PLATELET CYTOSOLIC FREE CALCIUM AND ECHOCARDIOGRAPHIC LEFT VENTRICULAR MASS IN ESSENTIAL HYPERTENSION AND PRIMARY HYPERPARATHYROIDISM

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ABBREVIATIONS

SHR	-	spontaneously hypertensive rat
SHRSP	-	stroke prone spontaneously hypertensive rat
WKY	-	Wistar-Kyoto rat
Ca^{2+}	-	ionized calcium
$[Ca^{2+}]i$	-	free cytosolic calcium
$[Ca^{2+}]e$	-	extracellular calcium
IP_3	-	inositol trisphosphate
AVP	-	arginine vasopressin
AMP	-	adenosine monophosphate
ATP	-	adenosine trisphosphate
PTH	-	parathyroid hormone
LV	-	left ventricle

RELEVANT PRESENTATIONS AND PUBLICATIONS

Presentations to Learned Societies

Dominiczak AF, Morton JJ, Semple PF. Cytosolic free calcium in platelets in essential hypertension. International Symposium on Mechanism and Treatment in Essential Hypertension, May 1988.

Dominiczak AF, Morton JJ, Semple PF. Platelet cytosolic free calcium in essential hypertension. British Hypertension Society, September 1988.

Dominiczak AF, Boyle IT, Dargie HJ, Semple PF. Blood pressure, left ventricular mass and free cytosolic calcium in primary hyperparathyroidism and essential hypertension. British Cardiac Society, November 1988.

Dominiczak AF, Lyall F, Morton JJ, Boyle IT, Murray G, Semple PF. The relationship between intracellular calcium, blood pressure and left ventricular mass in primary hyperparathyroidism. European Society of Hypertension, June 1989.

Publications

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SUMMARY

Force development in cardiac and vascular muscle is directly related to the concentration of calcium ions in the cytosol. Sustained hypertension is predominantly maintained by an increase in total peripheral resistance and attention has therefore focused on the possibility that changes in intracellular calcium in myocytes of resistance vessels are important in the pathogenesis of essential hypertension in man. Apart from vascular tone, free calcium in cells also stimulates cell proliferation and growth and increased concentrations of calcium ions in cardiac and vascular smooth muscle could mediate the hypertrophy that characteristically occurs in response to an increase in wall tension.

Studies of cytosolic free calcium concentrations in vascular smooth muscle cells have not yet been performed in man. Instead, blood platelets, which are accessible cells that have a calcium-dependent contractile apparatus and a complement of membrane receptors not unlike those in vascular smooth muscle, have been used to study cellular calcium handling. It has been proposed that membrane control of intracellular free calcium is abnormal in hypertension, and that this abnormality is not limited to the contractile cells of the cardiovascular system. This hypothesis has generated a considerable volume of experimental work which has extended to studies of extracellular calcium metabolism and the possible therapeutic role of dietary calcium manipulation.

A high prevalence of hypertension has been described in patients with primary hyperparathyroidism, a disease that is

characterised by increased parathyroid hormone concentrations and chronic extracellular hypercalcaemia.

Two related studies have been carried out to determine levels of free calcium in platelets and the relationship of these levels to arterial pressures in patients with essential hypertension, primary hyperparathyroidism and normal control subjects.

The first study examined resting and stimulated free calcium concentrations in platelets loaded with the fluorescent probe Quin 2 from 30 patients with essential hypertension and from 30 age-matched controls. The median cytosolic free calcium in platelets in the hypertensive group was 93.3 nmol/l and values were not significantly different from those in the control group (87.8 nmol/l, 95% CI - 12 to 5.8, P=0.5). Arginine vasopressin caused a transient increase in platelet free calcium in all subjects. In the presence of 1 mmol/l extracellular calcium the increase was significantly higher in the control subjects than in the hypertensive patients (P=0.005). In the absence of extracellular calcium, arginine vasopressin caused much smaller increases and there was then no difference between the responses of the two groups. Platelet free calcium concentrations were measured again in 13 patients after 8 weeks of treatment with either verapamil (n=6) or atenolol (n=7). The reductions in systolic pressure after drug treatment were correlated with the changes in cytosolic free calcium concentrations ($r=0.75$, $p<0.01$).

Compared with results of previous studies in essential hypertension, the differences in platelet calcium were quite

small. The lack of adequate matching for age and body weight as well as inclusion of patients with malignant hypertension in early reports may account for some of the differences. Since an increase in free calcium concentration is a necessary part of the process of activation, the higher platelet calcium concentrations in some studies may reflect changes that occurred after blood sampling as a result of an enhanced tendency to activation and aggregation. The rise in cell calcium after arginine vasopressin appeared to be primarily dependent on calcium influx and this response was reduced in the hypertensive group.

The second study examined blood pressure, left ventricular mass, platelet cytosolic free calcium, renin-angiotensin system, catecholamines and atrial natriuretic peptide in 23 patients with untreated primary hyperparathyroidism, 30 normotensive control subjects and 23 control subjects matched for age, sex and blood pressure. In 12 patients measurements were repeated after parathyroidectomy.

Patients with primary hyperparathyroidism had significantly elevated blood pressures compared with age-matched control subjects and 22% were hypertensive (WHO grades I and II). Blood pressures were little changed six to twelve months after correction of the hyperparathyroidism by surgery. Despite chronic extracellular hypercalcaemia, intracellular free calcium concentrations were lower in patients with hyperparathyroidism than in controls matched for age, sex and blood pressure (median concentrations 81.5 nmol/l vs 93 nmol/l, 95% CI 0.1 to 20.1; $P<0.05$) and values tended to increase after parathyroidectomy. There was an inverse

correlation between platelet calcium and diastolic blood pressure in the hyperparathyroid group ($r=-0.46$, $P<0.05$). The median peak concentrations of calcium after vasopressin were lower in the hyperparathyroid group than in the control subjects (95% CI 76 to 1551, $P<0.001$). Chronic hypercalcaemia or parathyroid hormone excess may lower intracellular calcium in some tissues, either by stabilizing the membrane and reducing calcium influx or by increasing cyclic AMP.

Left ventricular mass index was increased in the hyperparathyroid group as compared to control subjects matched for age, sex and blood pressure (123 g/m^2 vs 100 g/m^2, 95% CI -36.1 to -3.1, $P=0.03$). Parathyroidectomy was associated with a small reduction of the left ventricular mass index (123.5 g/m^2 vs 104 g/m^2, 95% CI -46.5 to 2.5; $P=0.1$). There was some evidence that the degree of left ventricular hypertrophy was out of proportion to the blood pressure suggesting that extracellular calcium or parathyroid hormone may have direct effect on cardiac myocytes. The plasma concentrations of the components of the renin-angiotensin system, catecholamines and atrial natriuretic peptide were similar in patients with hyperparathyroidism and the two control groups and apart from small changes in aldosterone, they were not affected by parathyroid surgery. There was little evidence to implicate either the renin-angiotensin or the sympathetic nervous systems in the pathogenesis of the high blood pressure.

The increased cardiovascular mortality that has been described in primary hyperparathyroidism may result from hypertension and left ventricular hypertrophy. Further prospective studies are required to establish whether cardio-

vascular mortality is prevented by parathyroidectomy.

CHAPTER 1 - REVIEW

"A small quantity of calcium added to saline solution with small amounts of potassium chloride, makes a good artificial circulating fluid and the ventricle will continue beating perfectly for more than four hours". [Sidney Ringer, 1883 (1)].

The importance of calcium ions to the function of vascular muscle and nerves has been recognised since the time of Ringer. Over the past 20 years there has been increasing interest in the role of calcium in the pathogenesis of hypertension. Arterial pressure is determined by cardiac output and peripheral resistance: calcium ions affect both components (2). Calcium directly affects cardiac output and peripheral vascular resistance by stimulating muscle contraction and changing responsiveness to and release of vasoactive substances. Force development in cardiac and vascular muscle is directly related to the concentration of calcium ions in the cytosol (3). Sustained hypertension is predominantly maintained by an increase in total peripheral resistance and attention has therefore focused on the possibility that changes in intracellular calcium in myocytes of resistance vessels are important in the pathogenesis of essential hypertension in man.

Section 1 - Evidence for and against calcium deficiency in genetic hypertension

It may seem paradoxical that so much consideration has been given to the possibility that genetic hypertension is

associated with calcium deficiency. This section examines the evidence.

1.1.1. Epidemiology

The first suggestion that there might be a link between calcium deficiency and high blood pressure came from clinical studies of essential hypertension (5,6). This was followed by evidence of an inverse relationship between calcium intake and blood pressure in a population study (4). Paradoxically, it had also been found that the concentration of total calcium in serum was positively correlated with blood pressure (7) but the relationship has not been evident in all studies (8,9). Interpretation of such studies can be difficult and this was apparent when two independent groups of investigators (4,10) drew different conclusions from their analyses of the same data, from the first National Health and Nutrition Examination Survey (1971-1973).

Epidemiological surveys of diet are notoriously difficult to perform. The method of dietary recall by questionnaires is often inaccurate. In relation to hypertension, factors that affect preference for or avoidance of dietary products may independently affect blood pressure. In some surveys, possible differences in other nutrients and elements like potassium were not completely excluded (4). Epidemiologic studies can only document an association but do not establish causality.

1.1.2. Increased urinary calcium excretion

There is a general agreement that some patients with essential hypertension have increased rates of calcium ex-

cretion or hypercalciuria relative to sodium excretion (5,7,9,11,12). Similar findings have been described in spontaneously hypertensive rats (SHR)(13,14). The renal excretion of calcium and sodium is linked and dietary intake of sodium is a factor which influences calcium excretion (15). Some studies in the SHR have concluded that an intestinal mechanism is responsible for the hypercalciuria present in this animal model (16), but such increased absorption of calcium is not firmly established (17).

1.1.3. Reduced serum ionized calcium

Serum concentrations of ionized calcium have been found to be reduced in hypertensive subjects (6,8,9,18) and SHR (13) but these differences have not been confirmed by later studies (5,12,19). Confounding variables like pH and food intake were not always controlled in the earlier studies (12,16). Resnick et al (18) has examined serum ionized calcium concentrations in hypertensives subdivided into low-renin, normal-renin, and high-renin subgroups. Low-renin patients seemed to show lower levels of serum ionized calcium than normal-renin and high-renin groups. Hypertensive patients with high values of plasma renin activity had higher serum levels of ionized calcium.

1.1.4. Calcium regulating hormones in essential hypertension

Serum parathyroid hormone concentrations are probably slightly increased in middle aged subjects with established hypertension (5,11). Concentrations of intact parathyroid hormone also seem to be raised in young subjects with mildly

raised blood pressure and correlate with the level of pressure (20). These observations have generated the hypothesis that enhanced parathyroid gland activity is implicated in the development of essential hypertension. Intact parathyroid hormone concentration is probably a more sensitive index of activity of the parathyroid gland than total immunoreactive parathyroid hormone concentration and unaffected by changes in renal clearance of the metabolites of parathyroid hormone which may be present in chronic hypertension (21).

Increased activity of the parathyroid gland may be an appropriate response to a renal calcium 'leak' or to a low dietary intake of calcium: both have been reported in subjects with hypertension (4,5,11). Parathyroid gland secretion may be affected by the slight reduction of serum ionized calcium concentrations which has been found in genetic hypertension in animals. Parathyroid activity may also be influenced by the sympathetic nervous system (22) and increased sympathetic tone may be present in early essential hypertension (23).

The two other calcium regulating hormones calcitonin and 1,25-dihydroxyvitamin D_3 have not been studied as extensively as parathyroid hormone. One study from New York (24) described abnormalities in calcium regulating hormones linked to renin-sodium status and consistent with the observed differences in ionized calcium levels. Calcitonin levels in plasma were higher in hypertensive subjects with high renin activity than in low-renin and normal-renin groups of normotensive controls but levels of 1,25-dihydroxyvitamin D_3 and parathyroid hormone were higher in the low-renin group. There

was a significant inverse relationship between circulating 1,25 vitamin D_3 levels and plasma renin activity and a direct relationship between calcitonin levels and plasma renin activity.

Most of these correlations depend on inclusion of patients with high-renin essential hypertension. In Scottish experience and except for patients with renovascular disease, high plasma renin concentrations are unusual in essential hypertension, occurring only in young patients with severe disease (12).

1.1.5. Decreased intestinal calcium absorption

In vivo perfusion studies across small intestinal segments of the anaesthetised SHR and experiments using isolated, everted duodenal sacs have described decreased calcium absorption in the hypertensive animals (17). Contrary results have also been reported (25) and in situ duodenal ^{45}Ca uptake in conscious SHR has even been found to be greater than in Wistar-Kyoto (WKY) control animals (16). Most findings are not compatible with the notion of calcium mal-absorption in the SHR.

1.1.6. Oral calcium supplementation - conflicting findings

McCarron and Morris (27) reported that one gram of elemental calcium caused a small but significant fall in blood pressure in hypertensive patients after eight weeks of treatment. In normotensive subjects oral calcium supplementation also seemed to cause a small fall in blood pressure (26,28). Studies in hypertensive rats have shown that a large increase in calcium intake does lower blood

pressure (14,30,31) but this effect is abolished when reductions in phosphate balance are prevented (32), which tends to suggest that reduced phosphate is more important than increased calcium. Direct effects of phosphate deficiency on vascular resistance have been described (33). A large retrospective study of blood pressure in hyperparathyroidism failed to show any correlation between blood pressure and serum calcium concentration, but did show a significant inverse relation with serum phosphate concentration (34).

More recent carefully controlled studies by Capuccio et al (29) and Siani et al (35) have not confirmed earlier results of McCarron and Morris. Both studies found that increases in calcium intake for four weeks in mild to moderate essential hypertension did not lower blood pressure. Studies on calcium supplementation in hypertension are at present inconclusive and there is not yet enough evidence to justify a recommendation for calcium supplementation as a mode of treatment.

1.1.7. Hypertensive effects of acute hypercalcaemia

It is well established that acute hypercalcaemia increases arterial blood pressure in man and in experimental animals (36-39). Since calcium ions have been shown to increase cardiac muscle contractility *in vitro* (40) and vascular smooth muscle contraction (41), it has been suggested that the hypertensive response is mediated by a combination of increased cardiac output and increased peripheral resistance. *In vivo* studies have established that

there may be an increase in cardiac output in the initial phase of acute hypercalcaemia (42) but the hypertension is then maintained by an increase in vascular resistance (38,39). Berl et al (39) studied hypercalcaemia in the conscious rat achieved by a 30 minute infusion of calcium chloride. This resulted in increased mean arterial pressure and peripheral vascular resistance (measured using microspheres) but no change in cardiac index. The pressor response to acute hypercalcaemia did not seem to be mediated by vasopressor hormones or attenuated by vasodepressor hormones since inhibition of the renin-angiotensin system, catecholamines, vasopressin, prostaglandins and parathyroid hormone did not alter the pressor response to calcium. It seems likely that there is a direct action of calcium ions on smooth muscle and perhaps cardiac muscle, since pretreatment with the calcium channel blockers verapamil and nifedipine blocks the pressor response (39).

1.1.8 Chronic hypercalcaemia

In 1958 Hellstrom et al (43) reported an increased incidence of hypertension in patients with primary hyperparathyroidism and subsequent studies have mostly confirmed this observation (34,44,45). Initially it was suspected that renal damage caused by hypercalcaemia explained this association and renal damage probably does contribute to hypertension in some severe cases. But differences in pressure were still present when patients with creatinine values of 150 μmol/l or more were excluded (44). Blood pressure elevation may be caused directly either by hypercalcaemia or by parathyroid hormone excess. Blum et al have described reversible hyper-

tension caused by the hypercalcaemia of hyperparathyroidism, vitamin D toxicity and calcium infusion (46). Hypertension associated with hypercalcaemia of malignancy has also been described (47).

The common occurrence of hypertension in hyperparathyroidism is probably not the explanation for the association of hypercalcaemia with high blood pressure in epidemiological studies. Bulpitt et al (48) found a positive correlation between total serum calcium concentration and systolic blood pressure in the Whitehall study and this link between blood pressure and calcium is supported by Kesteloot and Geboers (7), who showed an independent and highly positive correlation between serum calcium concentrations and both systolic and diastolic pressure in Belgian soldiers. Diuretic treatment was not recorded, but the Renfrew study (49) has supported the relation between calcium and blood pressure in a population who were not taking these drugs.

Overall, epidemiological, clinical and experimental studies do not provide a firm evidence for the presence of calcium deficiency in all forms of genetic hypertension. Variables such as age, sex, race or renin-sodium profile may contribute to conflicting results obtained by different authors. The calcium deficiency hypothesis requires careful testing with intervention studies of adequate size and duration before calcium supplementation can be regarded as adequate therapy for hypertensive patients.

It is agreed that extracellular and intracellular calcium homeostasis is abnormal in the spontaneously hypertensive rat and probably also in essential hypertension in

man. To further understand how these abnormalities might be translated to increased peripheral vascular resistance it is necessary to consider the mechanisms that regulate the intracellular calcium concentration.

Section 2 - Regulation of the intracellular calcium concentration

1.2.1. Calcium and the contractile proteins

The concentration of calcium ions in vascular smooth muscle cells is the major determinant of contractile activity and free cytosolic calcium is sometimes referred to as "activator calcium" (41). Electron microscopy reveals that the contractile mechanism of vascular smooth muscle is composed of thick (14.5 nm) and thin (6.4 nm) filaments. Shortening and force generation is produced in a manner similar but not identical to the classic sliding filament model of skeletal muscles (50). Filaments do not change in length but slide in parallel alignment so that the length of the cells tends to shorten. As in skeletal muscle, the thin filament consists of the proteins actin and tropomyosin, whereas the thick filament consists of myosin. In both types of muscle force develops as a result of a cycling motion of a bridge extending from the myosin molecule that alternately attaches to and detaches from the actin molecule (51).

Cycling or activation of the myosin bridge occurs when a light chain of the myosin molecule is phosphorylated by the enzyme myosin light chain kinase (MLCK). There is an invariable relationship between myosin light chain phos-

phorylation and the mechanical response (54). Calcium activates MLCK in the presence of calmodulin, and is thus the physiological activator of smooth muscle contraction (53). Using aequorin to monitor intracellular calcium concentration, Rembold and Murphy (54) have demonstrated the relationship between intracellular calcium concentration, myosin light chain phosphorylation, and cross bridge cycling rate. These investigators have provided a full understanding of the mechanism of contraction of vascular smooth muscle and have made it clear that this contraction is regulated by the concentration of calcium in the myoplasm.

1.2.2. Cellular calcium pools, calcium channels, pumps and exchangers

A simple generalised representation of cellular Ca^{2+} compartmentalisation and flux systems is given in Figure 1. The processes that regulate the concentration of the activator calcium in vascular smooth muscle cells include mechanisms that affect the movement of calcium across the plasma membrane and mechanisms that sequester and release calcium from the sacroplasmic reticulum (51,52,55). As early as 1963, Bohr found it possible to differentiate contractile responses to calcium from these two sources (57) after observing that the response of the rabbit aorta was comprised of a fast and slow component. The early fast component depended on intracellular calcium release, and therefore could occur in the absence of external calcium; the subsequent slow component depended on calcium influx through the membrane (57,58).

Calcium for the fast component of the contraction is

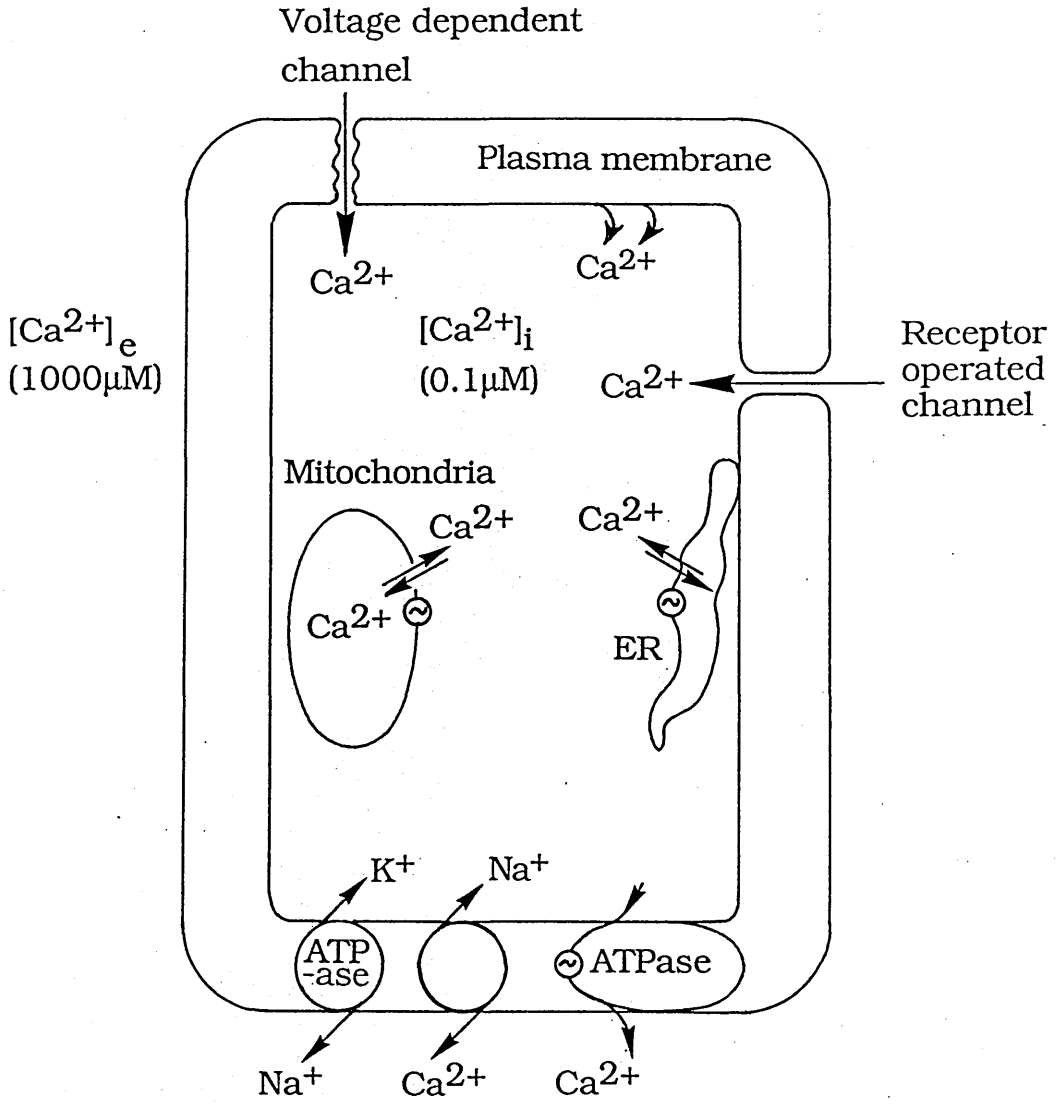


Figure 1 Cellular Ca^{2+} pools and autoregulation of cystolic Ca^{2+} concentration

$[Ca^{2+}]_e$ and $[Ca^{2+}]_i$ indicate extracellular and intracellular Ca^{2+} concentrations, respectively. Arrows indicate direction of Ca^{2+} flux (or Na^+ and K^+ as specified). ATP requiring transport systems are indicated by ~ . ER - endoplasmic reticulum (dense tubular system). Modified from reference 56.

released from the sacroplasmic reticulum when a constrictor agonist activates its specific receptors in the plasma membrane. Somlyo and Somlyo have named this process pharmaco-mechanical coupling (59). It can occur without a change in membrane potential. The role of an intracellular source of calcium in response to physiological agonist is demonstrated by the observation that vascular smooth muscle contracts when stimulated with noradrenaline in a calcium-free medium (58). The signal for calcium release is transmitted to the sacroplasmic reticulum either by calcium-induced calcium release (60) or by inositol trisphosphate (61).

