesium has a stabilising effect not only on the cell membrane but also on various subcellular organelles. The stabilising effects depend mainly on its structural role (Durlach, 1988a). The complexing of magnesium with phospholipids reduces membrane fluidity and lowers membrane permeability with parallel polarising electrostatic effects (Gunther, 1981). Alteration of the lipid fluidity of the bilayers may induce a conformational change in the ATPase complex (Sadri et al, 1983; Li et al, 1987).

Magnesium also induces structural and functional changes on the receptor and mitochondrial inner membranes (Ligeti and Horvath, 1980; Mollevanger Leo and De Grip, 1984). It is estimated that 70-80% of membrane proteins are intrinsic proteins which are partially buried in the hydrophobic portion and partially in contact with the aqueous phase (Yang et al, 1988). Thus, the structure may be modulated by magnesium (Gunther, 1981). Magnesium also binds to nucleic acids (Vernon, 1988).

9.1.2.4 Effects of decreased magnesium concentrations on ATPase activity and cell membrane function

In magnesium deficient cells, the permeability of plasma membranes increases. Cells become loaded with calcium and sodium and loose potassium and phosphorous (McIntyre and Davidsson, 1958; Siegel et al, 1981). At the same time, the cell depolarises (Siegel et al, 1981). The effects are greater in muscle and heart compared to liver and kidney (Whang and Welt, 1963; Martindale and Heaton, 1964).

Extracellular or intracellular magnesium deficiency results in decreased activity of the ATPase pumps (Altura and Altura, 1985b). Na^+-K^+-ATPase and Ca^{2+}-ATPase are 'activating' enzymes i.e. they move cations across the cell membrane (Gunther, 1981). The ATPase of myosin is normally an inhibitory enzyme (Durlach, 1988a). Consequently inhibition of these pumps by magnesium deficiency will

result in impaired transmembrane cation transport. Decreased Na^+-K^+-ATPase activity leads to intracellular sodium accumulation with decreased concentration of intracellular potassium (Altura and Altura, 1985a; Durlach, 1988b). Impaired Ca^{2+}-ATPase activity is associated with decreased calcium efflux and consequent increased intracellular calcium (Altura and Altura, 1985a; 1985b). Decreased myosin-ATPase would result in enhanced actin-myosin coupling and increased contractility.

Thus, magnesium deficiency induces changes in cellular ion levels which in turn influences various ionic interactions. For example, increasing intracellular sodium alters the distribution of the cytoplasmic calcium by influencing the Na^+ - Ca^{2+} exchange mechanism (Blaustein, 1977). Also the level of intracellular calcium which has already been increased by the hyperpermeability of the plasma membrane is increased even more by decreased Ca^{2+} transport out of the cell (Altura and Altura, 1985a). Furthermore, the sarcoplasmic reticular and mitochondrial Ca^{2+}-pumps would be inhibited, resulting in decreased calcium influx into the organelles and increased cytoplasmic calcium concentrations (Gunther, 1981). Part of the calcium taken up in magnesium deficiency may be stored in the intracellular calcium stores (Gunther, 1981). In magnesium deficient dogs, using electron-microscopic examination, electron-dense particles (calcium phosphate) were found in the sarcoplasmic reticulum of muscle cells (Gunther, 1981).

In summary experimental evidence indicates that magnesium deficiency leads to:-

i) decreased Na^+-K^+-ATPase activity resulting in increased intracellular sodium concentrations,

ii) decreased plasma membrane Ca^{2+}-ATPase activity resulting in intracellular calcium accumulation,

iii) decreased sarcoplasmic reticulum and mitochondrial Ca^{2+}-ATPase activity, resulting in decreased calcium uptake by the organelles and increased cytosolic calcium,

iv) decreased myosin-ATPase activity resulting in myosin-actin coupling,

v) increased cell membrane permeability to ions resulting in increased intracellular sodium and calcium and decreased potassium and magnesium concentrations,

vi) cell membrane depolarisation and

vii) increased binding of calcium to the cell membrane.

In vascular smooth muscle, specific membrane magnesium sites can act physiologically to control and regulate entry and exit of calcium (Altura and Altura, 1982). Magnesium has thus been described as a physiological or natural calcium ion antagonist (Iseri and French, 1984).

Since all the above effects of magnesium deficiency are important in vascular smooth muscle contractility and tone, magnesium may be considered a regulator of vascular smooth muscle and may play a role in the development and control

of essential hypertension.