Insight has been gained into the role played by the plasma membrane in the regulation of intracellular calcium concentration, role that is largely dependent on permeability to calcium which in turn is governed by the status of specific protein channels in the lipid bilayer. Current concepts of the entrance of calcium into the cell depict it as occurring through four different types of channels (52,55,56). This classification of the channels is based on the specificity of both their mechanisms of activation and the actions of various calcium entry blockers (55,62). 1) One type of channel referred to as the "calcium leak channel" appears to be present in all types of biological membranes composed of phospholipids, cholesterol and protein. It is active in the absence of membrane stimulation. 2) A potential sensitive channel becomes increasingly available to the calcium ion when membranes are depolarised. 3) A third type of channel is activated when agonists (eg angiotensin II or vasopressin) react with their membrane receptor, the so-

called receptor-operated channel. This channel has a specific role in activating the phosphoinositide system. 4) It is now apparent that a fourth type of calcium channel may be activated by the stretch of vascular smooth muscle (63).

Van Breemen et al (55) have compared the rates of calcium influx in vascular smooth muscle from resistance vessels of WKY and SHR through three of these channels. Through the leak channel, the receptor-operated channel, and the potential-sensitive channel, the influx was greater in smooth muscle from the SHR than it was in that from the WKY. More detailed characterisation of the calcium channels of vascular smooth muscle has been possible with the use of the patch-clamp technique. Rusch and Hermsmeyer (64) have compared the whole cell calcium currents of smooth muscle from the azygos veins of WKY and SHR. They noted that in both types of cells two categories of currents were observed: one transient and one long-lasting. Although the total calcium current did not differ between the cells from these two sources, the relative proportion of transient and long-lasting calcium currents was significantly different between WKY and SHR cells. The transient current was greater in WKY cells, whereas the long-lasting current was greater in cells from SHR. Since the long-lasting current delivers the calcium, which is thought to regulate contraction, it is possible that these differences between neonatal animals of the two strains account for the difference in peripheral resistance.

Cellular calcium homeostasis is a remarkable achievement in that a 5000 fold to 10000 fold concentration gradient is maintained across the plasma membrane (65). The concentration

of ionized calcium in the cell cytosol is normally between 100 to 200 nM and in the extracellular fluid about 1 mM. This gradient is maintained by a low natural permeability of the plasma membrane to calcium and by at least two ATP-dependent mechanisms by which calcium is pumped out of the cell. The first is Ca^{2+}/Mg^{2+}-ATPase (66) and the second is Na^{+}/Ca^{2+} exchanger which is driven by the sodium gradient across the membrane, then maintained by Na^{+}/K^{+}-ATPase (67). In general, the Na^{+}/Ca^{2+} exchange mechanism is a high-capacity, low-affinity system, which can move relatively large quantities of calcium out of the cell per unit of time. In contrast, $Ca^{2}+/Mg^{2+}$-ATPase is a high-affinity, low-capacity system, which fine-tunes the intracellular calcium concentration (67). Both are regulated by changes of calcium ions concentration in the cell. When the calcium concentration rises, the efficiency of both efflux pathways increases.

The existence of a sodium/calcium exchanger in vascular smooth muscle has not been universally accepted. Using electrophysiological technique, Hermsmyer and Harder (68) were unable to find evidence of a Na/Ca exchanger in basilar and caudal arteries from stroke prone spontaneously hypertensive rats (SHRSP) and normotensive WKY rats. In contrast, Matlib et al (69) have observed that calcium uptake by sarcolemmal vesicles of mesenteric arteries from SHR and WKY is dependent upon sodium loading. The activity of the exchanger appeared slightly greater in membrane vesicles from SHR, but the difference was not statistically significant. A review of the exchanger in genetic hypertension (70) concluded that despite the strong possibility that blood vessels do possess

a Na^+/Ca^{2+} exchange mechanism, and the attractiveness of this exchange as a mechanism linking sodium with the increase of vascular resistance, there was little direct evidence for a pathogenetic role.

In contrast, significant abnormalities of Ca^{2+}-ATPase or calcium extrusion pump have been described in hypertension. Kwan et al (71) compared the ATP-dependent calcium accumulation into inside-out sarcolemmal vesicles from mesenteric arteries of normotensive and hypertensive rats. This measure of active calcium extrusion was reduced in both SHR and mineralocorticoid-induced hypertension. Postnov and Orlov (76), in support of their hypothesis that the membrane abnormality in hypertension is generalised to all tissues, presented evidence that the calcium uptake by plasma membrane vesicles from rat brain is 40% less in SHR than WKY.

The other organelles of central importance to calcium homeostasis are mitochondria. An efficient calcium pump drives Ca^{2+} out of the cytosol into the mitochondrial matrix, where calcium is stored largely in non-ionic calcium-phosphate complex (65). There is also a calcium leak or efflux pathway whereby calcium can leave the mitochondria and return to the cytosol. In times of excessive cellular calcium uptake, the rate of mitochondrial uptake becomes much greater than the rate of efflux, and the organelles then accumulate calcium which protects the cell against overload or intoxication (65).

1.2.3. Calcium and the plasma membrane

Not only is the plasma membrane equipped with mechanisms

that regulate intracellular concentrations of calcium ions, but the function of the membrane is itself regulated by calcium. The intracellular environment is protected from calcium overload by a mechanism whereby extracellular calcium concentration affects cell membrane permeability to calcium (52,62). This inactivation of calcium channels by increasing concentrations of calcium can be demonstrated by the relaxation of vascular smooth muscle that occurs when extracellular calcium concentration is increased above physiological levels (72). The decrease in membrane permeability caused by the increase in calcium concentration has been described as "membrane stabilization". It is a generalised effect which alters sodium and potassium fluxes in lymphocytes (73) and vascular smooth muscle cells (74) in genetic and mineralocorticoid hypertension (Figure 2).

At any calcium concentration, the flux is greater through the membranes from hypertensive rats than through membranes from normotensive rats. This has been interpreted as evidence that calcium has less of a stabilizing influence in the membrane from the hypertensive rat (62,73). This interpretation is in accord with observations that less calcium is bound to isolated cell membranes from genetically hypertensive than from normotensive rats or humans (75,76). At a molecular level, Kowarski et al (77) found a significant reduction of an "integral membrane calcium-binding protein" (IMCAL) in various tissues of the SHR as compared to those from WKY. It has been suggested that the impaired ability of calcium to stabilize the membrane might be a consequence of the deficiency of calcium binding sites in the membrane in

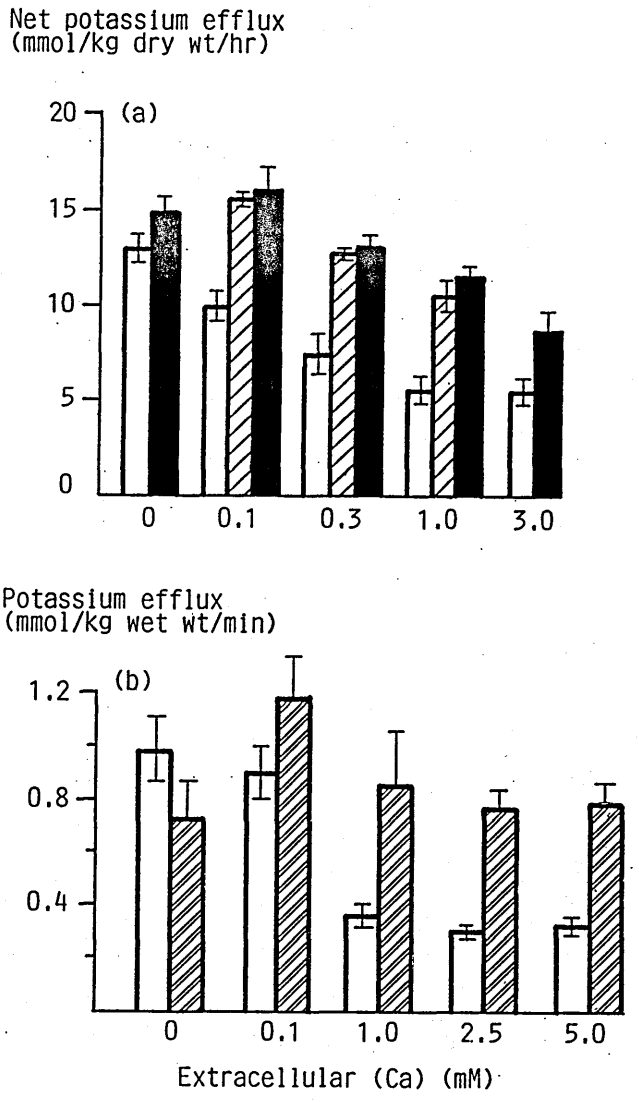


Figure 2 Effects of varying external calcium concentrations on net potassium efflux

(a) Lymphocytes from WKY (□), SHR (▨) and SHRSP (■). Bars, means ± SEM; from reference 73 by permission.

(b) Aortic smooth muscle from DOCA hypertensive (▨) and hormotensive (□) rats; data reported in tabular form in reference 74.

Efflux values from both tissues decrease with increasing calcium concentrations. The magnitude of this stabilising effect of calcium is less in aortic smooth muscle from DOCA-hypertensive rats and in lymphocytes from SHR and SHRSP compared to normotensive controls.

hypertension (62,75,76). This unifying hypothesis has been outlined by Bohr and Webb (62) in a recent review and is summarised in Figure 3.

1.2.4. Calcium as a second messenger

Intracellular calcium serves as a second messenger for the control of a variety of cell functions, including secretion, contraction, cell division and differentiation, and potassium and sodium permeability (78). Although it has been assumed for some time that Ca^{2+} stabilized the plasma membrane, the key Ca^{2+} pool in terms of contractile function is the small pool of free Ca^{2+} in the cell cytosol, $[Ca^{2+}]i$. The concentration of Ca^{2+} in this pool rises during contractile response, and the fall precedes the relaxation process (79). Moreover it has recently become apparent that this messenger or coupling function of Ca^{2+} is not confined to excitable tissues, but is a universal one. Ca^{2+} serves as a second messenger in the evocation of the specific response of almost every type of differentiated cell by its appropriate extracellular messenger, which may be hormone, circulating metabolite, or neurotransmitter (79).

The discovery of cyclic adenosine monophosphate (cAMP) and the elucidation of its role as a second messenger in the action of peptide and amine hormones (80,82) coincided with the identification of Ca^{2+} as a coupling factor between excitation and response in excitable tissues (81). For a short period, it was thought there were two quite different models of cell activation: in excitable tissues Ca^{2+} coupled stimulus to response and in non-excitable tissues cAMP served this function. However, by the late 1960s it had become clear

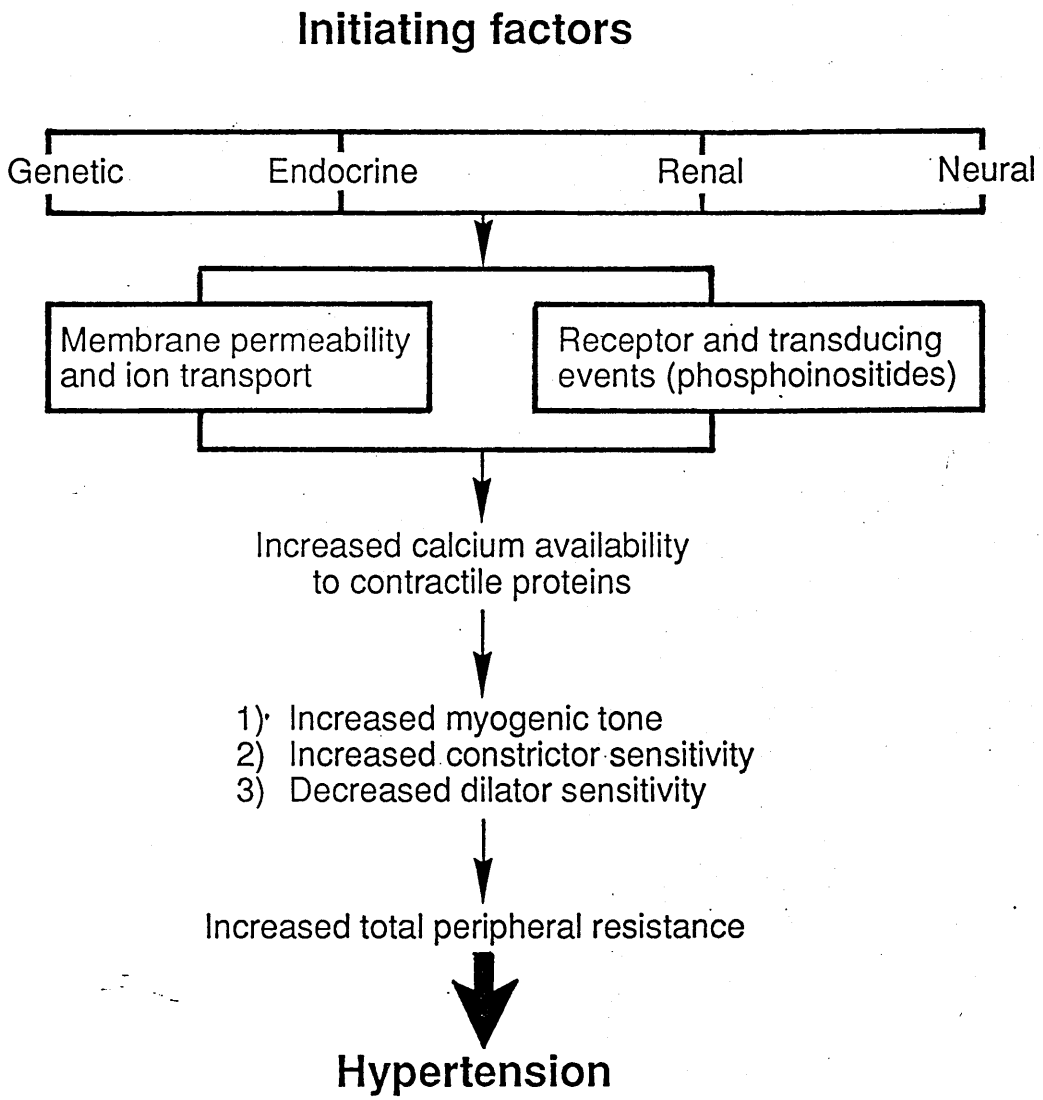


Figure 3

Involvement of the plasma membrane of the vascular smooth muscle cell in hypertension. (From reference 62 with permission).

that a considerable overlap existed in the functions of these two intracellular messengers (79,83). It was also shown that adenyl cyclase and cAMP were abundant in neural tissues (83). As knowledge of the messenger roles of Ca^{2+} and cAMP has grown, it has become apparent that both act together in regulation of cell function.

For calcium mediated effects agonist/receptor binding causes both calcium release from an intracellular pool (plasma membrane and/or endoplasmic reticulum) and increased entry of extracellular calcium into the cell (51,55,82). This is followed by a sharp, but transient, rise in the $[Ca^{2+}]i$ then followed by a fall to near basal values. Then, despite the transient rise in $[Ca^{2+}]i$, there is a sustained calcium influx and enhancement of cellular response (83). This augmented influx is nearly balanced by a high rate of calcium efflux, so that there is only a small rate of net accumulation of cellular calcium (83). The mitochondria serve as an intracellular "sink" for calcium and the non-ionic intramitrochondrial pool of calcium provides a means of stabilizing $[Ca^{2+}]i$ at any desirable level (65).

Small changes in $[Ca^{2+}]i$ cause dramatic changes in cell function. Cell activation could be described as a process of information flow from surface to interior (79). This process will be outlined as it applies to the vascular smooth muscle cell. Binding of the agonist to its specific membrane receptor represents the first step in the hormone mediated contractile process (Figure 4). Each pressor hormone has a specific type of receptor capable of initiating contraction (84). The activation signal generated by receptor occupancy

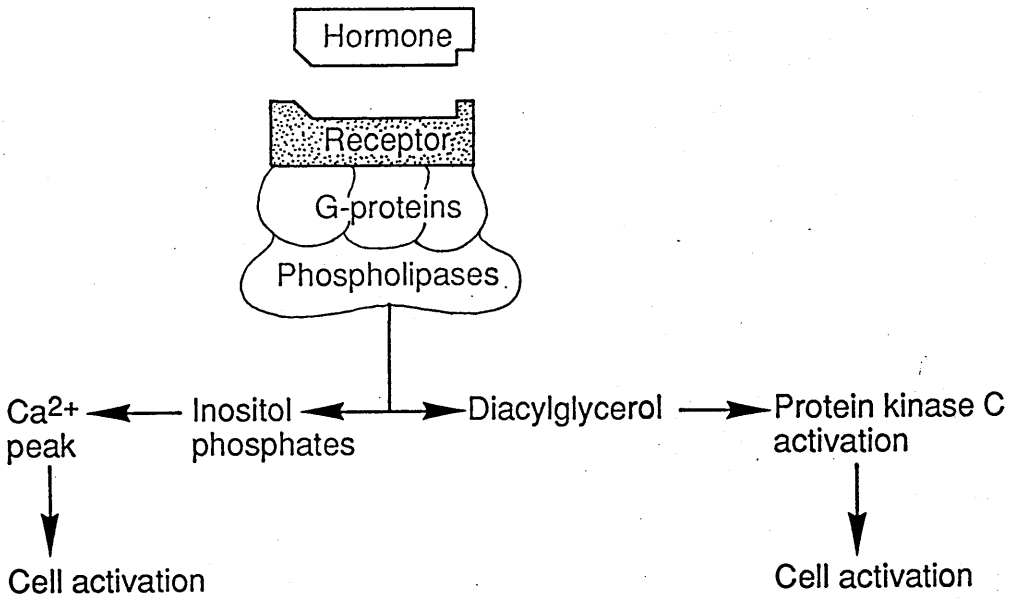


Figure 4

Schematic view of the cell activation mechanisms by pressor hormones. (From reference 84).

is transmitted to a group of guanine nucleotide-binding proteins, the so-called G proteins which act as intermediaries between the receptor and intracellular effector events. The signal initiated by hormone receptor binding is transmitted by G proteins to a group of membrane associated phospholipases (85). Hormone-activated phospholipase C acts on a particular inositol pool confined to the plasma membrane by hydrolysing inositol bisphosphate. This leads to the production of the inositol trisphosphate (IP_3) which enters the cytosol. At the time diacylglycerol is also produced but remains within the plane of the membrane (65).

It has been established that IP_3 elicits the release of Ca^{2+} from the endoplasmic reticulum (65,84). The release of calcium ions from intracellular stores is the major cause of the cytosolic Ca^{2+} rise in response to stimulation. A minor component is said to be contributed by Ca^{2+} influx from the extracellular space and a metabolite of IP_3, IP_4 is possibly a stimulator of this influx (84). A rise in the concentration of Ca^{2+} in the cell cytosol leads to an association of Ca^{2+} with receptor protein(s). The most ubiquitous and thoroughly studied of calcium receptor proteins is calmodulin (86). During cell activation $[Ca^{2+}]i$ rapidly rises from 0.1 μM to 1-4 μM, which is sufficient to shift calmodulin controlled response elements to a state of high activity (79). Another of these elements is the ATP driven Ca^{2+} pump in the plasma membrane (66). The mechanisms of $[Ca^{2+}]i$ normalization after the transient peak involve both Ca^{2+} efflux to the extracellular space and re-uptake of Ca^{2+} by the endoplasmic reticulum (52,65).

The second branch of vascular smooth muscle cell activation is dependent on protein kinase C. Activation of this enzyme by diacylglycerol causes vascular smooth muscle contraction that reaches a plateau after about 40 minutes. This contraction occurs without a $[Ca^{2+}]i$ peak and corresponds to the phase of latch-bridge formation, which is a state of contraction of the actomyosin complex that is long-lasting and consumes little energy (87). The molecular phenomena related to this contractile process are incompletely understood. Protein kinase C produces phosphorylation of several proteins, probably including actomyosin, myosin light-chain kinase, ionic channels and enzymes involved in the energy production and consumption. This phosphorylation results in structural modifications favouring the maintenance of prolonged contraction. The two pathways of vascular smooth muscle activation are not mutually independent and contraction observed in the presence of pressor hormones results from a combination of Ca^{2+}-calmodulin and protein kinase C-mediated mechanisms.