9.2 AIMS

The aims of this study were:-

2.1 To determine the activity of cell membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase in essential hypertension.

2.2 To assess the relationships between the major cations, and specifically magnesium, and cell membrane Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities in hypertensive patients and

2.3 To determine whether transmembrane ion transport differs between black and white hypertensive subjects.

9.3 SUBJECTS AND METHODS

9.3.1 Subjects

Seventy six hypertensive patients and 78 normotensive subjects were studied. Inclusion criteria, medical examination and blood pressure measurement techniques are described in chapter 5.

Venous blood was obtained from the antecubital vein, without cuff compression.

9.3.2 Methods

9.3.2.1 Isolation of platelets

Forty millilitres of venous blood was obtained by venipuncture as described in chapter 5.3.2. Platelet rich plasma (PRP) was prepared by centrifugation of the blood at 160 x g for 15 minutes and a platelet pellet obtained by centrifugation at 600 x g for 15 minutes. The platelet pellet was washed in a washing buffer containing 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 5 mM glucose, 20 mM Hepes - NaOH (pH7,4), as described in Section 6. The platelet pellet was gently suspended in the washing buffer and contaminating erythrocytes and leukocytes removed by centrifugation at 200 x g for 5 minutes. The platelet containing supernatant was further centrifuged at 600 x g

for 15 minutes at room temperature. The washed platelet pellet was suspended in 1 ml of buffer.

A full blood count using an automated technique the (Technicon H-I system) was performed. Contamination of the washed platelet preparation by erythrocytes and leukocytes was less than 0,001% and 0,10% respectively.

Preparation of platelet membranes

Preparation of platelet membranes was performed using the technique described by Resink et al (1986). The washed platelets were suspended in ice-cold lysis buffer. The buffer contained 5 mM ethylenediaminetetraacetic acid (EDTA), 5 mM Tris-hydrochloride (pH 7,0 at 20°C) and frozen overnight. After thawing at room temperature, the platelet lysate was centrifuged at 39000 x g for 10 minutes at 4°C. A Beckman ultracentrifuge (TL - 100; Beckman instruments Inc California) was used. The lysed pellet was washed three times by resuspension in lysis buffer and centrifuged at 39000 x g for 10 minutes at 4°C. A washed platelet membrane pellet was obtained.

The membrane pellet (0,1 - 0,6 mg protein) was finally resuspended in 1,0 ml of storage buffer. The storage buffer contained 130 mM KCl, 0,5 mM $MgCl_2$, 1,0/um $CaCl_2$, 2,0 mm dithiothreitol and 20 mM N-2-hydroxyethylpiperazine -N^1-2-ethane-sulfonic acid (Hepes) - NaOH (pH 7,4) and

maintained at 0°C until used the following day.

Platelet membranes were prepared within two days of blood collection. Assay procedures, involving the membranes, were performed a day after the membranes had been isolated.

9.3.2.2 Isolation of erythrocytes

Ten millilitres of venous blood was obtained as described in chapter 5.3.2. The anticoagulant used was lithium heparin. Erythrocytes were separated from heparinised blood samples by centrifugation at 450 x g for 10 minutes. The plasma was aspirated and the buffy coat discarded. The erythrocytes were washed three times with the NaCl buffer as described in chapter 8.3.2.1.

Preparation of erythrocyte membranes

Isolation and preparation of erythrocyte membranes were performed according to the method described by Ringel et al (1987). The washed erythrocytes were lysed in 10 volumes of 1 mM EDTA/Tris (pH 7,6) and centrifuged at 50,000 x g for 15 minutes using a Beckman ultracentrifuge. Membrane washing in washing buffer was repeated until the membranes were haemoglobin-free. For membranes to become haemoglobin-free, they were washed four tc five times.

The washed membranes were finally suspended in 1,0 ml of

0,9% NaCl buffer. The membranes were stored overnight at 0°C.

9.3.2.3 Determination of protein concentrations in the platelet and erythrocyte membranes.

Aliquots of the membrane suspensions were solubilised in 0,2% Triton x-100 (60 minutes at room temperature).

Protein was determined according to a modified method of the Lowry technique (see Appendix K).

The protein concentration of the membrane pellet (suspended in 1 ml storage buffer) varied between 0,1 and 0,3 mg. The protein concentration of 50 µl of suspension ranged between 5,0 and 15,0 µg.

9.3.2.4 Determination of cell membrane adenosinetriphosphatase (ATPase) activity

The method employed to measure ATPase activity in cell membranes was based on the principle:

$$ATP + H_2O \xrightarrow[Mg^{2+}]{ATPase} ADP + P_i$$

The amount of P_i released per ATP molecule was measured according to the methods of Taussky and Shorr (1953). (Appendix L).

The activities of Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase in platelet and erythrocyte membranes were determined. The methods for ATPase measurements were based on those described by Niggli et al, (1981); Resink et al, (1986); Ringel et al, (1987) and Rahman et al, (1986). (See Appendix M).

9.4 RESULTS

One hundred and fifty four subjects were studied - 104 blacks (52 normotensives and 52 hypertensives) and 50 whites (24 hypertensives and 26 normotensives). The normotensive and hypertensive groups were matched for age, height and weight. The clinical characteristics of the groups are presented in chapter 8.5.1. (Table 8.1).

9.4.1 ATPase activity

9.4.1.1 Black group (Table 9.1, Figures 9.1; 9.2)

9.4.1.1 (i) Na^+-K^+-ATPase activity

In the hypertensive group erythrocyte and platelet membrane Na^+-K^+-ATPase activity was significantly lower compared to the normotensive group ($p = 0,0001$). Although platelet Na^+-K^+-ATPase activity was greater than that of erythrocyte membrane activity this was not significant.

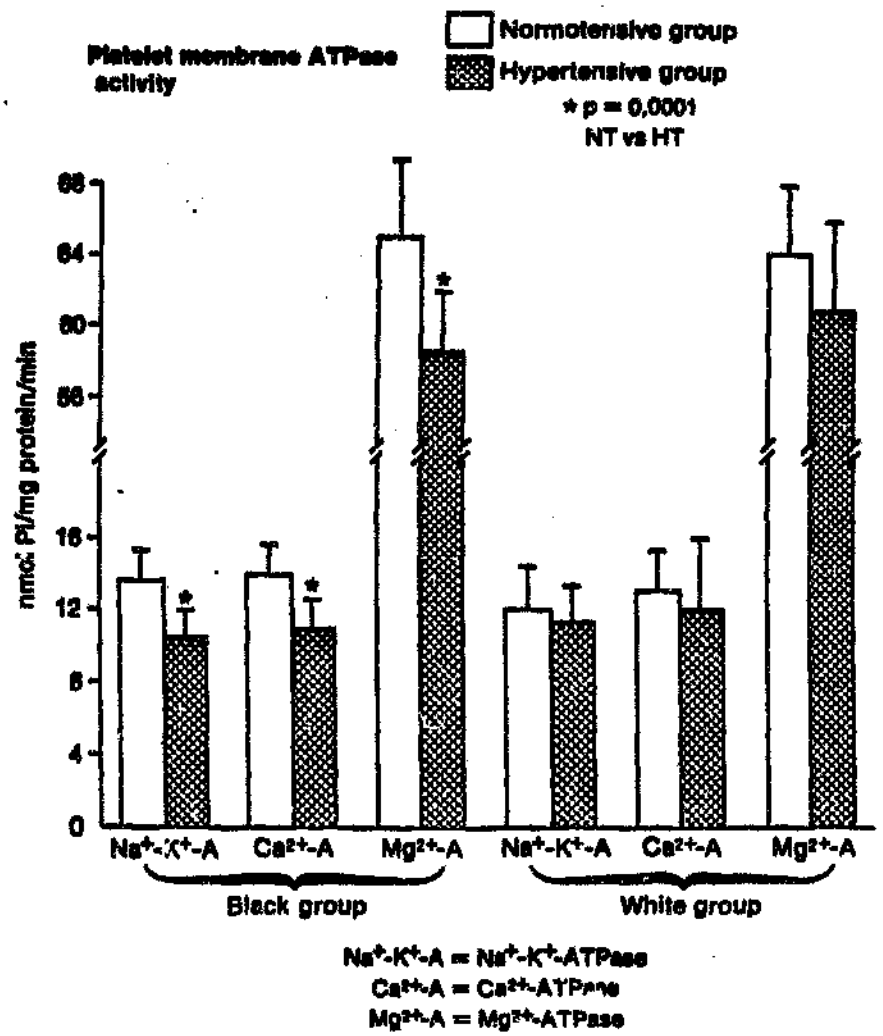


Figure 9.1:
Platelet membrane ATPase activity in the black and white normotensive and hypertensive groups.

Table 9.1: Erythrocyte and platelet membrane ATPase activity in the black group.