Section 3 - Methods of measurement of intracellular free calcium

1.3.1. Photoproteins (aequorin and obelin)

Aequorin is a 2000-dalton photoprotein from the marginal photocytes of the jellyfish, Aequorea forskalea (88). In the presence of calcium ions the protein emits photons and light which then can be detected by a suitable photomultiplier tube. The main advantage of aequorin as an indicator of

ionized calcium are its sensitivity and lack of calcium-buffering. A major disadvantage is the difficulty of incorporating such a protein in the cell interior (89). In 1975 Campbell and Dormer (90) succeeded in incorporating another photoprotein obelin into red blood cell ghosts using osmotic shock. Later Morgan and Morgan (91) used ethylene-glyco-bis (beta-aminoethylether) - N,N' - tetra-acetic acid (EGTA) to increase vascular smooth muscle cell membrane permeability to aequorin. Snowdowne and Borle (89) described the method of incorporating aequorin into the cells by hyposmotic shock treatment. Later McNeil and Taylor (92) developed two methods for entrapping the photoprotein in cultured fibroblasts. In the first, single cells were individually micro-injected and ultrasensitive light detection was then employed to measure the resulting weak luminescence. In the second, aequorin was entrapped using "loading by scraping" technique in thousands or millions of cells to provide a larger total luminescent signal, and without the need for microinjection. The last method is probably particularly useful in mammalian cells grown in tissue culture (92).

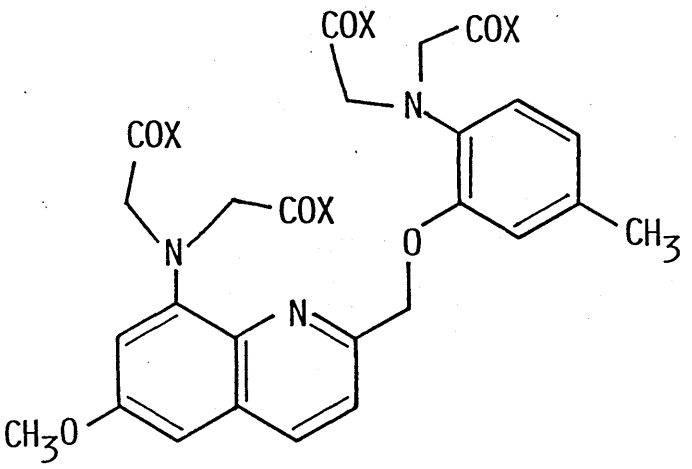
1.3.2. Fluorescent probes

Quin-2 is one of a series of fluorescent calcium indicators described by Tsien (93) for measuring and manipulating cytosolic free calcium. As an indicator, Quin 2 has advantages over previously available compounds and some drawbacks which are discussed later. Entry of indicators into intact cells is by permeation with subsequent hydrolysis of the tetra-acetoxymethyl esters (94,95,97). This advance allowed the first measurements of $[Ca^{2+}]i$ in a wide range of

cells.

Quin 2, as shown in Figure 5, is a tetracarboxilic acid with 1:1 stochiometry of calcium binding and a chelation claw resembling that of EGTA (94). Unlike EGTA, affinity for calcium is not significantly dependent on pH in the physiological range since the binding of calcium ions is not dependent on proton displacement. As a result, Quin 2 takes up and releases calcium more quickly than does EGTA and can follow increases in $[Ca^{2+}]i$ more rapidly than aequorin (99). Quin 2 fluorescence has an excitation peak near 340 nm and a measured emission peak near 490 nm (94,95). On binding one calcium ion, the fluorescence intensity of Quin 2 increases some 6.2 fold. Loading cells with Quin 2 is accomplished by incubation of the hydrophobic ester Quin 2/AM (Figure 5) with a suspension of cells and only some of the added Quin ends up inside the cells (between 15% and 30%). Because Quin 2 fluorescence is rather weak, the optimum concentration of dye in cells is between 0.3 and 1 mM. This represents a compromise between concentrations of dye that cause least buffering of calcium and the requirement for an adequate signal (94,99).

Loading of Quin 2 into most mammalian cells is readily achieved and available evidence suggests that nearly all the trapped dye is in the cytoplasm (95,98). The incorporation of a calcium chelator can affect calcium handling. Effects of Quin 2 on cellular function may be caused by calcium buffering or by toxic effects of by-products of hydrolysis of Quin 2/AM. The most toxic is formaldehyde but most cells appear to cope with the low concentrations that are generated



quin2 X = O^-

quin2/AM X = OCH_2OCOCH_3

Figure 5

Structure of Ca^{2+} indicator Quin2 and its acetoxymethyl ester Quin2/AM.

(98). In many preparations loading of Quin 2 is reported not to affect indices of cell function. Examples of known adverse effects include: a lowering of ATP concentration, an increase in lactate production, and some degree of mitogenic stimulation in murine lymphocytes (98,99). Other effects noted have included activation of phosphorylase in hepatocytes (100) and inhibition of glycolysis in human red cells with increased permeability to calcium (101).

It is possible to calculate the average resting $[Ca^{2+}]i$, for a Quin 2-loaded cell suspension. The question is often raised as to whether the incorporation of a significant calcium buffering will alter the resting calcium level (99). Tsien et al (97,98) showed that in the presence of normal external calcium, the incorporation of extra buffering into the cytosol should not per se influence steady state calcium level since this is set by the permeability of the membrane and the properties of the calcium pump. If cells are loaded in the absence of external calcium then the chelation of calcium by the incorporated Quin 2 does depress $[Ca^{2+}]i$.

In many preparations, stimulus-evoked changes in $[Ca^{2+}]i$ can be easily measured and simple experimental manipulations have allowed investigation of the sources and nature of calcium influx (99). For example, it is assumed that a signal seen in the presence of very low external calcium results from the discharge of an internal pool. A signal seen in the presence of external calcium is assumed to result from combined effects of discharge from an internal pool and influx across the plasma membrane. Influx can be inferred from the difference. The extra calcium buffering introduced by the

Quin 2 inevitably alters the pattern of evoked changes in $[Ca^{2+}]i$ (94,98). The presence of Quin 2 probably has two effects on $[Ca^{2+}]i$ caused by short-lived increases in plasma membrane permeability: the rate of rise of $[Ca^{2+}]i$ is slowed and the peak reached is reduced (96).

Although Quin 2 remains the most widely used fluorescent probe for clinical studies, a new generation of calcium indicators have been synthesised (102). The most commonly used of the new generation of fluorophores is Fura 2 which has a 30 fold brighter fluorescence than Quin 2, shows wavelength shifts upon calcium binding and has a relatively low binding capacity for free calcium. Four significant advantages of Fura 2 over Quin 2 have been identified (103). Firstly, the Kd of Fura 2 enables intracellular Ca^{2+} concentrations to be determined at concentrations of fluorophore that have only half the buffering capacity of Quin 2. Secondly, the higher fluorescence intensity of Fura 2 means that at equal signal: autofluorescence ratios, Quin 2 has 60 times more buffering capacity than Fura 2. A further advantage of the lower intracellular Fura 2 concentrations is the reduced accumulation of formaldehyde, acetate and hydrogen ions in loaded cells. Lastly, the spectral shift on binding Ca^{2+} enables both the Ca^{2+}-free and Ca^{2+}-bound forms of the indicator to be monitored, thus providing a check that the fluorescent signals resulted from changes in Ca^{2+} binding and eliminating the possibility of artefactual changes in fluorescence.

Another, less widely used calcium-responsive fluorescent dye is Indo 1. Advantages include the 10-fold or greater

difference in emission intensity at 400 nm between the chelated and the unbound species. The frequencies used for Indo 1 are further separated from cell autofluorescence signals than is the case for Fura 2. The dye undergoes a change in emission (instead of excitation) spectrum upon chelation (104).

The newer agents, Fura 2 and Indo 1, may provide better information on intracellular calcium fluxes than Quin 2 and further developments can be expected in this area.

Section 4 - Cytosolic free calcium in hypertension

1.4.1. Platelets

Erne et al (105) was the first to report that the intracellular ionized calcium concentrations in platelets, measured with the fluorescent probe Quin 2, were higher in patients with essential hypertension than in normotensive subjects. They also reported very close correlations between cytosolic free calcium concentrations and blood pressure ($r = 0.883$ for systolic and 0.931 for diastolic; $p<0.001$). Platelets are easily available in man and share many properties with vascular smooth muscle cells. Both cell types contain a calcium-dependent contractile system that couples hormone signals from various membrane receptors such as alpha-2-adrenoceptors or serotonergic receptors with contractile mechanisms (106). Activation in platelets causes shape change and aggregation (107). Platelets also show some alterations of calcium-dependent functions in essential hypertension. The examples include: increased aggregation due to adenosine diphosphate or adrenaline, enhanced shape change

after serotonin and increased secretion of beta-thromboglobulin (108). In vascular smooth muscle cells from spontaneously hypertensive rats there is also enhanced calcium-influx-dependent vasoconstriction (41,51,62).

Erne et al (105) concluded that the observed relationship between intracellular calcium concentration and blood pressure provided further evidence for the pathogenic role of intracellular free calcium in causing vasoconstriction in essential hypertension. Specifically, they suggested that defects in membrane permeability and/or alterations in ionic membrane pump mechanisms led to increased intracellular sodium and calcium concentrations. Further insights into possible causes of increased platelet free calcium in essential hypertension were obtained by Lindner et al (109). Incubation of platelets from normotensive subjects in plasma ultrafiltrate from patients with essential hypertension caused a marked increase in the cytosolic calcium concentration in these cells but no significant changes in cytosolic calcium were observed when normal platelets were incubated in plasma from normotensive subjects. High cytosolic calcium concentrations which occurred in cells from patients with essential hypertension were lowered into the normal range when platelets were incubated in plasma ultrafiltrate from normotensive subjects. The findings supported the concept that a circulating factor (or factors) in hypertension is responsible for the increased cytosolic calcium concentration in platelets.

In 1967 and on the basis of their classic parabiotic experiments, Dahl and colleagues (110) proposed that a

humoral factor explained the rise in blood pressure in salt-sensitive hypertensive rats. Haddy and Overbeck (111) later suggested that a circulating sodium-potassium-pump inhibitor was involved in the pathogenesis of volume-expansion hypertension. This substance, like ouabain, is a sodium-potassium ATPase inhibitor (112,113,126). Inhibition of active sodium transport then causes a rise in intracellular calcium, and the subsequent development of increased tone and raised blood pressure. Inhibition of the sodium pump may result in a small reduction in membrane potential and increased calcium influx through voltage dependent calcium channels (114). As an alternative, a rise in cell sodium could increase cell calcium by activation of the sodium/calcium exchanger in the plasma membrane (115). Thus, circulating humoral factors that primarily affect intracellular sodium content cause increases in cytosolic calcium concentration in platelets.

Although subsequent studies have tended to confirm the higher levels of intracellular calcium in platelets in essential hypertension (116-119), the differences in free calcium concentration observed in these studies have been smaller than reported by Erne et al (105). One possible weakness of the original study was the lack of adequate matching for age and body weight between hypertensive and control subjects. Severity of hypertension might also be relevant. The initial series (105) included a few hypertensive emergencies; platelet turnover is increased in malignant hypertension and thrombocytopenia associated with microangiopathy then occurs quite frequently (120).

Lechi et al (119) measured platelet cytosolic calcium

concentrations in response to various agonists. Adrenaline, noradrenaline and angiotensin II assayed over the concentration range of 10^{-8} to 10^{-5} mol/l, had no effect on $[Ca^{2+}]i$ in either normal or hypertensive subjects. Stimulation with thrombin resulted in a concentration-dependent increase in $[Ca^{2+}]i$ which was due mainly to calcium influx from the extracellular space and to a lesser extent to calcium release from intracellular stores. Furthermore, the $[Ca^{2+}]i$ increase in the presence of 1 mmol/l external calcium was significantly higher in the hypertensive patients than in the control subjects at all concentrations of thrombin tested. In the absence of extracellular calcium, release from intracellular stores induced by thrombin was similar in hypertensive and normotensive subjects. The difference between the two groups appeared to be in calcium influx from the extracellular space. Thus, the increased response of platelets to thrombin in hypertensive subjects could involve a receptor-operated mechanism linked to increased transport of calcium across the cell membrane. This interpretation is in line with cell membrane abnormalities present in arterial hypertension and expressed as impaired homeostasis of intracellular calcium (62,76).

There is experimental evidence that membrane cholesterol and its oxidised derivatives are critical determinants of calcium fluxes through biomembranes. In human erythrocytes, it has been shown that transmembranous calcium influx through the calcium channel correlates with the proportion of cholesterol in the membrane (121). Locher et al (122) has measured effects of cholesterol on intracellular free calcium

concentration in human platelets. An increasing proportion of cholesterol incorporated into platelet membrane caused a significant rise in intracellular calcium which led to the hypothesis that membrane cholesterol contributed to the regulation of cellular calcium metabolism.

In the spontaneously hypertensive rat, studies on free calcium levels in platelets have given contradictory results. Bruschi et al (123) reported significantly elevated $[Ca^{2+}]i$ in platelets of 8- and 20-week old SHR in comparison with WKY but no difference in $[Ca^{2}]+$ at 4 weeks. On average, the difference in levels between SHR and WKY showed a tendency to increase with age and reflected the divergence of arterial pressure. In contrast Zimlichman et al (124) could find no differences in baseline levels of cytosolic calcium or in the kinetics of changes in $[Ca^{2+}]i$ between the SHR and WKY rats studied at a time when highly significant differences in average blood pressure were obtained between the groups. They concluded that the results were inconsistent with the hypothesis that intracellular calcium in platelets at rest or in response to agonists could be determined by the same factors that cause high blood pressure in SHR. They hypothesised further that increased platelet aggregability described in SHR was related to the abnormalities in intracellular mechanism triggered by intracellular calcium rather than responsiveness of cytosolic calcium *per se*.

1.4.2. White blood cells

Several studies have examined intracellular calcium concentrations in white blood cells in human and experimental

hypertension with conflicting results. Shore et al (125) measured free calcium in mononuclear leucocytes of normotensive and hypertensive subjects but could find no significant difference between leucocyte $[Ca^{2+}]i$ in the two groups.

Bing et al (127) studied the relationship between intracellular concentrations of calcium and sodium in white blood cells and blood pressure in patients with essential hypertension, normal controls and subjects with contrasting family histories of hypertension. They failed to show elevation of $[Ca^{2+}]i$ in leucocytes of untreated essential hypertensive patients compared with those of normotensive subjects with or without a family history of high blood pressure. In contrast, there was a significant positive correlation between $[Ca^{2+}]i$ and blood pressure in subjects with normal pressures and negative family histories, but the relationship was lost if subjects with a positive family history or hypertensive patients were included. Again there did not seem to be any relationship between $[Na^{+}]i$ and $[Ca^{2+}]i$ in leucocytes.

Oshima et al (128) studied intracellular calcium, sodium and potassium in the lymphocytes in essential hypertension. Intracellular calcium and sodium were significantly increased in lymphocytes of hypertensive patients, $[Na^{+}]i$ and $[Ca^{2+}]i$ were correlated in both groups. $[Ca^{+}]i$ and $[Na^{+}]i$ were both inversely related to plasma renin activity in the hypertensive group, but not related to blood pressure. These findings, discrepant with other findings in white blood cells in essential hypertension, may be accounted for by characteristics of the hypertensive group such as ethnic

origin or renin profile. Differences in dietary sodium intake could be relevant. Changes in salt intake alter $[Ca^{2+}]i$ in lymphocytes (129): if salt intake was higher in one of the groups, the difference in $[Ca^{2+}]i$ could be diminished. A close positive correlation observed between $[Na^{+}]i$ and $[Ca^{2+}]i$ in the study by Oshima et al (128) indicates a link between calcium handling and sodium transport across the cell membrane in patients with essential hypertension and normotensive subjects. The definition of the cell type as lymphocytes was different from the earlier studies.

There is more agreement on calcium levels in white blood cells of genetically hypertensive rats. Bruschi et al (130) were first to report that cytosolic free Ca^{2+} in lymphocytes of spontaneously hypertensive rats was increased as compared to WKY control rats. Increasing the extracellular Ca^{2+} concentration 10-fold to 10 mM induced only a modest rise of intracellular free Ca^{2+} concentration. The extent of this rise was comparable in cells from SHR and WKY. Decreasing extracellular Ca^{2+} to about 100 nM by the addition of EGTA caused a 20% fall of cytoplasmic calcium and this reduction was similar in hypertensive and normotensive rats. Homeostasis of intracellular calcium was maintained despite extreme variations of external calcium. This could reflect low membrane permeability to calcium ions or effective compensatory mechanisms such as activation of membrane Ca^{2+}-ATPase or uptake into intracellular stores. The important point is that the SHR-WKY difference was maintained at any given concentration of extracellular calcium. Furspan and Bohr (73) measured free intracellular calcium concentration

in lymphocytes from stroke prone spontaneously hypertensive rats (SHRSP) and Wistar-Kyoto control rats (WKY) using Quin 2. Like Bruschi et al they found higher concentrations of intracellular free calcium in lymphocytes from the SHRSP than in those from the WKY. The same laboratory has presented evidence for increased membrane permeability to sodium and potassium in genetic hypertension (131). Net passive influx of sodium and efflux of potassium were higher in lymphocytes from SHRSP than in lymphocytes from WKY.

A subsequent study by Furspan and colleagues revealed a genetic association between net potassium efflux of lymphocytes and blood pressure when these traits were followed in the progenies (F_2 and backcross) of WKY and SHRSP matings (132). They postulated that the change in potassium permeability is secondary to a primary genetic defect of altered calcium handling by the lymphocyte membrane, which results in an elevated intracellular free calcium concentration. This elevation in turn activates the calcium-sensitive potassium efflux channels. Further evidence of a membrane abnormality in lymphocytes was found in the observation that in these cells from WKY, SHR and SHRSP, the net sodium influx varied inversely with the external calcium concentration. At all concentrations of calcium tested, the net sodium and potassium fluxes were higher in the lymphocytes from the SHR and SHRSP than in those from WKY (73). The greater fluxes were interpreted as consistent with a reduced calcium-binding capacity of the plasma membranes of lymphocytes from SHR and SHRSP. This hypothesis has been discussed in detail in section 2 (1.2.3).

1.4.3. Vascular smooth muscle cells

For the study of intracellular calcium in man platelets or leucocytes have the advantage of convenience, but the disadvantage that levels in these blood cells may not reflect levels in vascular myocytes. Changes in electrical activity of the cell membrane or interaction of pharmacological agents with membrane receptors cause either a decrease or increase in cytosolic calcium concentration and thereby alter the contractile state of the myocyte (3,51,52,55,59).

A key issue in the study of genetic hypertension is the putative role of alterations in cellular Ca^{2+} metabolism in the maintenance of an increase in basal vascular tone or reactivity (51,52,62,133,134). Several lines of evidence support this contention. Cauvin et al (55,135) measured $^{45}Ca^{2+}$ influx in vascular smooth muscle from the resistance vessels of SHR and WKY (Figure 6). The influx was greater through the "leak channel", the receptor operated channel and the potential sensitive channel in the smooth muscle from the SHR than it was in that from the WKY. In the SHR, Mulvany et al found that mesenteric resistance vessels showed exaggerated contractile responses to noradrenaline compared with the normotensive WKY rats (136). This increased contractility seemed to result from the increase in both Ca^{2+} leak and activity of excitable Ca^{2+} channels as indicated in Figure 6. Bukoski et al (137) demonstrated a similar enhanced influx of radiolabelled Ca^{2+} into cultured vascular smooth muscle cells from SHR.

Other evidence comes from observations of a decreased capacity of subcellular membrane fractions of aortic (138)

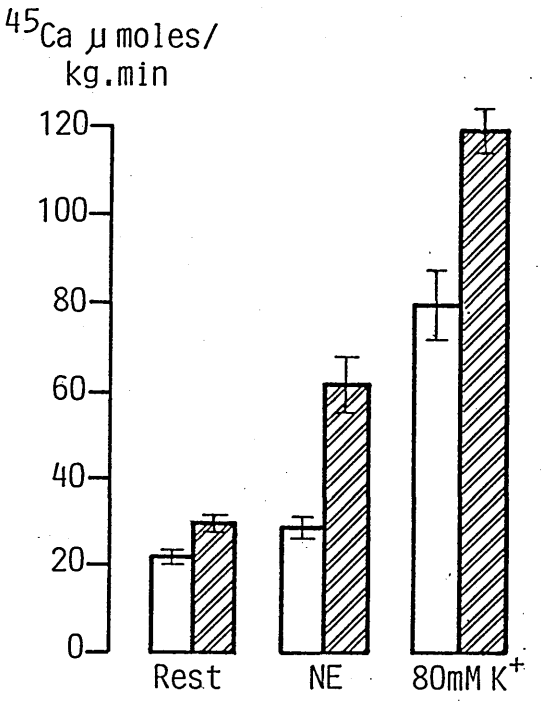


Figure 6 $^{45}Ca^{2+}$ influx in the resistance vessels

Ca^{2+} influx was measured over a 90-sec. period in vessels exposed to Ca^{2+} - labelled physiologic salt solution (PSS) (rest) or Ca^{2+}-PSS +10^{-4}M noradrenaline (NE) or 80mM K^+-PSS, and then washed for 20 min. in ice-cold Ca^{2+}-PSS containing 2mM EGTA. Resting Ca^{2+} influx and Ca^{2+} influx in the presence of NE or 80mM K^+ were significantly elevated in the SHR vessels as compared to those of the WKY rat ($P<0.05$) when expressed as Ca^{2+} flux per unit vessel weight. (From reference 55 with permission.)

and mesenteric arteries (139) to bind Ca^{2+} in the presence of ATP which may result in impairment of control of high intracellular calcium concentrations in smooth muscle from the hypertensive rat.