	Black group		
	Normotensive blacks	Hypertensive blacks	
Variable			p value Normotensive vs Hypertensive
Erythrocyte membrane			
Na^+-K^+-ATPase	11,3 ± 1,92	7,4 ± 1,52	0,0001*
Ca^{2+}-ATPase	11,4 ± 1,52	9,9 ± 1,72	0,0008*
Mg^{2+}-ATPase	60,8 ± 5,10	56,8 ± 5,8	0,001*
Platelet membrane			
Na^+-K^+-ATPase	14,9 ± 3,0	10,6 ± 2,20	0,0001*
Ca^{2+}-ATPase	15,0 ± 2,61	11,4 ± 2,81	0,0001*
Mg^{2+}-ATPase	65,9 ± 8,1	58,8 ± 9,0	0,0001*

ATPase activity is expressed as:-

nmol/mg membrane protein/minute at 37^0C

* = significant difference

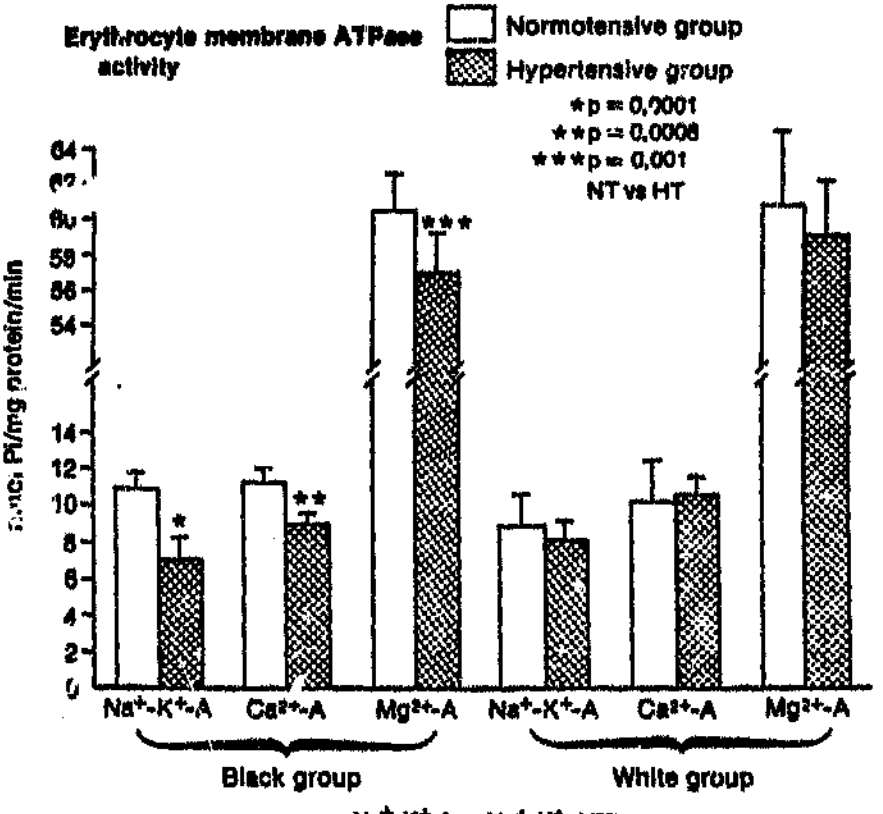


Figure 9.2:

Erythrocyte membrane ATPase activity in the black and white normotensive and hypertensive groups.

Table 9.2: Cell membrane ATPase activity in male and female black normotensive and hypertensive groups.

	Black group			
	Normotensive blacks		Hypertensive blacks	
	Female (n = 26)	Male (n = 26)	Female (n = 30)	Male (n = 22)
Erythrocyte membrane				
Na^+-K^+-ATPase	12,0 ± 1,77	10,7 ± 1,86	7,43 ± 1,50	7,41 ± 1,59
Ca^{2+}-ATPase	12,0 ± 1,63	10,9 ± 1,23	9,87 ± 1,67	9,95 ± 1,83
Mg^{2+}-ATPase	59,4 ± 4.22	62,0 ± 5,57	55,7 ± 6,39	58,3 ± 4,66
Platelet membrane				
Na^+-K^+-ATPase	13,62 ± 2,31	15,9 ± 3,15	10,43 ± 2,59	10,8 ± 1,72
Ca^{2+}-ATPase	15,0 ± 2,89	15,0 ± 2,40	11.67 ± 3,29	11,0 ± 2,10
Mg^{2+}-ATPase	67,3 ± 6,56	64,9 ± 9,12	58,5 ± 8,90	59,4 ± 9,33

ATPase activity is expressed as:-

nmol/mg membrane protein/minute at 37^0C

Table 9.3: Erythrocyte and platelet membrane ATPase activity in the white group.