However, direct studies of resting, unstimulated free cytosolic calcium in vascular smooth muscle cells have given contradictory results. Compared with control animals, it has been variously reported that intracellular Ca^{2+} of the SHR is elevated (140), depressed (141), or unchanged (142,143). Nabika et al (142) measured cytosolic free calcium concentrations in cultured aortic smooth muscle cells obtained from 5 week old (prehypertensive) SHR and WKY rats. Cell suspensions were prepared and loaded with Quin 2/AM. The mean resting values of $[Ca^{2+}]i$ were almost identical in WKY and SHR (114 ± 6 nM [SEM] and 116 ± 5 nM, respectively). The same study analysed intracellular Ca^{2+} responses to two potent vasoconstrictor substances: angiotensin II and arginine-vasopressin (AVP). These peptides bind to specific receptors on vascular smooth muscle cells and cause Ca^{2+} entry or Ca^{2+} mobilization from intracellular stores. Angiotensin II caused concentration-dependent $[Ca^{2+}]i$ increases but there were no significant differences between the responses of smooth muscle cells from WKY and SHR. AVP caused smaller $[Ca^{2+}]i$ increases than angiotensin but vascular smooth muscle cells from SHR showed greater responses than controls. The cells used in the experiments described were obtained from prehypertensive rats: the possibility that high $[Ca^{2+}]i$ is a consequence of high blood pressure is not excluded. Humoral or neuronal factors not present in tissue culture may be

necessary to maintain high $[Ca^{2+}]i$ in SHR cells. The physiological significance of enhanced responsiveness to AVP is doubtful because the concentration of AVP was much higher than circulates in plasma (144). However, Hamley et al (145) found an immunoreactive vasopressin-like peptide in the vessel wall and suggested that AVP might control vascular tone as a neurotransmitter. They proposed that AVP may be present in peripheral nerve endings of vascular wall at concentrations higher than those found in plasma and that enhanced responsiveness to AVP could contribute to the pathogenesis of hypertension.

Bukoski et al (143) measured basal and agonist-stimulated concentrations of intracellular Ca^{2+} in resistance vessels of 12 week old hypertensive and normotensive rats. The measurements were done in isolated vessel segments using Fura 2/AM and a fluorescence microscope interfaced with a dual excitation wavelength fluorometer. They found no difference in resting concentrations of intracellular calcium in isolated arterioles of the SHR as compared to those of the WKY. When the vessels were stimulated by noradrenaline, there was a difference in the transient increase in Ca^{2+} concentration with arterioles of SHR exhibiting significantly lower response to 1 and 10 μM norepinephrine. The results did not support the hypothesis that increased sensitivity of vascular smooth muscle of the SHR to catecholamines or an increase in resting tone of resistance vessels is the result of an inherently greater level of intracellular Ca^{2+} (143).

Sugiyama et al (140) measured the absolute values of $[Ca^{2+}]i$ in single vascular smooth muscle cells using the UV-

laser and Ca^{2+}-sensitive probe Indo-1. This system has several advantages: 1) $[Ca^{2+}]i$ is calculated not from the fluorescence intensity but from the fluorescence ratio; the photobleaching of Indo-1 and intracellular Indo-1 concentration have no effect on the calculation of $[Ca^{2+}]i$. 2) Use of a laser to excite the dye allows the low energy excitation to produce a high intensity of fluorescence from low concentrations of intracellular Indo-1. Using this method the mean $[Ca^{2+}]i$ in 8 and 12 week old SHR were significantly higher than those in 8 and 12 week old WKY, but intracellular calcium concentrations were not higher in four week old (prehypertensive) SHR. They also studied intracellular free calcium in vascular smooth muscle cells from WKY and SHR in primary culture, and in the 6th passage. In the 6th passage cultured vascular smooth muscle cells, the haemodynamic effects of the high blood pressure in SHR on $[Ca^{2+}]i$ in vivo should have disappeared. However, intracellular calcium concentrations were the same in primary culture as in passaged cells and the increase in $[Ca^{2+}]i$ in SHR was maintained. These results are consistent with the idea that maintenance of $[Ca^{2+}]i$ levels of vascular smooth muscle cells in SHR is genetically regulated and that high levels are not a consequence of high blood pressure.

The results of measurements of $[Ca^{2+}]i$, in platelets, white blood cells and vascular smooth muscle cells in hypertensive man and in spontaneously hypertensive rat are summarised in Table 1.

Developments in methods of measuring cytosolic free calcium should allow more precise estimations of resting and

TABLE 1

Resting free cytosolic calcium $[Ca^{2+}]i$ in platelets, white blood cells and vascular smooth muscle cells in hypertensive man and in the spontaneously hypertensive rat*

Cell type	Source of tissue	Method	Result	Ref No
Platelets	Man	Quin 2	↑	105
Platelets	Man	Quin 2	↑	109
Platelets	Man	Quin 2	↑	116
Platelets	Man	Quin 2	↑	117
Platelets	Man	Quin 2	-	118
Platelets	Man	Quin 2	↑	119
Platelets	SHR/WKY	Quin 2	↑	123
Mononuclear leucocytes	Man	Quin 2	-	125
Leucocytes	Man	Quin 2	-	127
Lymphocytes	Man	Quin 2	↑	128
Lymphocytes	SHR/WKY	Quin 2	↑	130
Lymphocytes	SHR/WKY	Quin 2	↑	73
VSMC	SHR/WKY	$^{45}Ca^{2+}$influx	↑	135
VSMC	SHR/WKY	$^{45}Ca^{2+}$influx	↑	137
VSMC	SHR/WKY	Quin 2	-	142
VSMC	SHR/WKY	Fura 2	-	143
VSMC	SHR/WKY	Indo 1	↑	140

* Arrows indicate direction of difference from normotensive controls: (-) indicates that there were no differences between hypertensive and normotensive subjects or SHR and WKY. Vascular smooth muscle cells - VSMC

stimulated intracellular calcium in intact living cells under physiological conditions, and may eventually allow simultaneous measurements of $[Ca^{2+}]i$ and contractile activity.

1.4.4. Effects of blood pressure lowering treatment

Effects of antihypertensive therapy on free cytosolic calcium concentration in human platelets have been described (105,109,118,146). Erne et al (105) initially reported that antihypertensive treatment with calcium-channel blockers, beta-adrenoceptor blockers and thiazide diuretics all resulted in a fall in levels of cytosolic calcium. When all treated patients (n=33) were analysed, the treatment-induced changes in platelet calcium levels correlated with the changes in systolic and diastolic pressures. Lindner et al (109) also observed that the intracellular free calcium concentration in platelets decreased into the normal range during successful treatment of hypertension with either a calcium-channel blocker (nicardipine) or an angiotensin-converting enzyme inhibitor (lisinopril). In addition, when platelet calcium was measured repeatedly over a range of blood pressures in the same patients, these values were directly correlated in each treatment group. Lenz et al (118) examined the acute effects of nifedipine and captopril on free intracellular platelet calcium in patients with essential hypertension. Both drugs lowered blood pressure within 60 minutes but platelet calcium was only lowered by nifedipine and not by captopril. There was no correlation between blood pressure reductions and falls in platelet calcium. The authors speculated that calcium changes observed

during chronic antihypertensive therapy (105,109) might be a consequence rather than a cause of the blood pressure reduction. McVeigh et al (146) studied platelet free intracellular calcium levels during a placebo-controlled study of the effects of increasing doses of thiazide diuretic in mild essential hypertension. After 8 weeks' therapy 125 and 150 µg of cyclopenthiazide produced significant decrements of blood pressure compared with placebo but 50 µg had no effect. Platelet $[Ca^{2+}]i$ levels decreased to similar extent in each of the three treatment groups, as well as in the placebo group. No correlation between decrease in blood pressure and cytosolic calcium was evident. These findings were inconsistent with the hypothesis that changes in platelet $[Ca^{2+}]i$ could be determined by the same factors that control blood pressure.

Various classes of blood pressure-lowering drugs have been shown to lower free cytosolic calcium concentration in platelets. The data available are not consistent about the relationship between changes in intracellular calcium and blood pressure. It is too early to say if changes in intracellular calcium are a cause or a consequence of changes in blood pressure.

1.4.5. Effects of dietary calcium supplementation

Epidemiological studies, summarised in Section 1, have led to the hypothesis that dietary deficiency of calcium has a role in the pathogenesis of essential hypertension. Some intervention studies have found that an increased dietary intake of calcium has a blood pressure-lowering effect in

essential (27) and experimental hypertension (14). It remains uncertain whether normalization of extracellular ionized calcium is a necessary part of this effect. In contrast to extracellular ionized calcium, intracellular free calcium, which tends to be elevated before treatment, decreases as blood pressure is lowered by several drugs (105). McCarron et al (147) has proposed that dietary calcium supplementation acts by correcting an imbalance between extracellular and intracellular free calcium concentrations thereby causing vascular smooth muscle to become more efficient or effective in handling intracellular free calcium.

Furspan et al (31) made comparisons of the effects of feeding normal (1%) or high (2.5%) calcium diets to SHRSP for nine weeks. Initial values for sodium and potassium fluxes, intracellular ionized calcium and blood pressure were elevated in the SHRSP compared to the WKY. The high calcium diet caused a significant increase of extracellular ionized calcium accompanied by a normalization of sodium and potassium fluxes, $[Ca^{2+}]i$ and blood pressure. Felodipine treatment had the same effect (148).

The mechanisms linking dietary calcium manipulations with blood pressure are not well defined and further studies are needed to examine whether dietary supplements of calcium normalize intracellular free calcium. The paradox that blood pressure is lowered both by increased calcium intake and calcium channel blockers may then be resolved.

Section 5 - Parathyroid hormone and blood pressure regulation

1.5.1. Parathyroid hormone; structure and biological

actions

Parathyroid hormone is an 84 amino-acid single chain polypeptide. The amino-acid sequences of bovine, porcine and human parathyroid hormone (PTH) are defined (149). The N-terminal 27 amino-acids seem to be essential for biological activity but full activity requires the segment 1 to 34. The determinant of secretion of parathyroid hormone is the plasma concentration of calcium ions. A physiological increase in PTH secretion appears to depend in the short-term on release of stored peptide. There is little evidence that PTH secretion is controlled at transcriptional or translational levels. Short-term changes in secretion involve calcium effects on degradation of PTH in parathyroid cells and long-term changes are mediated by hyperplasia or atrophy of the glandular tissue (150). Parathyroid hormone in plasma measured by radioimmunoassay is heterogenous. The main fragment of the hormone has a molecular weight near 6000 and corresponds to the C-terminal portion of the molecule; there are also amino-terminal fragments which may have biological activity, as well as small amounts of the complete 84 amino-acid mature hormone. Most assays measure the dominant C-terminal fragment and therefore reflect long-term changes in hormone secretion or removal rather than rapid physiological or pathological variations (22,151). Novel assays based on monoclonal antibodies which measure intact 1-84 parathyroid hormone are under development (21,152).

Parathyroid hormone acts on kidney, bone and intestinal tract. In the kidney the hormone has three separate effects; enhancement of phosphate excretion, increased reabsorption of

calcium and promotion of conversion of 25-hydroxycholecalciferol to the highly active 1,25-dihydroxycholecalciferol. These combine to cause an increase in the plasma concentration of calcium ions. The second messengers are cAMP and intracellular calcium (153,154). Parathyroid hormone causes not only an increase in intracellular cAMP, but also spillover of cAMP into the urine (155). Evidence that an increase in intracellular calcium is involved is less clear. In bone, PTH enhances osteoclastic activity (150). In addition to this action which produces bone destruction, there is some evidence that PTH can have an anabolic effect on bone so that net bone mass is increased. The cellular mechanisms involved in the action of the parathyroid hormone on bone presumably resemble those in the kidney: increase in cAMP, perhaps associated with an increase in intracellular calcium. The action of parathyroid hormone in the intestinal tract is an indirect one, exerted via the increased renal production of calcitriol. The net effect is to enhance absorption of calcium and phosphorus (153).

1.5.2. Actions of parathyroid hormone in the cardiovascular system

Studies from several centres have suggested that the cardiovascular system is another target for parathyroid hormone action (156-159). Charbon (156) and later Pang et al (160) have shown that injections of parathyroid extract or synthetic bovine PTH (1-34) cause a rapid reduction in systemic blood pressure in dogs. This hypotensive effect is reproducible, dose dependent (161) and caused by systemic

vasodilation. Both purified bovine PTH (1-84) and its synthetic amino-terminal fragment (1-34) PTH increase coronary, renal, hepatic, gastric and pancreatic blood flow in the dog (162,163). Bolus injection or short-term infusion of parathyroid extract, (1-84) PTH and (1-34) PTH (160,164) decrease blood pressure in a variety of normotensive anaesthetised vertebrates. Studies in spontaneously hypertensive rats, in the two-kidney one-clip model of hypertension and in deoxycorticosterone and salt hypertension in rats showed that acute administration of (1-84) and (1-34) PTH reduces blood pressure (165,166). The vasodilatory action has been demonstrated in the perfused rat hindlimb and isolated rabbit aorta preparation and it is not blocked by propranolol, phentolamine, atropine or promethazine (163). Indomethacin, a cycloxygenase inhibitor, seems to reduce the hypotensive action of PTH (159), which implicates vasodilatory prostaglandins. The only specific agents which inhibit these vasodilator effects are synthetic analogues of PTH which act as competitive antagonists, at least in the kidney (158).

PTH interacts with the smooth muscle cells of vascular tissue via stimulation of specific membrane receptors. Nickols (167) showed that PTH relaxed precontracted aortic strips, increased cAMP in cultured vascular smooth muscle cells and stimulated adenylate cyclase in membrane preparations in a dose-dependent manner.

Besides vasodilation, PTH administration causes an increase of heart rate in anaesthetised dogs which is not a result of baroreceptor activation (158). Crass et al (168) have described concentration-dependent vasodilation of the

coronary artery. At higher doses, there is stimulation of left ventricular isometric contractile force and an increase in spontaneous heart rate. PTH also increases the frequency at which rat heart cells beat in culture and stimulates cAMP production (157).

1.5.3. Cyclic AMP and intracellular calcium - synarchic second messengers for the parathyroid hormone

Increased entry of calcium into vascular smooth muscle normally causes vasoconstriction (2,3,41,51-55,57-59), but PTH probably does not enhance entry of calcium into smooth muscle cells (159). Data from Pang et al (163,171) suggest that the hormone blocks entry of calcium. (1-34) PTH appeared to reduce the low-affinity lanthanum-resistant pool of calcium in the tail artery of the rat and in mesenteric artery of the chicken.

In more recent studies, Pang et al (personal communication) examined the effect of the bovine PTH fragment (1-34) on the L voltage-dependent calcium channel currents of the rat tail artery smooth muscle cells, using the patch clamp technique. In the majority of cells studied, the L channel currents were inhibited by PTH and this inhibition could be reversed by calcium channel agonist BAY-K 8644. The authors interpreted these data as suggesting that the mechanism of vasodilation produced by PTH may be mediated by the inhibition of L voltage-dependent calcium channels in vascular smooth muscle.

Rasmussen (154) has proposed that PTH acts through two second messenger systems, namely cAMP and intracellular free

calcium. This dichotomy underlies the conflicting findings and conclusions concerning the cellular responses to the hormone. In smooth muscle the acute actions of PTH are primarily mediated by cAMP, which acts in two ways: firstly, it stimulates phosphorylation of myosin light chain kinase (169) and secondly, it lowers intracellular ionized calcium concentrations (170,171). Both processes promote muscle relaxation.

Part of the effect of PTH on the renal proximal tubule is also mediated through cAMP, and in this regard both PTH and cAMP inhibit the Na+/H+ exchanger at the apical portion of this segment of the nephron (172). These observations raise an intriguing possibility concerning the role of PTH in salt-sensitive essential hypertension. Elevated serum concentrations of this hormone may serve to counteract the vasoconstriction associated with increased activity of the Na+/H+ exchanger in renal proximal tubules of salt sensitive individuals. Resnick et al (18) showed that patients with lower serum ionized calcium are primarily low-renin hypertensives. It is of interest that black and older patients with essential hypertension, who compromise a major portion of this group, manifest high levels of serum PTH (173,174). Moreover, a higher salt intake is associated with an increase in serum PTH concentrations in hypertensive blacks (175). Aviv (176) has hypothesised that PTH elevation in salt sensitive essential hypertension is a compensatory process, attenuating both the hyperactive Na+/H+ antiport in the proximal tubule and the increased peripheral vascular resistance. Hypertension, observed in primary and secondary

hyperparathyroidism, may not be directly related to elevated PTH levels but to other primary metabolic abnormalities.

1.5.4. Primary hyperparathyroidism

Primary hyperparathyroidism is due to oversecretion of parathyroid hormone. The pathogenesis is not understood. Most patients have overactivity of a single gland which contains an adenoma. It has been suggested that adenoma may be a consequence of parathyroid hyperplasia because the constituent cells are polyclonal (177). This question has been re-examined by Arnold et al (178), who came to the alternative view that adenomas are often, if not always composed of a clone of cells. Firstly, they found evidence that the parathyroid hormone gene had undergone a clonal rearrangement; secondly, clonality was inferred from the evaluation of X chromosomes in parathyroid adenomas obtained from women. A clonal abnormality implies that the underlying disturbance in DNA structure originated in one cell as a result of a somatic mutation (179).

In contrast to sporadic adenomas, hereditary disturbances or germ-line mutations that cause primary hyperparathyroidism are almost always associated with generalised hyperplasia of the parathyroids. Familial hypocalciuric (benign) hypercalcaemia is such a condition possibly due to impaired feedback of calcium on secretion of the parathyroid hormone. Heterozygotes with a single abnormal gene have life-long hypercalcaemia and normal or slightly hyperplastic parathyroid glands; homozygotes have life-threatening hypercalcaemia and severe parathyroid hyperplasia which are both evident at birth (180). In type I familial multiple endocrine

neoplasia, parathyroid hyperplasia is associated with benign pituitary tumours and benign or malignant islet-cell tumours of the pancreas. The mutant gene has been localised to a region on chromosome 11 (181). In type II familial multiple endocrine neoplasia, parathyroid hyperplasia is associated with phaeochromocytoma and with benign or malignant proliferation of calcitonin-containing cells of the thyroid gland. The mutant gene has been mapped to a region near the centromere of chromosome 10 (182).

The clonality of parathyroid adenomas has important clinical implications. It provides a basis for the hypothesis that the pathogenesis of adenoma is fundamentally different from that of hyperplasia. A diagnosis of adenoma usually implies that family screening is not necessary, that subtotal parathyroidectomy with its increased risk of permanent hypoparathyroidism need not be performed, and that the risk of postoperative recurrent hyperparathyroidism is low (179). The concept that adenoma results from somatic gene mutation is supported by the suggestion that parathyroid adenoma may be induced by irradiation (183).

In 1934, and only ten years after the first operation for removal of parathyroid tumour, Albright et al (184) defined the condition in a paper entitled "Hyperparathyroidism - a common and polymorphic condition as illustrated by seventeen proved cases in one clinic". There has been increasing awareness that primary hyperparathyroidism is not rare and often occurs as a relatively mild condition lacking the florid renal and skeletal manifestations found in the original cases. The introduction

of multiple channel autoanalysers into clinical practice has greatly increased the apparent incidence of the disorder (45,185-188). Current estimates of the prevalence of primary hyperparathyroidism in hospital populations are 100-200 per 100,000 (185,186). A report from Sweden (189) found that the figure in adult workers was as high as 520 per 100,000. In Rochester, Minnesota, the addition of calcium to the 12 unit serum biochemistry profile increased the number of known cases of primary hyperparathyroidism four-fold (45). The same study gave an age-adjusted rate of 27.7 per 100,000 with an annual incidence of 188 per 100,000 in women over 60. By extrapolating, it was argued that there might be 35,000 to 86,000 new cases of primary hyperparathyroidism per year in the United States. Mundy et al (190) reported similar findings from an urban community survey in England. The annual incidence in Birmingham was calculated at 25 cases per 100,000; and this figure if true for England as a whole, gave an annual incidence of 10,000.

Primary hyperparathyroidism is a disease of protean manifestations (191). In its mildest form there are no symptoms or signs, and the diagnosis is only suspected after routine determinations of serum calcium. Symptomatic disease develops insidiously over a period of years. Renal colic is the most common symptom occurring in 25-35 per cent of cases, with nephrocalcinosis occurring less often. Stones are usually composed of calcium oxalate and less commonly of calcium phosphate. In a few patients the condition progresses rapidly, with marked hypercalcaemia, debility, bone pain and sometimes pathological fractures. Bone disease in hyperpara-

thyroidism is now quite rare but may present with bone pain, pathological fractures, bone cysts, or localised swellings of bone described as "epulis" of the jaw or "brown tumours" of bone, which comprise accumulated osteoclasts, osteoblasts and fibrous tissue (192). Peptic ulcer and chronic pancreatitis occur with increased frequency. Hyperparathyroidism as a component of the multiple endocrine neoplasia type I (MEN I) syndrome may be associated with the Zollinger-Ellison syndrome (193). Neurological abnormalities in hyperparathyroidism include emotional lability, slow mentation, poor memory, depression and neuromuscular abnormalities. Some signs and symptoms in hyperparathyroidism may be secondary to hypercalcaemia per se. Polyuria, polydipsia and constipation are common. Other less frequent abnormalities include "band keratopathy", pruritus, subconjunctival deposits of calcium, and ectopic calcification of lungs, kidneys, arteries and skin (184,191). An association between primary hyperparathyroidism and high blood pressure has been suggested by several studies (43-45,194,195) and will be discussed in detail in chapter 1.5.4.

Radiographic evidence of hyperparathyroidism includes subperiosteal resorption (best recognised in the phalanges and distal portions of the clavicles), generalised osteopenia or osteoporosis, demineralisation ("salt and pepper pattern" of the skull), bone cysts or brown tumours (192). Hypercalcaemia accompanied by hypophosphataemia is the typical feature of primary hyperparathyroidism. The increased calcium concentration in serum reflects the action of parathyroid hormone on the kidney and the skeleton and stimulation of

$1,25(OH)_2$ vitamin D_3 production resulting in increased calcium absorption from the gut. About half of all patients with hypercalcaemia detected in the hospital clinics have primary hyperparathyroidism, and hypercalcaemia detected in surveys of healthy populations is caused by primary hyperparathyroidism in most instances (186). PTH causes hypophosphataemia by increasing renal clearance of phosphate, once used as a diagnostic test. Laboratory methods that now contribute to the diagnosis include radioimmunoassay of PTH, urinary clearance of cAMP, and determination of vitamin D metabolites in plasma. Concentrations of PTH in plasma are elevated in hyperparathyroidism and low or undetectable in other forms of hypercalcaemia. Some aspects of cAMP as a second messenger for parathyroid hormone have been discussed in the previous chapter. Renal tubular cells express specific membrane receptors for PTH and the increase in intracellular cAMP in response to PTH is reflected in the appearance of this nucleotide in the urine (155).

Surgical correction of primary hyperparathyroidism results in correction of hypercalcaemia in most cases. There are several methods of localising abnormal parathyroid tissue before surgery. Computerised axial tomography, ultrasonography or radiothallium scanning are said to have 60-90% success rates in previously unoperated cases (196,197). Angiography and selective venous catheterisation should be reserved for instances of recurrence or persistence of hyperparathyroidism after initial neck exploration (198). Purnell et al (199) identified four groups of patients with primary hyperparathyroidism, based on large clinical experience at

the Mayo Clinic. The first category comprised patients with severe hypercalcaemia and bone or renal disease. Surgical removal of abnormal parathyroid tissue is clearly indicated in this group and an accomplished parathyroid surgeon can achieve this in 90 per cent of patients not previously operated on. In the second category are patients who have had unsuccessful surgical treatment or recurrence of hyperparathyroidism after a successful operation. In this group the problem is to locate the abnormal parathyroid tissue, preferably before surgery. The third and largest category includes patients with so called "asymptomatic" or "biochemical" disease, with mild hypercalcaemia (serum calcium less than 2.75 mmol/l), high PTH concentrations, no radiological evidence of bone disease, normal renal function and without urinary tract stones. The best treatment for these patients is not decided (199,200). The fourth category includes patients who are unfit for surgery or in whom surgery has repeatedly failed and who are candidates for medical treatment.

The preferred surgical approach is to search one side of the neck first. If an adenoma and a normal gland on that side are proved by biopsy (frozen sections), the adenoma is removed and the wound closed. If a lesion is not found then the procedure is repeated on the other side until all four glands are identified (191). If several glands are hyperplastic then all parathyroid tissue should be removed apart from about 50 mg of the least affected gland. Autotransplantation of this 50-100 mg tissue to the arm is favoured by many surgeons (201,202). If abnormal parathyroid tissue is

not found then mediastinal exploration at a later date should be considered. Before this is carried out, detailed studies to localise the abnormal tissue should be carried out (203).

Treatment of asymptomatic hyperparathyroidism remains a matter of controversy (199,200,204-207). The natural history of symptomless hyperparathyroidism is often benign. Purnell et al (199) studied 141 asymptomatic patients with serum calcium concentrations below 2.75 mmol/l and normal renal function. At follow-up, 12 years after the beginning of the study (208), 33 patients (23%) had been treated by operation because they no longer fulfilled the criteria: eight had shown a rise in serum calcium concentration; six had developed active stone disease; four had developed bone disease; nine had psychological and various other factors and in six renal function had decreased. The study could not identify features predictive of a favourable outcome or factors predictive of serious and irreversible complications such as renal insufficiency or hypertension. Sampson et al (200) followed 68 patients with mild disease for a mean period of four and a half years (range 1-15 years) and failed to show any significant decline of renal function or worsening of hypercalcaemia. Only three patients from this series were referred for parathyroid surgery when serum calcium concentration rose above 3 mmol/l, and another three developed a similar degree of hypercalcaemia. In this subgroup the rise in calcium was gradual and there were no instances of hypercalcaemic crises or symptoms. The authors suggested conservative management for patients over the age of 55 years with mild asymptomatic disease. Again, factors

predictive of severe disease were not identified.

Most patients with uncomplicated hyperparathyoidism are women past the menopause, a group already at high risk of bone loss which may be accelerated further by hyperparathyroidism (45,209). Oestrogen treatment may ameliorate some consequences of hyperparathyroidism as well as preventing bone loss (207,210). Coe et al (207) reviewed available information about oestrogen treatment and concluded that evidence about the efficacy of this long-term medical treatment compared with surgery was lacking.

Palmer et al (211) reported results of a 14-year follow-up of 172 persons identified in a health survey as having mild to moderate hypercalcaemia. A matched normocalcaemic group from the same population was followed up for comparison. The all-cause mortality was higher in the hypercalcaemic group, which was mainly due to higher mortality from diseases of the circulatory system. The hypercalcaemic group had significantly higher systolic and diastolic blood pressures and serum concentrations of urate. There was a tendency for serum concentrations of glucose and cholesterol to be higher but these differences were not significant. In this study PTH concentrations were unknown but it was assumed that the majority of patients had primary hyperparathyroidism. There are other reports, describing better defined populations of patients with primary hyperparathyroidism, which have confirmed Palmer's findings. Risk factors for cardiovascular disease were more frequent in patients with primary hyperparathyroidism than in controls (34,212-214) and association between hyperparathyroidism and

cerebrovascular or ischaemic heart disease has also been recorded (215,216).

Although such findings suggest that patients with hyperparathyroidism are at excess risk of cardiovascular disease, the reversibility of this risk by successful parathyroid surgery has not been established.

1.5.5. Hypertension and the parathyroid gland

An association between hypertension and hyperparathyroidism was first described by Hellstrom et al (43) in 1958. Hypertension which was defined as a blood pressure above 150/100 mmHg was present in 70 per cent of a series of 105 patients with primary hyperparathyroidism. The degree of hypertension seemed to correlate with indices of renal impairment and in the majority of cases the impairment of renal function antedated the rise in blood pressure. In this early series, high blood pressure and its sequelae were responsible for about half of the mortality.

Surprisingly little progress has been made in understanding the nature of the association between hyperparathyroidism and hypertension since the time of Hellstrom. The prevalence of hypertension has been variously estimated at 10% to 60% of patients with primary hyperparathyroidism (34,45,192,212,213). Some of this variation reflects differences in methods of blood pressure measurement, diagnostic criteria and severity of hyperparathyroidism. Studies by Rosenthal and Roy (212) and Avioli (217) indicated that hyperparathyroidism is ten times more frequent in patients with otherwise "essential" hypertension than in the general

population. The known but rare links between hyperparathyroidism and other endocrinopathies, including phaeochromocytoma, Cushing's syndrome, primary aldosteronism and acromegaly, do not explain the association (218).

Daniels and Goodman (34) in a large retrospective analysis of 120 patients with surgically proved primary hyperparathyroidism found that the mean serum calcium concentration in the 49 patients with high blood pressures was almost identical to levels in the 71 patients with normal pressures. Significant correlations between serum concentrations of calcium and systolic or diastolic blood pressures were not found. Salahudeen et al (195) came to the same view and also failed to find a fall in blood pressure after parathyroidectomy despite correction of hypercalcaemia and high parathyroid hormone levels.

It has been proposed that hypertension in hyperparathyroidism results from increased activity of the renin-angiotensin system. Brinton et al (194) reported that plasma renin activity was elevated in four out of seven patients with hyperparathyroidism and hypertension but normal in five patients with normal blood pressures. Blood pressure and renin returned to normal following removal of parathyroid adenomas in three hypertensive patients with hyperparathyroidism and were significantly improved in a fourth. However, calcium infusion does not stimulate renin secretion in vivo (37) and blood pressure does not fall after saralasin infusion (219). The most recent study (195) reported an increase in the mean ambulant renin above values in normotensive controls but not exceeding levels in patients with

essential hypertension. These results and the absence of change after parathyroidectomy were interpreted as providing strong evidence against a role for renin or aldosterone in the hypertension of hyperparathyroidism.

Vlachakis et al (220) reported that patients with primary hyperparathyroidism had higher plasma concentrations of catecholamines and increased vascular reactivity to infused noradrenaline, but subsequent studies (221,222) have not confirmed this finding. Richards et al (222) could find no relationship between changes in catecholamines and changes in blood pressure or plasma levels of calcium after surgery. Despite the inherent problems in using concentrations of noradrenaline in venous plasma as an index of the activity of the sympathetic nervous system (223), the results of most studies do not support the contention that increased sympathetic tone or responsiveness to noradrenaline are responsible for the high arterial pressure in primary hyperparathyroidism.

Is it then possible that renal damage is of importance in the pathogenesis of hypertension in hyperparathyroidism as originally suspected (43)? Later studies have questioned the relationship and found that hypertension was not invariably associated with impairment of renal function (212,213). Daniels and Goodman (34) could find no difference between the hypertensive and normotensive group in respect to mean values of serum creatinine, urea or creatinine clearance. The most recent study of the relationship between hypertension and renal dysfunction in primary hyperparathyroidism by Salahudeen et al (195) found a significant inverse relation-

ship between blood pressure and ^{51}Cr-EDTA clearance which persisted after successful surgical treatment.

Hypertension in patients with primary hyperparathyroidism might be heterogeneous in origin. The mean age at the time of diagnosis of patients with hyperparathyroidism is about 50 years (34,43,45). In the general Caucasian population at this age, the prevalence of essential hypertension may be as high as 10-20%. Therefore, a number of patients with hyperparathyroidism and high blood pressure may have hypertension unrelated to hyperparathyroidism. Richards et al (222) have suggested that such coincidences might obscure true instances of hyperparathyroid specific hypertension. This conclusion was based on a single observation of a pronounced fall in blood pressure in one of eight patients studied in detail before and after parathyroid surgery; a pressure fall that coincided with the largest falls in plasma concentrations of noradrenaline, renin and cortisol.

There have been conflicting reports on the overall blood pressure response to successful surgical treatment. Only one group has reported cure of hypertension in all cases (213). Daniels and Goodman (34) reviewed data from five studies on the effect of surgery on blood pressure. Blood pressure returned to normal postoperatively in 23 out of 83 patients studied (28%), which again raised the possibility that there is a subgroup who develop hypertension as a direct consequence of hypercalcaemia. Worsening of hypertension after surgery has also been reported in some patients (43,187,224). The majority of studies have reported no change in blood pressure for groups of patients studied before and after

parathyroidectomy (195,221,222,225,226). In addition Posen et al (226) found no difference in the prevalence of hypertension in patients cured of hyperparathyroidism compared with those having no surgery or unsuccessful surgery.

A randomised prospective study of the value of surgery in hyperparathyroidism has not been performed. It is probably premature to advise that hypertension is an indication for surgery in uncomplicated mild hyperparathyroidism.

Section 6 - Left ventricular hypertrophy

1.6.1. Calcium and cell proliferation

Growth factors and other mitogens stimulate quiescent, nonproliferating cells in culture to re-enter the cell cycle and become committed to DNA synthesis. Although changes in intracellular pH and Ca^{2+} have been observed in many systems, it is not yet clear which of these ionic events are necessary steps in the pathway leading to DNA synthesis (227,228). The biochemical steps that drive quiescent cells out of G_0 and through G_1 to S phase are the key to understanding the mitogenic pathway (229). Evidence has been presented that early calcium increases associated with intracellular acidification are necessary steps for this transition (230-232). Subsequent mitogen-induced alkalinisation of the cell by activation of the Na^+/H^+ exchanger is probably independent of changes in intracellular Ca^{2+} (231).

It is interesting that contractile agonists and growth factors share many properties. Both have been reported to mobilize intracellular calcium (230,233), increase inositol trisphosphate turnover (230,234,235) and activate Na^+/H^+

exchanger (231,232,236). Evidence has been presented that platelet derived growth factor (PDGF) induces not only cell proliferation but also vasoconstriction of smooth muscle cells (237). Similarly angiotensin II has been shown to stimulate growth in cultured vascular smooth muscle cells (238,232). Kawahara et al (239) presented evidence that angiotensin II can induce expression of the proto-oncogene c-fos through activation of protein kinase C and Ca^{2+} mobilization in cultured rat aortic vascular smooth muscle cells. Similar processes have been described earlier for well-established growth factors such as platelet derived growth factor and epidermal growth factor (235). In line with these findings, calcium channel blockers such as nifedipine appear to slow the rate of PDGF-induced DNA synthesis and proliferation of smooth muscle cells (240).

It has been assumed that calcium influx in cardiac myocytes contributes to hypertrophy and it has been suspected that hypertrophic cardiomyopathy could be related to abnormal regulation of cytosolic calcium. In a Syrian hamster model of hereditary cardiomyopathy the concentration of calcium in myocytes was elevated (241), verapamil treatment prevented or delayed development of the condition (242,243) and the density of dihydropyridine binding sites was increased (244-246). Chronic administration of a calcium ionophore to pregnant rats has caused hypertrophy and disarray of myocardial myofilaments in the offsprings, histological findings similar to those found in hypertrophic cardiomyopathy (247).

Clinical trials have shown that diastolic dysfunction in hypertrophic cardiomyopathy in man is ameliorated by

verapamil (248,249), which also relieves some of the symptoms (250). Increased numbers of dihydropyridine binding sites have been shown in the myocardium of affected patients (251). This increased density of voltage-sensitive calcium channels in the heart and the resultant abnormal calcium kinetics may explain the adverse effects of cardiac glycosides on ventricular function in this condition (252).

Much less is known about the more common form of cardiac hypertrophy that results from high blood pressure.

1.6.2. Left ventricular hypertrophy in essential hypertension

Although a variety of factors modulate the rate or extent of myocardial hypertrophy, increased haemodynamic loading itself appears to be the primary factor responsible for compensatory hypertrophy of the pressure- or volume-overloaded heart in adults (253,254). In beating or quiescent isolated cardiac preparations, enhanced protein synthesis has been shown to result from increases in preload or afterload (255). Isolated rabbit papillary muscles have demonstrated a rapid increase in protein synthesis when stretched (256). Kent et al (257) showed that in isolated cardiac muscle of the ferret, stretch-induced activation of cation channels resulted in sodium influx, activation of the Na^+, K^+ -ATPase, and enhanced protein synthesis. The indirect effects of stretch-dependent sodium influx might include increased intracellular pH mediated by Na^+/H^+ exchanger, and increased intracellular calcium promoted by Na^+/Ca^{2+} exchanger. All these processes might contribute to cellular growth. Hormonal

stimulation by angiotensin II or noradrenaline acting through myocyte membrane receptors could also induce intracellular messengers and cause ventricular hypertrophy (258,259).

Studies with echocardiography have shown increases in left ventricular mass in young persons between 12 and 20 years of age with only borderline elevations of arterial pressure (260). Mahoney et al (261) suggested that this increase in ventricular mass in childhood may predict subsequent hypertension and its complications. Echocardiography has been especially useful in the study of left ventricular hypertrophy because it provides a wide range of information about anatomic variables (wall thickness, chamber dimension) as well as function of the ventricle during systole and diastole. Devereux and Reichek (262) have shown that left ventricular mass as determined by echocardiography in life is closely correlated with weight measured at postmortem examination. In adults with mild to moderate essential hypertension the prevalence of left ventricular hypertrophy by echocardiography is an order of magnitude greater than the prevalence found using electrocardiography. It has become clear with more sensitive methods that as many as 40 to 50% of patients with relatively mild hypertension have increased ventricular mass (263). In most of these patients function of the left ventricle at rest and measured by echocardiogram or radionuclide cineangiogram is generally normal or even supranormal (264,265).

Left ventricular hypertrophy in hypertension has beneficial and detrimental effects (266,267). Several studies have shown increased incidence of ventricular dysrhythmias in

patients with electrocardiographic left ventricular hypertrophy (268-270). Data from the Framingham study (270,271) and from the Glasgow Blood Pressure Clinic (272) have implicated left ventricular hypertrophy as an independent risk factor for cardiovascular mortality. A recent study from Glasgow by McLenachan et al (273,274) demonstrated that potentially serious ventricular arrhythmias were much more frequent in fifty treated hypertensive patients with electrocardiographic evidence of left ventricular hypertrophy than in the fifty hypertensive controls without ECG abnormalities. Patients with echocardiographic (but not electrocardiographic) evidence of left ventricular hypertrophy had more ventricular couplets and more runs of ventricular tachycardia of less than five complexes than normotensive controls. It appeared that increased ventricular mass predisposed to ventricular ectopy.

Clinical and experimental studies on regression of left ventricular hypertrophy are of particular interest. Several questions have to be asked: Can established left ventricular hypertrophy be reversed by antihypertensive drug therapy? Does such reversal reduce the risk? Is it possible to distinguish lack of progression with time from true regression (275-277)? Recent experimental and clinical evidence have demonstrated that some antihypertensive agents (eg, methyldopa, angiotensin converting enzyme inhibitors and most calcium antagonists) decrease left ventricular mass and wall thickness, whereas others (eg, diuretics, hydralazine, minoxidil and alpha-adrenergic receptor blockers) do not (278-281). It is not yet known if reducing cardiac size to

normal with pharmacological agents removes the risk associated with left ventricular hypertrophy. Studies in spontaneously hypertensive rats suggest that the proportion of collagen in the ventricular wall after regression of hypertrophy is still greater that normal (282). It is possible that antihypertensive therapy permits normal cardiac performance by inducing new forms of myocardial protein synthesis (283,284).

1.6.3. Left ventricular hypertrophy in endocrine hypertension and primary hyperparathyroidism

Cardiac hypertrophy has been reported in most series of patients with acromegaly (285-288). Extreme cardiomegaly with heart weights between 1000-1300 g has been reported in the absence of other causes of cardiac enlargement such as hypertension, valve disease and overt myocardial ischaemia (289,290). The term "acromegalic heart disease" has been used to describe this condition (291). Generalised enlargement of organs and hypertrophy of tissues is a prominent feature of acromegaly, but detailed studies by Lie and Grossman (288) showed that enlargement of the heart was sometimes disproportionate. Data from animal studies suggest that growth hormone excess causes increased DNA synthesis in cardiac myocytes and increased heart size. It is not certain whether this is a direct action of the hormone itself, an effect of somatomedin, or other mechanisms, such as ion fluxes across the cell membrane (292). A reversible action of growth hormone on cardiac contractility has also been described in man (293).

Using echocardiography, Mather et al (294) demonstrated

increased left ventricular mass but normal values for indices of left ventricular function measured by non-invasive methods. They also suggested that cardiac mass might decrease with successful treatment. Further echocardiographic studies have established that the majority of patients with acromegalic heart disease have concentric left ventricular hypertrophy. Less commonly, patients with acromegaly met the criteria for asymmetric septal hypertrophy and only very few fulfilled the criteria for hypertrophic cardiomyopathy (294,295).

Cardiac arrhythmias, including ventricular tachycardia refractory to drug treatment, have been described in acromegaly and were not improved by treatment. The latter finding is compatible with the view that fibrous tissue infiltration is responsible for dysrhythmias but increased endogenous myocardial catecholamine levels and a disease of small intramural vessels have also been implicated (291). The most recent data suggest that the impact of treatment of acromegaly upon acromegalic heart disease may be to slow its progression, but rarely to cause reversal (296).

In the case of phaeochromocytoma, it has been generally assumed that cardiac hypertrophy is the result of hypertension. However, long-term noradrenaline infusion in the adult dog, insufficient to increase systemic blood pressure, causes left ventricular hypertrophy and the angiographic and haemodynamic features very similar to hypertrophic cardiomyopathy (297). Little clinical evidence exists to suggest that the chronic catecholamine excess can cause hypertrophic cardiomyopathy, although this disorder has been described in

a patient with phaeochromocytoma (298). In contrast to cardiac abnormalities of acromegaly, the majority of reports of phaeochromocytoma-induced cardiomyopathy note that physiopathological effects are reversible after removal of the tumour (299).

Enlargement of the heart in hyperthyroidism has been recognised for many years but it is not yet clear if excess of thyroid hormone induces cardiac enlargement in human subjects without pre-existing cardiac disease. Cohen et al (300) have reported that thyroid hormone excess caused cardiac hypertrophy in mice. Conversely, thyroidectomy of young spontaneously hypertensive rats, during the prehypertensive phase, prevents the subsequent development of hypertension and causes a decrease in overall heart size and weight (301).

Thus in each of three disorders of endocrine function - growth hormone excess, catecholamine excess and thyroid hormone excess - cardiac hypertrophy may be the result of both abnormal wall stress and direct effects of growth factors. In each case hypertrophy may be preceded by changes in cellular calcium metabolism. The hypothesis that increased transmembrane calcium flux could be a common pathway in the pathogenesis of cardiac hypertrophy has been given some support by Symons et al (302) who studied cardiac hypertrophy in primary hyperparathyroidism. They described sixteen normotensive patients with primary hyperparathyroidism, of these five had hypertrophic cardiomyopathy, six had symmetric left ventricular hypertrophy and four had asymmetric septal hypertrophy. Only one patient in this group had no echocardio-

graphic abnormalities. In a further group of six patients with hypercalcaemia due to causes other than hyperparathyroidism, left ventricular wall thickness was normal in all cases. Moreover, parathyroid hormone concentration was raised in five of eighteen normocalcaemic patients with hypertrophic cardiomyopathy. The authors concluded that primary hyperparathyroidism was almost invariably associated with left ventricular hypertrophy in its various forms and that myocardial hypertrophy (including hypertrophic cardiomyopathy) was primarily associated with parathyroid hormone itself rather than with raised concentrations of extracellular calcium. As discussed previously (1.5.2.), parathyroid hormone has positive chronotropic effects on myocardial cells in culture, an action that is mimicked by calcium ionophores and blocked by verapamil (157). Parathyroid hormone has also been shown to have a positive inotropic effect on the rat heart at physiological concentrations (303). Since this action is also blocked by verapamil, it seems likely that it is related to the movement of calcium into the myocyte.