	White group		
	Normotensive whites	**Hypertensive whites**	
Variable			p value Normotensive vs Hypertensive
Erythrocyte membrane			
Na^+-K^+-ATPase	9,4 ± 1,74	8,3 ± 1,5	0,06
Ca^{2+}-ATPase	10,8 ± 2,61	10,9 ± 1,8	0,62
Mg^{2+}-ATPase	60,6 ± 4,90	59,2 ± 3,9	0,32
Platelet membrane			
Na^+-K^+-ATPase	12,1 ± 2,2	11,5 ± 2,6	0,36
Ca^{2+}-ATPase	13,3 ± 2,8	12,1 ± 3,4	0,14
Mg^{2+}-ATPase	64,1 ± 4,8	61,6 ± 5,1	0,30

ATPase activity is expressed as:-

nmol/mg membrane protein/minute at $37^{o}C$

Table 9.4: Cell membrane ATPase activity in male and female white normotensive and hypertensive groups.

	White group			
	Normotensive whites		Hypertensive whites	
	Female (n = 13)	Male (n = 13)	Female (n = 14)	Male (n = 10)
Erythrocyte membrane				
Na^+-K^+-ATPase	9,5 ± 1,56	9,3 ± 1,9	8,6 ± 1,6	7,9 ± 1,52
Ca^{2+}-ATPase	12,5 ± 2,50	9,2 ± 2,61	10,6 ± 1,65	11,4 ± 2,01
Mg^{2+}-ATPase	59,7 ± 5,76	61,5 ± 4,09	60,0 ± 4,46	57,9 ± 2,80
Platelet membrane				
Na^+-K^+-ATPase	13,0 ± 2,08	11,4 ± 2,21	11,6 ± 2,50	11,5 ± 2,95
Ca^{2+}-ATPase	65,4 ± 6,0	12,8 ± 2,31	12.1 ± 3,34	12,2 ± 3,37
Mg^{2+}-ATPase	13,9 ± 3,35	62,7 ± 2,83	60,5 ± 3,05	63,1 ± 6,98

ATPase activity is expressed as:-

nmol/mg membrane protein/minute at 37^0C

9.4.1.1 (ii) Ca^{2+}-ATPase activity

Erythrocyte and platelet membrane Ca^{2+}-ATPase activity was significantly depressed in the hypertensive group ($p = 0,001$ for erythrocyte Ca^{2+}-ATPase; $p = 0,0001$ for platelet Ca^{2+}-ATPase). Ca^{2+}-ATPase activity was higher in the platelet membranes compared to the erythrocyte membranes. The difference was not significant.

9.4.1.1 (iii) Mg^{2+}-ATPase activity

Mg^{2+}-ATPase activity (erythrocyte and platelet membrane), was significantly lower in the hypertensive group compared to the normotensive group ($p = 0,0008$ for erythrocyte Mg^{2+}-ATPase; $p = 0,0001$ for platelet membrane Mg^{2+}-ATPase). Platelet Mg^{2+}-ATPase activity was greater than erythrocyte Mg^{2+}-ATPase, but the difference was not significant.

9.4.1.1 (iv) ATPase activity in males and females

There were no significant differences for Na^{+}-K^{+}-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities between the black males and black females (Table 9.2).

9.4.1.2 White group (Table 9.3, Figures 9.1; 9.2)

They were no significant differences in ATPase activity (erythrocyte and platelet) between the white normotensive

and hypertensive groups. There were no differences in ATPase activity between the sexes (Table 9.4).

9.4.1.3 Combined black and white groups (Table 9.5; Figures 9.3; 9.4)

When the groups were combined, erythrocyte and platelet membranes Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were significantly lower in the hypertensive group compared to the normotensive group ($p = 0,0001$).

9.4.2 Comparison of ATPases between the sexes and groups

Significant differences of erythrocyte and platelet membrane Na^+-K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase activities between the normotensive and hypertensive, black and white, male and female groups are presented in tables 9.6-9.9. Platelet membrane Na^+-K^+-ATPase and Mg^{2+}-ATPase was significantly lower in the black hypertensive group compared to the white hypertensive group. In the black hypertensive subjects, erythrocyte Na^+-K^+-ATPase activity was significantly lower compared to the white hypertensive group.