Clinical evidence for a relationship between parathyroid hormone and ventricular hypertrophy (302), together with experimental evidence that hypertrophy and disarray of the cardiac myocytes are produced by substances which disturb membrane stability, such as triac and calcium ionophore (247,304), add another dimension to the understanding of pathogenesis of idiopathic ventricular hypertrophy. There are no data on reversibility of left ventricular hypertrophy after surgical removal of parathyroid adenoma. Such clinical data might allow better understanding of the link between

parathyroid hormone and ventricular hypertrophy.

Section 7 - Aims of thesis

Two closely related studies were carried out.

Study I

1. A comparison of resting platelet cytosolic calcium $[Ca^{2+}]i$ levels in patients with untreated essential hypertension and matched control subjects.
2. An assessment of $[Ca^{2+}]i$ responses to stimulation with vasoactive peptide arginine-vasopressin.
3. An examination of the relationships between platelet-free calcium, blood pressure and left ventricular mass index.
4. Determination of platelet $[Ca^{2+}]i$ concentrations in a subgroup of hypertensive patients after eight weeks of antihypertensive treatment.
5. Correlation of intracellular calcium levels with the activity of the renin-angiotensin system and plasma levels of catecholamines, atrial natriuretic peptide and parathyroid hormone.

Study II

1. An assessment of the prevalence of high blood pressure in a group of patients with primary hyperparathyroidism.
2. Determination of left ventricular mass index by echocardiography in the same group of patients and comparison of these indices with measurements in pair-matched controls (matched for sex, age and mean arterial pressure).
3. Measurements of resting free cytosolic calcium levels in platelets in primary hyperparathyroidism and of calcium

transients after treatment with vasopressin.

4. An examination of the effects on platelet-free calcium of the renin-angiotensin system, catecholamines, phosphate, parathyroid hormone and atrial natriuretic peptide in this condition.

5. Effects of surgery on blood pressure, left ventricular mass, intracellular-free calcium and other biochemical variables.

CHAPTER 2 - METHODS

Section 1 - Patients and study design

Eighty-three patients were studied and clinical details of these are described below:

2.1.1. Description of patients with essential hypertension and design of Study I

Patients with untreated essential hypertension (WHO grades I and II) and normal control subjects were studied. There were 30 patients with essential hypertension (17 males and 13 females) aged 25-67 years, and 30 normal control subjects (11 males and 19 females) aged 31-68 years. The majority of control subjects were either members of ancillary staff of the hospital or their relatives. Patients with essential hypertension had diastolic pressures above 95 mmHg (5 mins in sitting position) at two or three clinic visits. All subjects had normal serum concentrations of potassium and creatinine, and patients with secondary hypertension were excluded. The normal subjects had diastolic pressures less than 90 mmHg and no family history of hypertension in first degree relatives. All subjects gave informed consent and the protocol was approved by the Western Infirmary Ethical Committee.

On the morning of the study, subjects attended the department after fasting from 24.00 hours. Blood pressure was measured three times in subjects seated for 5 minutes, using a Hawksley random zero sphygmomanometer. An indwelling

cannula was then inserted in a forearm vein and after 30 minutes of recumbency 40 ml of blood was taken into sodium citrate for measurement of $[Ca^{2+}]i$ in platelets. Blood was also taken for measurements of serum concentrations of total calcium, ionized calcium and albumin, and plasma concentrations of parathyroid hormone, renin, angiotensin II, aldosterone, noradrenaline and atrial natriuretic peptide. Later the same morning two-dimensional and M-mode echocardiography were performed to estimate left ventricular mass. In thirteen patients all measurements were repeated after eight weeks of treatment with the calcium channel blocker verapamil (160-240 mg daily; n=6) or the beta-adrenoreceptor blocker atenolol (50-100 mg daily; n=7).

2.1.2. Clinical assessment of patients with primary hyperparathyroidism and design of Study II

Twenty-three patients with primary hyperparathyroidism were studied (4 men and 19 women), aged 27-76 years. Five patients had a history of at least one episode of renal colic, two further patients had radiological evidence of parathyroid bone disease. In the remaining sixteen the diagnosis was made after routine calcium measurement. All patients had persistently raised serum calcium concentrations with parathyroid hormone (PTH) values that were inappropriately high. Seventeen out of twenty-three patients had parathyroidectomy. In twelve patients a single parathyroid adenoma was removed and in four others the histology of the excised glands showed evidence of hyperplasia. In one patient an adenoma was not found during the first neck

exploration and a mediastinal location was suspected. Three patients declined to have surgery and in three others surgery has been deferred. The first comparison was made with a group of thirty normal control subjects described in 2.1.1. Because 20% of patients with primary hyperparathyroidism had high blood pressure (WHO grade I and II) a further control group was defined which allowed subjects to be matched for blood pressure. Each patient with primary hyperparathyroidism was matched for age, sex and mean arterial pressure (MAP) with a normal control subject or a subject from a group of thirty untreated hypertensive patients described in 2.1.1. The age, sex and blood pressure-matched control group consisted of twenty-three subjects (4 men and 19 women), aged 34 to 65 years. All subjects were on no medication and had normal serum concentrations of potassium and creatinine. The study protocol was approved by the Western Infirmary Ethical Committee and the subjects gave informed consent.

The protocol of this study was identical to the one described above (2.1.1). In twelve patients all measurements were repeated six to twelve months after parathyroidectomy.

Section 2 - Laboratory methods

2.2.1. Free cytosolic calcium concentration in platelets

The cytosolic free calcium concentration $[Ca^{2+}]i$ in platelets was measured using Quin 2; the method is a modification of the method first described by Tsien for lymphocytes (94). Platelet rich plasma was prepared by centrifuging citrated whole blood (1 ml 3.9 per cent sodium citrate and 9 ml blood) at 250 g for 5 min at room

temperature. Platelet rich plasma was then incubated for 30 min at 37^0C with 10 μmol/l Quin 2 acetoxymethylester (Quin 2/AM, Lancaster Synthesis Ltd, Morecambe, Lancs. UK). Ethyleneglycolbis (aminoethylether) tetra-acetate (EGTA 5 mmol/l; Fluka) was added and platelet-rich plasma then centrifuged at 250 g for 10 min. The supernatant was removed and the pellet resuspended in calcium-poor medium containing: 140 mmol/l NaCl, 1 mmol/l KCl, 1 mmol/l $MgCl_2$; 10 mmol/l glucose and 20 mmol/l 4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid, titrated to pH 7.3 at 37^0C. The cells in the suspension were counted in a Coulter Counter (Technicon H-1^{TH} system) and the concentration of cells was adjusted to 1.5 - $2x10^8$/ml. Calcium in the medium was restored to 1 mmol/l by adding calcium chloride. Fluorescence was measured with a Perkin-Elmer LS-3B spectrofluorometer at 339 nm excitation and 492 nm emission wavelengths. The calibration of intracellular fluorescence as a function of $[Ca^{2+}]i$ was performed as previously described (94) and $[Ca^{2+}]i$ was calculated from the equation:

$$[Ca^{2+}]i = 115\ (F-F_{min}/F_{max}-F)$$

where 115 represents the equilibrium dissociation constant (nmol/l) and F the fluorescence of the intact cell suspension. F_{max} represents the maximum fluorescence obtained after the cells were solubilized with digitonin (50 μmol/l), (Sigma). F_{min} represents the minimal fluorescence obtained in the presence of 2 mmol/l $MnCl_2$. Manganese displaces calcium from the chelator and gives a very low fluorescence signal. The leakage of Quin 2, examined by adding 2 mmol/l $MnCl_2$ to loaded platelets, after washing and resuspending in platelet

buffer was minimal (range 0.25 - 1 per cent in 2 hours). There was no difference between platelets from normotensive and hypertensive subjects. The intra-assay variability as measured by five experiments with 10-15 separate $[Ca^{2+}]i$ estimations on the same preparation of platelet rich plasma, was less than 10% and the day-to-day intrasubject variation, as measured by 21 estimations of $[Ca^{2+}]i$ within 10 days, was 10%. $[Ca^{2+}]i$ results are expressed as the mean of triplicate measurements.

2.2.2. Effects of stimulation with arginine-vasopressin (AVP)

The response of platelet $[Ca^{2+}]i$ to AVP ($[Arg^8]$ vasopressin; Sigma) was measured. Studies of the concentration-response relationship indicated that the response was maximal at an AVP concentration of 1 µmol/l. Accordingly, this concentration was used in the comparison of responsiveness measured in cells from hypertensive subjects, patients with primary hyperparathyroidism and normal controls. This response was measured in the presence of extracellular calcium (1 mmol/l) and when extracellular calcium was reduced to negligible concentrations (10-20 nmol/l) by adding 5 mmol/l EGTA.

2.2.3. Other biochemical variables

Ionized calcium concentrations corrected to pH 7.4 were measured using a Radiometer ICA-1 ionized calcium analyser as previously described (12). Total calcium and protein were measured on a SMAC-1 (Technician Instruments Corporation, Basingstoke, UK) according to the manufacturer's protocol.

Total serum calcium values were corrected for albumin concentration using the formula:

Calcium corrected = measured calcium + 0.02 (40 - serum albumin)

Serum parathyroid hormone (PTH) was measured with a two site immunometric assay for intact (1-84) human parathyroid hormone using monoclonal antibodies (152). Plasma active renin (305), angiotensin II (306), aldosterone (307) and atrial natriuretic peptide (308) were measured by radioimmunoassay and catecholamines by a radioenzymatic method (309).

Section 3 - Left ventricular mass

2.3.1. Two dimensional and M-mode echocardiography

Echocardiography was carried out using a Hewlett-Packard Ultrasound Unit with a 2.5 - MHz transducer. All echocardiograms were recorded by one person (AFD). Patients were studied in the left decubitus position with the transducer on the third to fifth intercostal space. Simultaneous visualisation of interventricular septal thickness (IVST), left ventricular internal dimension (LVID) and posterior wall thickness (PWT) was sought, just below the tips of the mitral valve leaflets. Echocardiograms which showed unambiguous high quality images of IVST, LVID and PWT with continuous interface lines were deemed technically adequate for study. Echocardiograms of two out of thirty hypertensive subjects (6%), four out of thirty control subjects (13%), two out of twenty-three hyperparathyroid patients (9%) and one out of twenty-three controls matched for age/sex/MAP (4%) were not of

adequate quality and were excluded from further analysis. It has been previously established that it is not possible to obtain technically satisfactory echocardiograms in up to 20% of hypertensive patients (310).

M-mode recordings were made on 6-inch (15 cm) light-sensitive paper with a paper speed of 50 mm/sec. The ECG was recorded simultaneously. The two-dimensional echocardiogram image was used to position the cursor appropriately. M-mode recordings were coded and analysed without the knowledge of the classification into patients and controls. Analysis was performed using a digitising tablet (Kontron Ltd) and a microcomputer. Left ventricular posterior wall thickness, interventricular septal thickness and left ventricular diastolic diameter were all measured distal to the tips of the mitral valve leaflets and at the peak of the R wave on the electrocardiogram. The measurement points were selected using the Penn convention (262). This method excludes the thickness of endocardial echoes from measurements of IVST and PWT and includes the thickness of endocardial echoes to measurements of LVID, as shown on Figure 7. Measurements were made over three consecutive cardiac cycles, and mean values were calculated. Figure 8 shows an M-mode recording from a normal subject.

2.3.2. Calculation of left ventricular mass

The method used in this analysis was the cube formula incorporating Penn convention as described by Devereux and Reichek (262). The formula is as follows:

$$\text{LV mass (g)} = 1.04\ [(\text{IVST}+\text{PWT}+\text{LVID})^3 - (\text{LVID})^3] - 13.6$$

where:

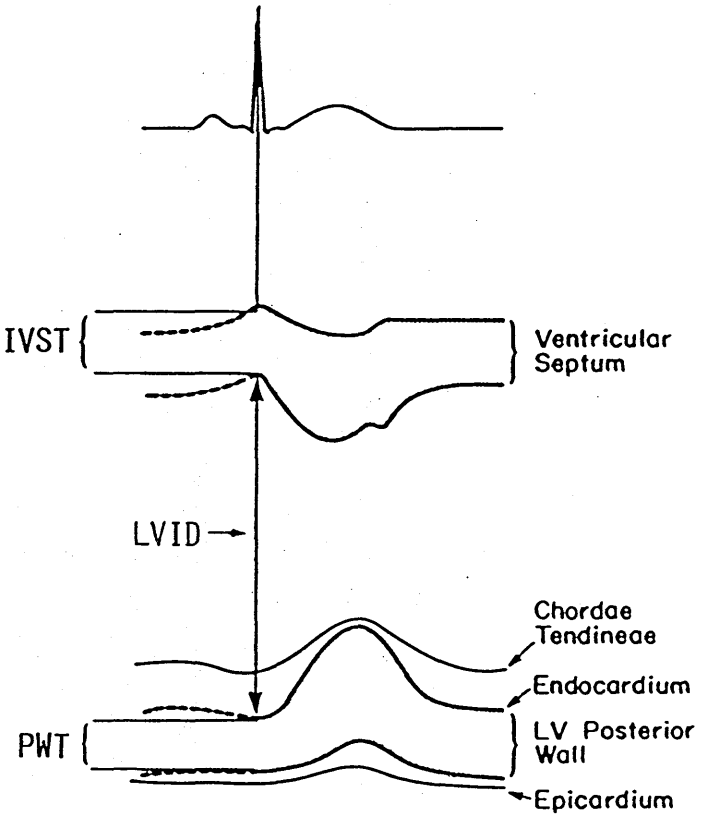


Figure 7 Schematic representation of M-mode echocardiographic left ventricular (LV) anatomic measurements (Penn convention).

Measurements are made at the peak of the electrocardiographic R wave with exclusion of endocardioal interface thickness from measurements of ventricular septal and posterior LV wall thickness. IVST = septal thickness, PWT = posterior wall thickness and LVID = left ventricular internal diameter. Modified from reference 262.

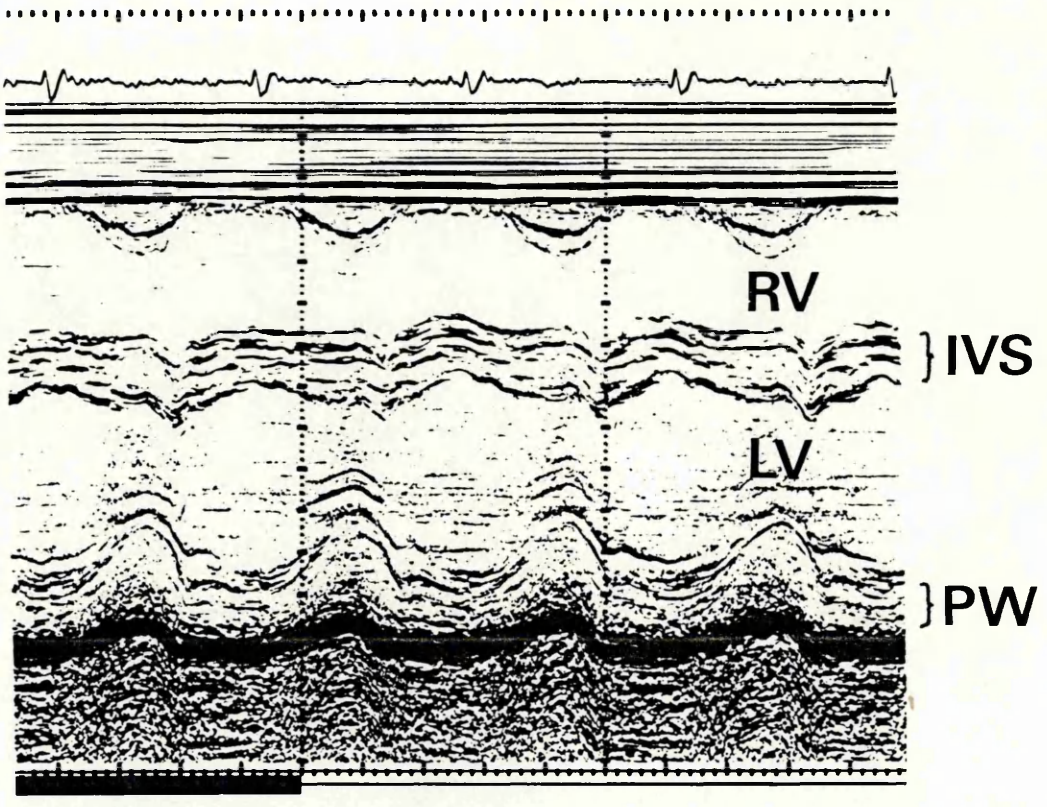


Figure 8

M-mode echocardiogram of a normal subject.

IVST = septal thickness excluding right and left ventricular endocardial echoes.

PWT = posterior wall thickness excluding endocardial and epicardial echoes.

LVID - left ventricular internal diameter including the septal and posterior wall endocardial echoes.

This method has been validated by comparison with anatomical left ventricular mass measurements (262). Measurements of left ventricular mass were then divided by body surface area to obtain left ventricular mass index (310).

$$\text{LV mass index } (g/m^2) = \frac{\text{LV mass } (g)}{\text{BSA } (m^2)}$$

2.3.3. Reproducibility of echocardiographic measurements

The reproducibility of echocardiographic measurements in a single subject was calculated by measuring left ventricular mass on ten occasions in the same healthy volunteer. The coefficient of variation was 2.9%. Interobserver variability of 4% was determined from calculations by two observers of left ventricular mass in 25 of the recordings.

Section 4 - Statistical analysis

2.4.1. Power calculation

It was estimated that twenty normal subjects and twenty hypertensive patients would be needed to give a 90% power to detect a difference in $[Ca^{2+}]i$ of 50 nmol/l at the significance level of 5%. For this calculation it was assumed that the difference in $[Ca^{2+}]i$ between hypertensive and normotensive subjects was in the range described by Erne et

al (105).

2.4.2. Statistical methods

Comparisons between groups were performed using Mann-Whitney U-test. Normally distributed variables are expressed as means, standard errors of mean and 95% confidence intervals. Other results are given as medians with 95% confidence intervals. Comparison of results before and after parathyroidectomy were made using Wilcoxon signed ranks test and correlations were calculated by the rank Spearman method. P values less than 0.05 were regarded as significant.

CHAPTER 3 - RESULTS

This chapter will be devided into two parts:

Section 1 - Results of Study I

3.1.1. Characteristics of patients with essential hypertension and control subjects

Clinical details are summarised in Table 2. The groups were well matched for age, body weight and height but there was a slight predominance of men in the hypertensive group. As expected, mean values of systolic and diastolic pressures were significantly higher in the hypertensive group. Left ventricular mass index was also significantly higher in the hypertensive patients (95% CI -55.6 to -21.8, $P<0.001$; Table 2).

Serum concentrations of total and ionized calcium, plasma concentrations of parathyroid hormone, renin, angiotensin II, aldosterone, catecholamines and atrial natriuretic peptide are summarised in Table 3. Serum concentrations of total and ionized calcium were similar in the two groups. Plasma concentrations of parathyroid hormone were significantly higher in the hypertensive group (95% CI -1 to -0.1, $P=0.013$; Table 3). Six of the hypertensive patients had low-renin essential hypertension as previously defined (311). Plasma concentrations of active renin, angiotensin II, aldosterone, atrial natriuretic peptide and adrenaline were similar in hypertensive patients and normal controls. Plasma noradrenaline concentrations were significantly higher in hypertensive subjects (95% CI -1.3 to -0.1, $P=0.02$; Table 3).

TABLE 2

Characteristics of hypertensive patients and normal controls*

	Hypertensive patients	Control subjects	95% confidence interval	P
n (sex)	30(17M/13F)	30(11M/19F)	-	-
Age (yr)	47 ± 3	49 ± 2	-4,10	0.34
Weight (kg)	75 ± 3	72 ± 2	-10,5.3	0.3
Height (cm)	168 ± 2	168 ± 2	-6,5	0.8
BSA (m^2)	1.84 ± 0.04	1.81 ± 0.03	-0.17,0.07	0.37
Systolic blood pressure (mmHg)	174 ± 3	125 ± 2	-56,-41	<0.001
Diastolic blood pressure (mmHg)	108 ± 2	78 ± 2	-34,-25	<0.001
Mean arterial pressure (MAP)	130 ± 2	94 ± 2	-40.7,-30.3	<0.001
Left ventricular mass index (g/m^2)	135 ± 5	99 ± 5	-55.6,-21.8	<0.001

* Results are given as mean ± SEM

TABLE 3

Extracellular calcium, parathyroid hormone, renin-angiotensin system and catecholamines in hypertensive patients and control subjects.*

	Hypertensive patients n=30	Control subjects n=30	95% confidence interval	P
Serum calcium corrected for albumin (mmol/l)	2.28	2.27	-0.04,0.02	0.69
Serum ionized calcium (mmol/l)	1.21	1.20	-0.05,0.04	0.54
Plasma PTH (units/l)	2.3	1.9	-1,-0.1	0.013
Plasma active renin (μ-units/l)	11	8	-6,0	0.09
Plasma angiotensin II (pmol/l)	6.6	4.7	-3,0.7	0.17
Plasma aldosterone (pmol/l)	166	166	-55,55	0.82
Plasma adrenaline (nmol/l)	0.2	0.25	-0.1,0.1	0.99
Plasma noradrenaline (nmol/l)	3.2	2.1	-1.3,-0.1	0.02
Plasma atrial natriuretic peptide (pmol/l)	38.0	39.5	-12,10	0.56

* Results are expressed as medians and 95% confidence intervals.

3.1.2. Resting and stimulated free cytosolic calcium in platelets $[Ca^{2+}]i$

The median $[Ca^{2+}]i$ in platelets in the hypertensive group was 93.3 nmol/l and values were not significantly different from those in the control group (87.8 nmol/l, 95% CI -12 to 5.8, P=0.5; Figure 9). Correlations between platelet $[Ca^{2+}]i$ and age, blood pressure, left ventricular mass index, serum concentration of calcium (total and ionized) or plasma concentration of renin were not significant. Median concentrations of $[Ca^{2+}]i$ in men and women in the control group were 92 nmol/l and 91 nmol/l, respectively (95% CI -11.7 to 11.1; P=0.62).

Preliminary experiments established that a concentration 1 umol/l of AVP caused the maximum increase of cytosolic free calcium concentrations (Figure 10). AVP caused an increase in $[Ca^{2+}]i$ in all subjects studied and representative transients obtained in platelet suspensions from hypertensive and control subjects at various extracellular calcium concentrations are shown on figure 11. In the presence of extracellular calcium (1 mmol/l) the $[Ca^{2+}]i$ increase was significantly higher in control subjects than in the hypertensive patients. In the presence of EGTA (5 mmol/l) and without added calcium, AVP at the same concentration caused much smaller increases in $[Ca^{2+}]i$ and there was then no difference between hypertensive and control subjects (Figure 12).

3.1.3. Effects of blood pressure lowering treatment

In the subgroup of patients who were treated for eight

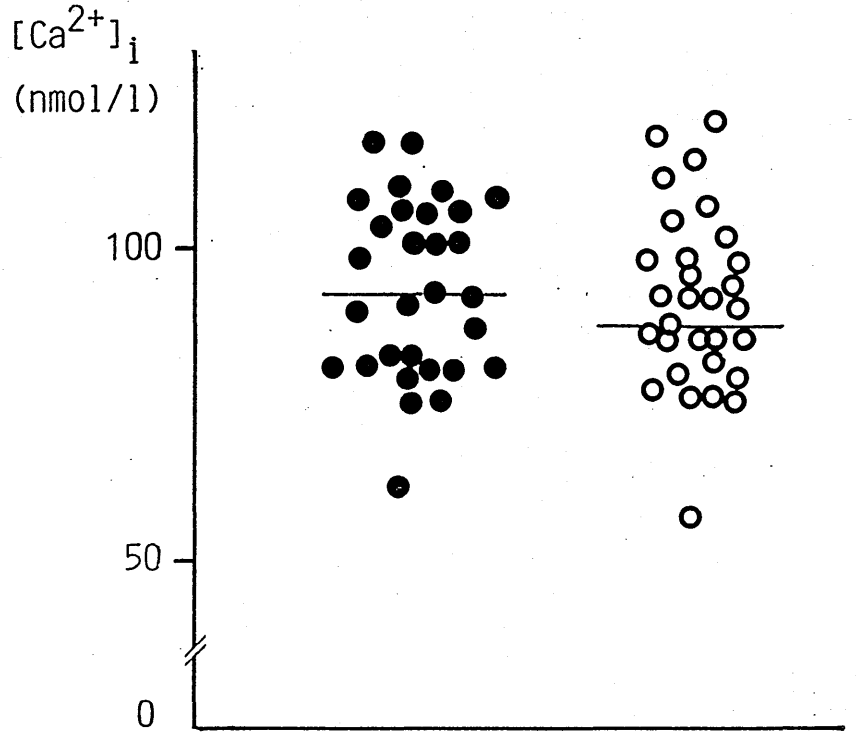


Figure 9

Scatter diagram of the $[Ca^{2+}]_i$ in 30 hypertensive patients (●) and 30 control subjects (o). Horizontal lines represent median values; 95% CI -12 to 5.8, P = 0.5.

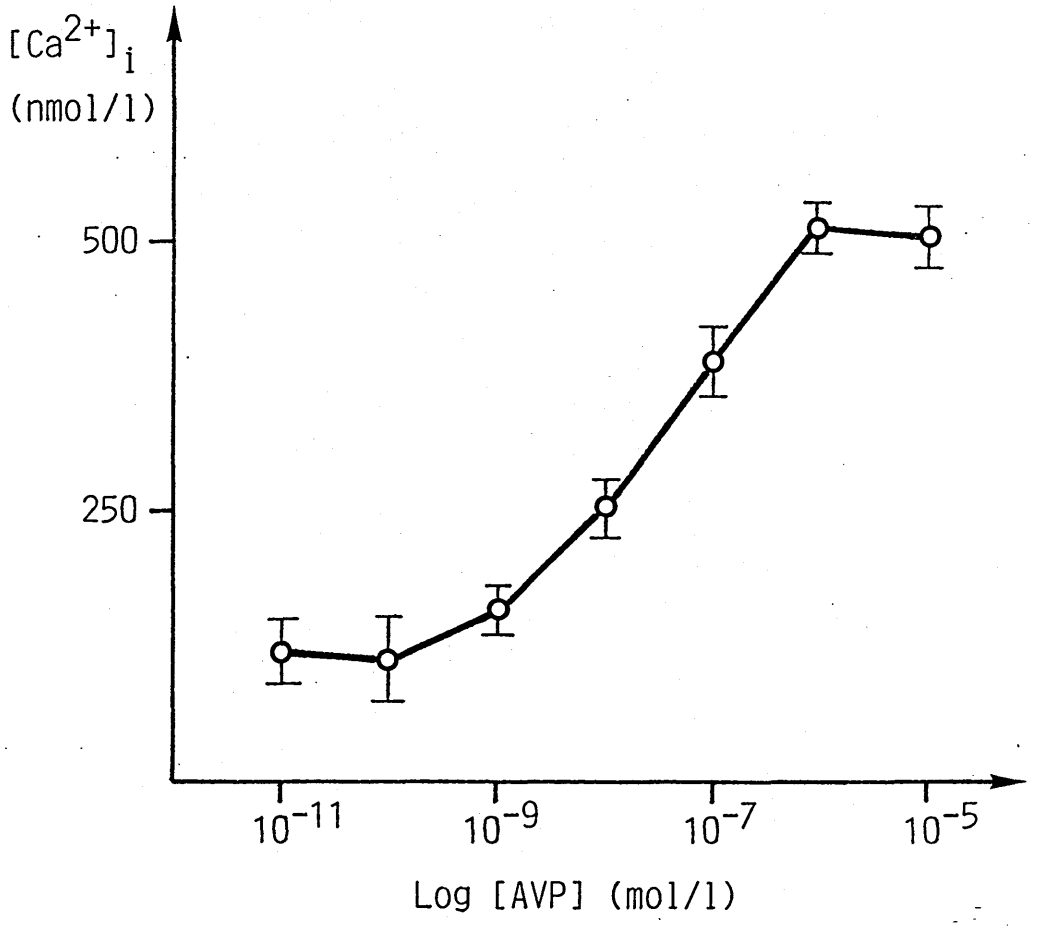


Figure 10

Concentration - response relationship between AVP and platelet $[Ca^{2+}]_i$ in cells from normal subjects in the presence of extracellular calcium (1mmol/l). Results are means of five experiments (± SEM).

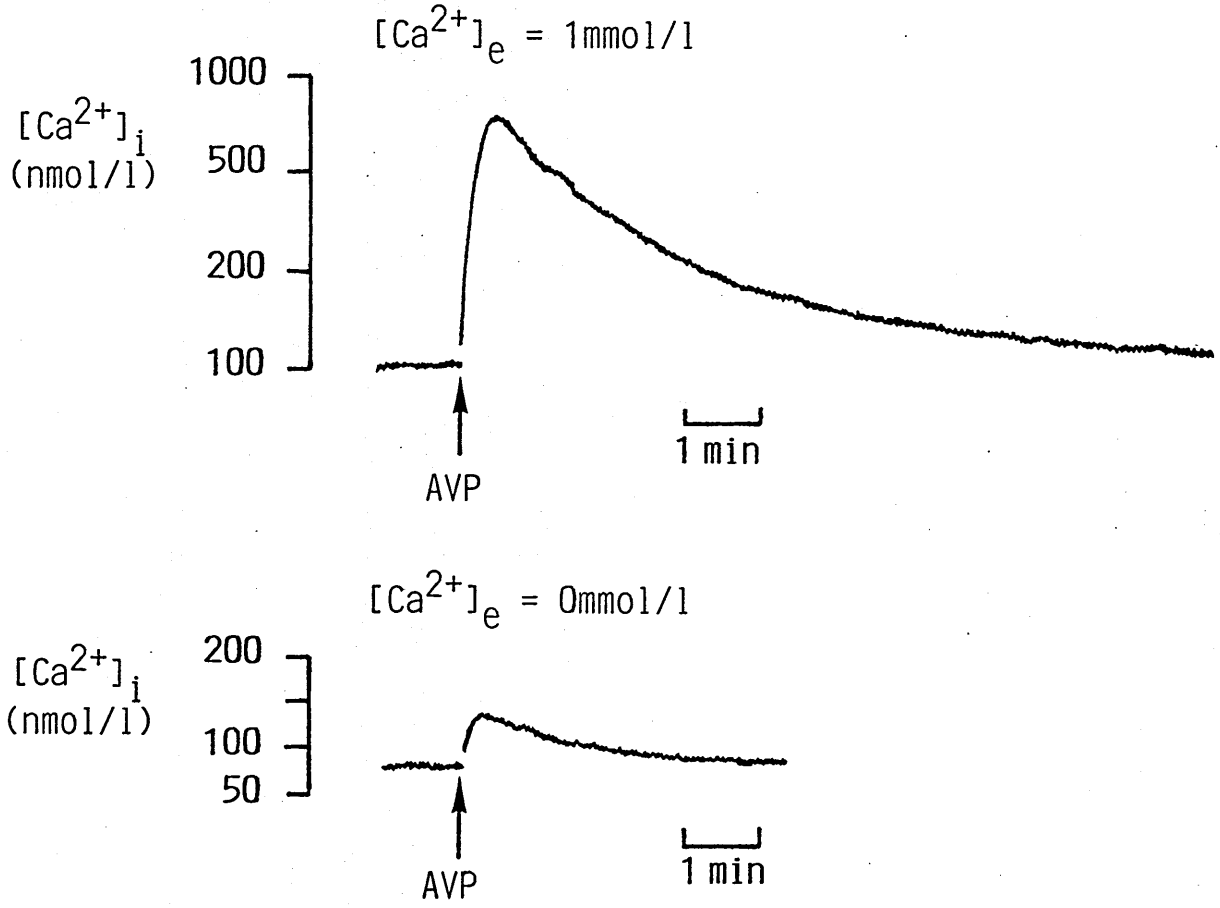


Figure 11

Effect of AVP on $[Ca^{2+}]_i$ in the presence (upper panel) and absence (lower panel) of external Ca^{2+}. AVP 10^{-6} mol/l was added as indicated. The appropriate calibration scales for $[Ca^{2+}]_i$ are shown on the left side of each fluorescence trace. Gaps in the recording show periods of addition.

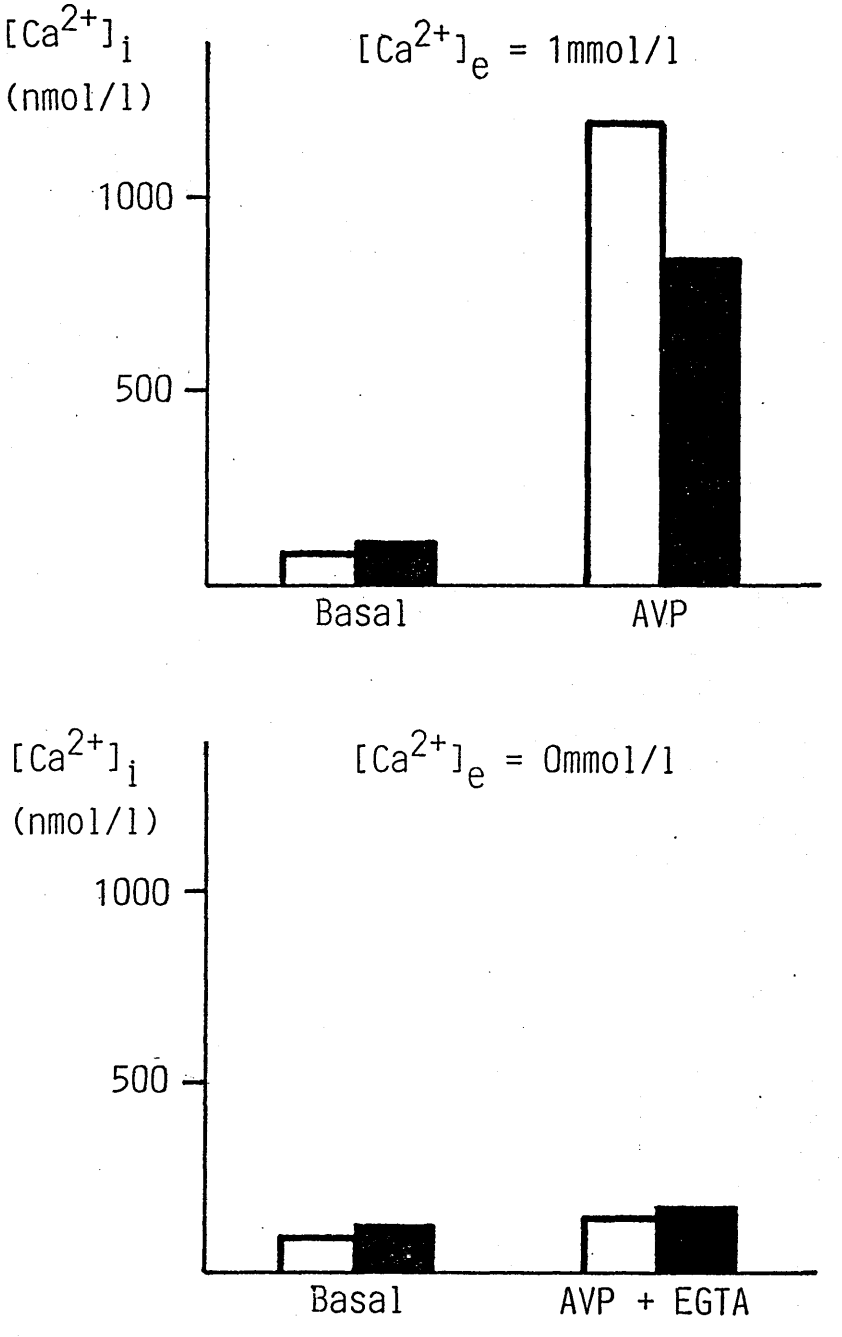


Figure 12

Comparison between the AVP-induced increase in $[Ca^{2+}]_i$ in hypertensive patients (■) and control subjects (□). Upper panel - in the presence of 1mmol/l external Ca^{2+} (95% CI 138 to 846, P = 0.004). Lower panel - in the absence of external Ca^{2+} (95% CI - 17.3 to 13, P = 0.82).

weeks with either verapamil (n=6) or atenolol (n=7), both systolic and diastolic pressures decreased significantly but this blood pressure-lowering effect did not result in changes in basal $[Ca^{2+}]i$ (Table 4). However, there was a significant positive correlation between changes of systolic pressure and changes in platelets $[Ca^{2+}]i$ for the whole group ($r=0.75$, $p<0.01$; Figure 13a). A similar but less significant positive correlation was found between changes of diastolic pressure and changes in platelet $[Ca^{2+}]i$, ($r=0.51$, $0.05 < P < 0.1$; Figure 13b). The AVP-induced increase in platelet $[Ca^{2+}]i$ was not affected by drug treatment (948 vs 1052 nmol/l, $P=0.6$). In the absence of extracellular calcium the $[Ca^{2+}]i$ transients were almost 10-fold lower, but drug treatment again had no effect: 132 nmol/l and 136 nmol/l before and after treatment, respectively.

Section 2 - Results of Study II

3.2.1. Characteristics of patients with primary hyperparathyroidism and control subjects and blood pressure results

Clinical details of 23 patients with primary hyperparathyroidism are summarised in Table 5. Two patients had asymptomatic bone disease with radiological evidence of subperiosteal bone resorption in the phalanges in one and a brown tumour in the second patient. Five patients gave a history of at least one episode of renal colic. Four out of 23 patients had a family history of hypertension in at least one first degree relative (Table 5).

TABLE 4

Effects of antihypertensive treatment on $[Ca^{2+}]i$ and blood pressure. Results from patients treated with verapamil and atenolol have been combined; n = 13.*

Variable	Value
$[Ca^{2+}]i$	
Before treatment (nmol/l)	102
After 8 weeks of treatment (nmol/l)	95
95% confidence interval	-13. 2.5
P	0.14
Systolic blood pressure	
Before treatment (mmHg)	169
After 8 weeks treatment (mmHg)	152
95% confidence interval	-29.5, -3.0
P	0.013
Diastolic blood pressure	
Before treatment (mmHg)	109
After 8 weeks treatment (mmHg)	91
95% confidence interval	-25.0, -9.5
P	0.002

*Results are given as medians and 95% confidence intervals.

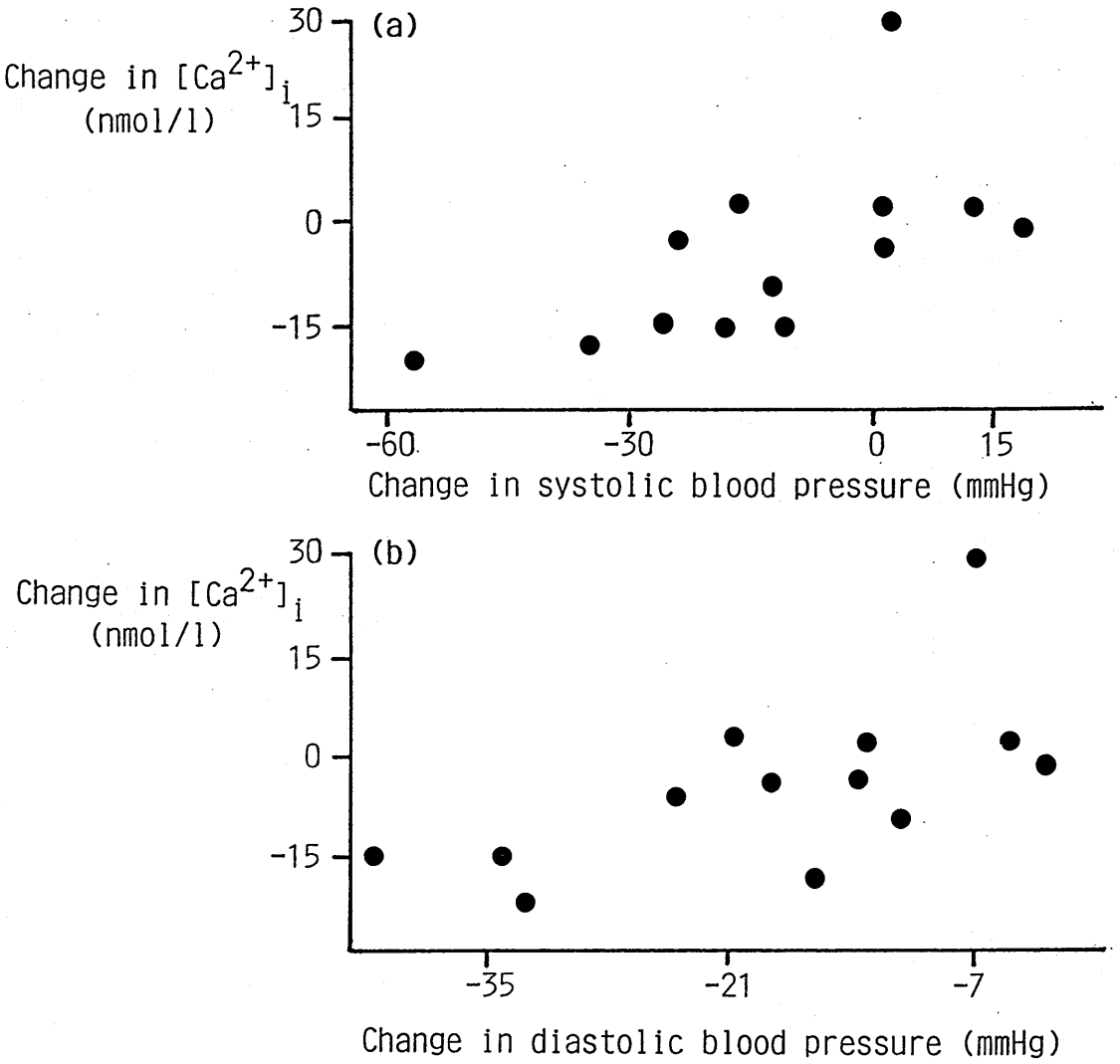


Figure 13

Correlations between changes in $[Ca^{2+}]_i$ and blood pressure observed in hypertensive patients (n = 13) during drug treatment. Results with verapamil and atenolol have been combined.

(a) Systolic blood pressure ($r = 0.75$, $P<0.01$).

(b) Diastolic blood pressure ($r = 0.51$, $0.05 <P<0.01$).

TABLE 5

Clinical findings in 23 patients with primary hyperparathyroidism

Case	Age (yrs)	Sex	Symptoms & Signs	Blood Pressure (mmHg)	Family history of hypertension	Serum calcium corrected for alb (mmol/l)	PTH (U/1)	Operative findings
1	39	F	Renal colic	134/76	-	2.64	4.7	Hyperplasia
2	53	F	-	125/92	+	2.80	8.4	Hyperplasia
3	68	F	-	150/77	-	2.82	8.6	Hyperplasia
4	62	F	-	151/87	-	3.05	16.7	Adenoma
5	64	F	-	146/116	-	2.69	3.9	Adenoma
6	33	M	-	117/89	-	2.67	8.9	Adenoma
7	45	F	-	140/87	-	3.07	4.7	Adenoma
8	31	F	Renal colic	113/76	-	2.60	11.3	Adenoma
9	33	M	-	118/81	-	2.74	5.4	Adenoma
10	43	F	-	146/92	-	2.75	3.2	-
11	52	F	-	110/64	-	2.64	11.3	Adenoma
12	27	F	-	121/61	-	2.75	2.9	-
13	59	F	-	149/75	-	2.58	6.3	-

Table 5 continued.

14	62	F	Renal colic	168/86	-	2.73	9.0	Adenoma
15	62	F	-	172/85	+	2.58	4.3	Adenoma
16	48	F	-	127/87	+	2.68	5.0	-
17	74	M	-	143/96	-	2.80	21.0	Mediastinal adenoma
18	76	F	-	129/70	Unknown	2.66	12.2	Adenoma
19	60	F	Renal colic	134/86	-	2.79	5.5	-
20	74	F	Hyperpara-thyroid bone disease	213/108	+	2.90	6.0	-
21	65	F	Renal colic	132/85	-	2.77	11.0	Adenoma
22	44	F	Hyperpara-thyroid bone disease	150/88	-	3.52	24.8	Adenoma
23	74	M	-	161/92	-	2.82	5.6	Hyperplasia

Patients with primary hyperparathyroidism were compared with a group of 30 normotensive controls who were matched for age and body weight and with a second control group which was further matched for sex and blood pressure (Table 6). The systolic, diastolic and mean arterial pressures were significantly higher in patients with primary hyperparathyroidism than in normotensive controls (Table 6, Figure 14) and five out of twenty-three patients (22%) were hypertensive using WHO criteria (grades I and II). In one patient there was a fall of 32 mmHg in systolic blood pressure eight months after parathyroidectomy, but there was no significant changes in blood pressure in the group as a whole (Figure 15).

3.2.2. Left ventricular mass index

Left ventricular (LV) mass index was significantly higher in the primary hyperparathyroid group than in either control group (Table 6, Figure 16) but did not correlate with platelet calcium, extracellular calcium, parathyroid hormone or blood pressure. However, there was a significant correlation between systolic blood pressure and left ventricular mass in the age, sex and blood pressure-matched control group ($r=0.48$, $P<0.05$, Figure 17a). A similar but less significant positive correlation was found in the same group between mean arterial pressure (MAP) and LV mass index ($r=0.4$, $P<0.1$; Figure 17b).

In twelve patients in whom LV mass index was measured six to twelve months after surgery, parathyroidectomy was associated with a small reduction in mass index of the left ventricle but this change was not significant ($P=0.1$, Figure 16).

TABLE 6

Characteristics of patients with primary hyperparathyroidism and control groups*

	1^{o} hyperparathyroid patients	Normotensive controls	P	Age/sex/MAP matched controls	P
n	23 (4M, 19F)	30 (11M, 19F)	-	23 (4M, 19F)	-
Age	54 ± 3 (SEM)	49 ± 2	0.9	54 ± 2	0.9
Weight	69 ± 3	72 ± 2	0.4	70 ± 4	0.8
Blood Pressure mmHg					
Systolic	139 ± 6	125 ± 2	0.004	141 ± 5	0.4
Diastolic	86 ± 3	78 ± 1	0.04	85 ± 3	0.8
Mean	104 ± 3	94 ± 2	0.003	104 ± 3	0.6
LV mass index g/m^2	123 ± 10	99 ± 5	0.03	100 ± 6	0.03

* Results are given as mean ± SEM

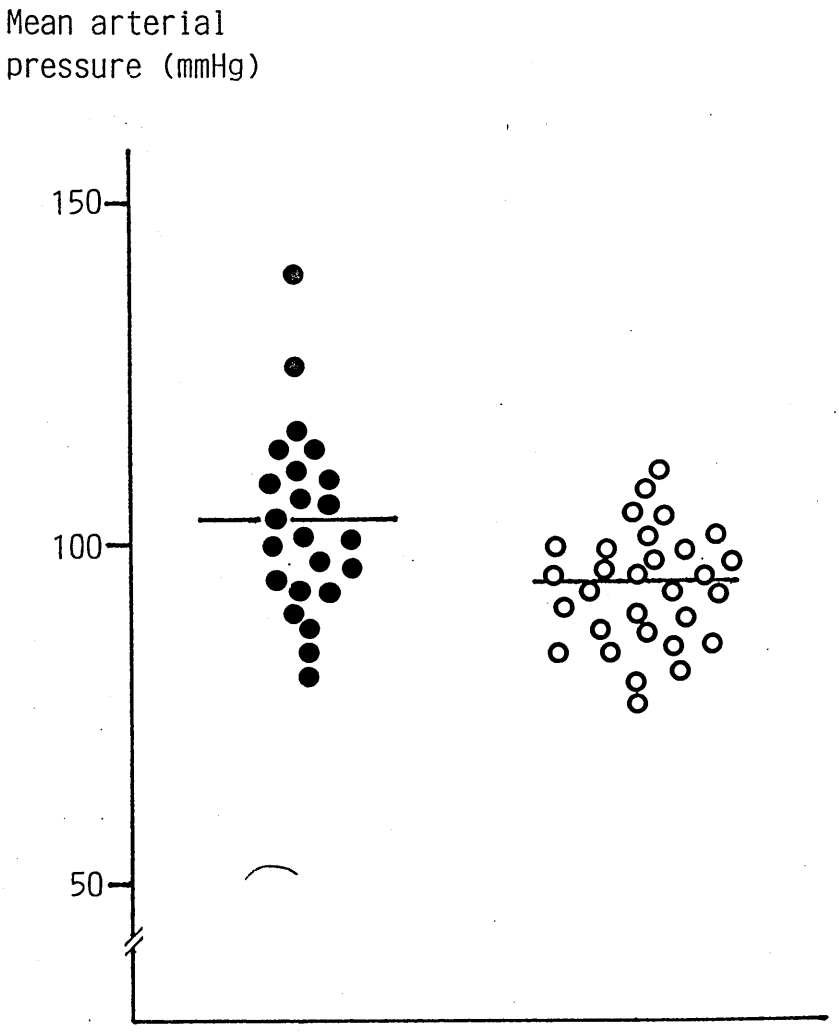


Figure 14

Scatter diagram of the mean arterial pressure in 23 patients with primary hyperparathyroidism (●) and 30 control subjects (o). Horizontal lines represent median values; 95% CI -15.3 to -3, P = 0.003.

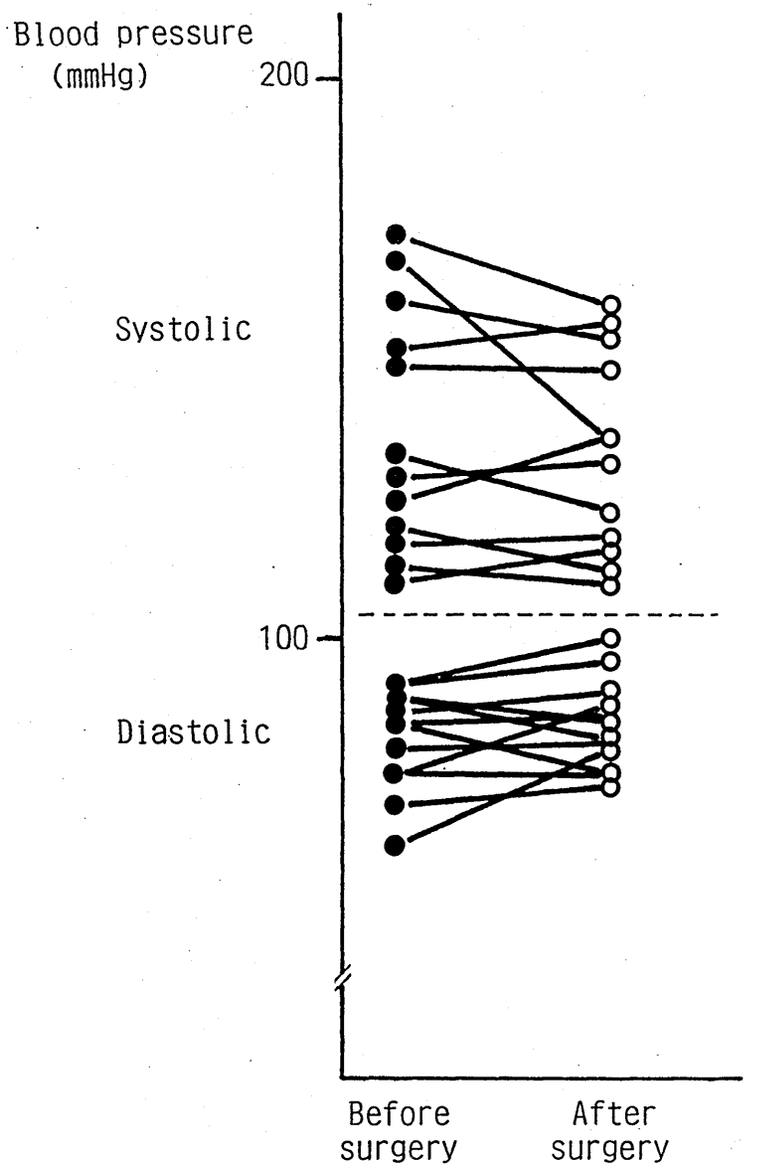


Figure 15
Blood pressure (systolic and diastolic) in 12 patients before (●) and after (o) parathyroidectomy. For systolic blood pressure: Δ = -3.3, 95% CI -10 to 3.5, P = 0.5. For diastolic blood pressure: Δ = +2.6, 95% CI -2 to 8, P = 0.33.

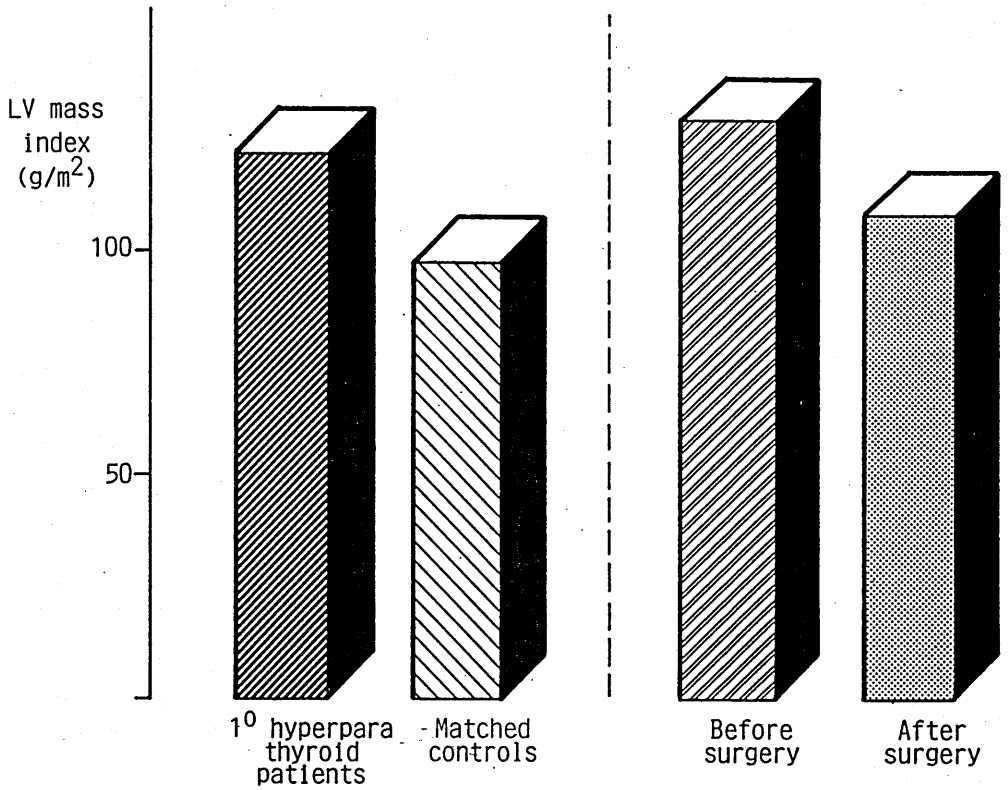


Figure 16 Left ventricular mass index

Left panel - comparison of LV mass index in 21 patients with primary hyperparathyroidism and 21 age, sex and mean arterial pressure matched controls; 95% CI -36.1 to -3.1, P = 0.03.

Right panel - comparison of LV mass index in 12 patients before and after parathyroidectomy; Δ =22.8, 95% CI -46.5 to 2.5, P = 0.1.

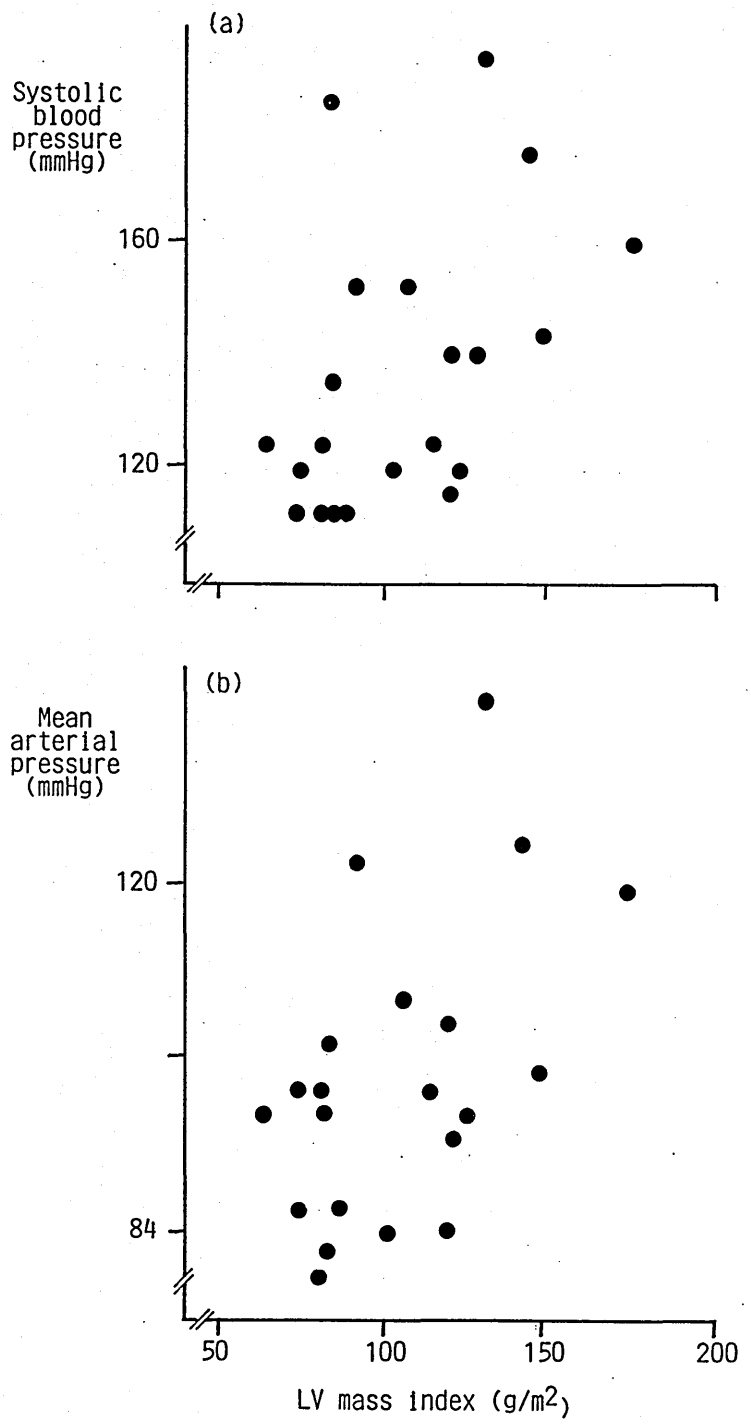


Figure 17

Correlations between blood pressure and LV mass index observed in matched control subjects.

(a) Systolic blood pressure ($r=0.48$, $P<0.05$).
(b) Mean arterial pressure ($r=0.40$, $0.05<P<0.1$).

3.2.3. Extracellular calcium, phosphate and parathyroid hormone

As expected, serum concentrations of calcium corrected for albumin, ionized calcium and plasma concentrations of parathyroid hormone were higher and serum concentrations of phosphate significantly lower in patients with primary hyperparathyroidism (Table 7, Figure 18). After parathyroid surgery these abnormal levels were corrected (Table 8).

3.2.4. Resting and stimulated free cytosolic calcium in platelets $[Ca^{2+}]i$; effects of parathyroidectomy

The median concentration of free cytosolic calcium in platelets in the hyperparathyroid group was significantly lower than in the blood pressure matched control group: 81.5 nmol/l and 93 nmol/l respectively, 95% CI 0.1 to 20.1, $P<0.05$ (Figure 19). There was an inverse correlation between $[Ca^{2+}]i$ and diastolic blood pressure in the hyperparathyroid group but not in the control group ($r=-0.46$, $P<0.05$; Figure 20). In eleven patients in whom $[Ca^{2+}]i$ was measured before and after successful parathyroidectomy there was a tendency of $[Ca^{2+}]i$ to increase at six to twelve months after surgery ($\Delta=8.9$, $P=0.068$, Figure 19).

Representative calcium transients in response to vasopressin are shown on Figure 21. The median concentrations of $[Ca^{2+}]i$ in response to AVP (1 μmol/l) were 136 nmol/l in the primary hyperparathyroid group and 1248 nmol/l in control subjects (95% CI 761 to 1551, $P<0.001$; Figure 22). This difference persisted when EGTA was added to the medium but was then much smaller: 110 nmol/l and 137 nmol/l in hyper-

TABLE 7

Extracellular calcium, phosphate and hormone concentrations*

	1° hyperparathyroid patients n=23	Age/sex/MAP matched controls n=23	95% confidence interval	P
Serum concentrations				
Calcium corrected for albumin (mmol/l)	2.73	2.29	-0.51,-0.37	<0.001
Ionized calcium	1.48	1.17	-0.38,-0.27	<0.001
Phosphate (mmol/l)	0.75	1.00	0.14,0.32	<0.001
Plasma concentrations				
PTH units/l	5.8	2.0	-6.9,-3.1	<0.001
Active renin (μ-units/l)	11.0	9.0	-7,3	0.5
Angiotensin II (pmol/l)	6.7	6.4	-3,1.8	0.6
Aldosterone (pmol/l)	222	167	-112,56	0.7
Adrenaline (nmol/l)	0.4	0.3	-0.2,0.1	0.4
Noradrenaline (nmol/l)	2.8	2.4	-1.2,0.4	0.2
Atrial natriuretic peptide (pmol/l)	40.5	34.0	-21,8	0.29

* Results expressed as medians and 95% confidence intervals

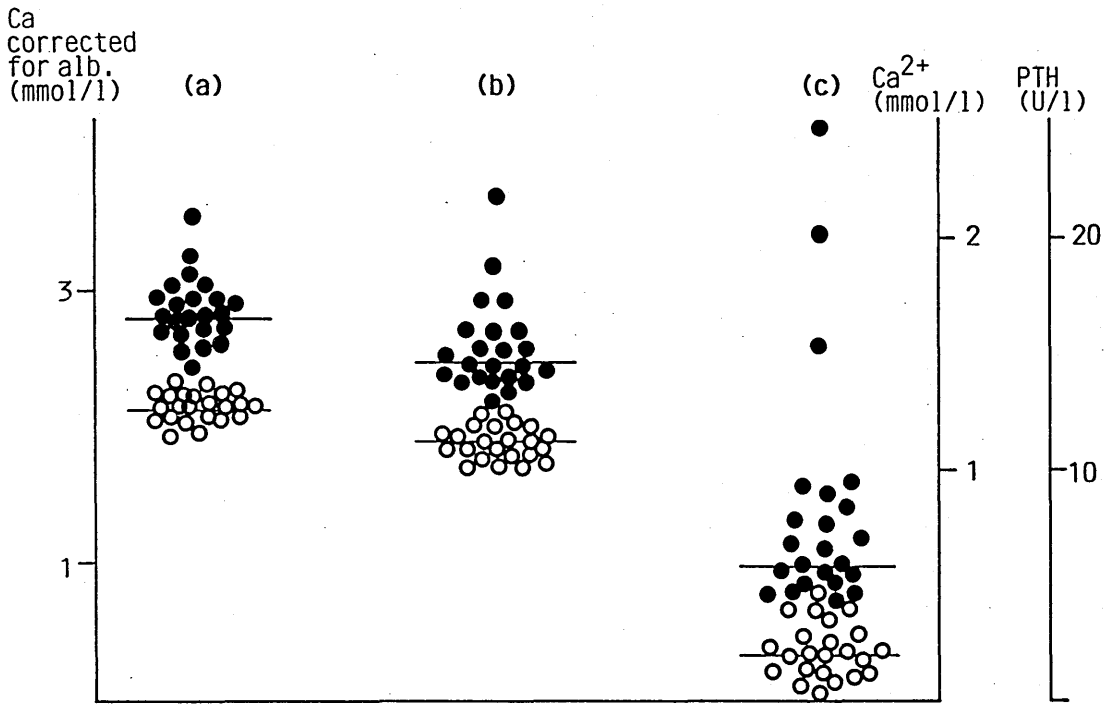


Figure 18

Scatter diagrams of the extracellular calcium and parathyroid hormone in 23 hyperparathyroid patients (●) and 23 matched control subjects (o).

(a) Calcium corrected for albumin - 95% CI -0.5 to -0.37, P<0.001.
(b) Ionized calcium (Ca^{2+}) - 95% CI -0.38 to -0.27, P<0.001.
(c) Parathyroid hormone (PTH) - 95% CI -6.9 to -3.1, P<0.001.

TABLE 8

Extracellular calcium, phosphate and hormone levels before and after parathyroidectomy*

	Untreated	After Surgery	95% confidence interval	P
Calcium corrected for albumin (mmol/l)	2.7	2.3	-0.6,-0.23	0.004
Ionized calcium (mmol/l)	1.50	1.25	-0.55,-0.27	0.004
Phosphate (mmol/l)	0.7	0.93	0.14,0.38	0.005
PTH (units/l)	8.4	3.85	-11.4,-2.2	0.006
Active renin (μ-units/l)	13.0	11.5	-12,8	0.26
Angiotensin II (pmol/l)	5.15	6.4	-1.3,3.6	0.41
Aldosterone (pmol/l)	224	308	0,280	0.042
Adrenaline (nmol/l)	0.35	0.25	-0.15,0.1	0.87
Noradrenaline (nmol/l)	2.75	2.60	-7.5,0	0.11
Atrial natiuretic peptide (pmol/l)	66	41	-48,12.5	0.31

* Results expressed as medians and 95% confidence intervals; (n=12).

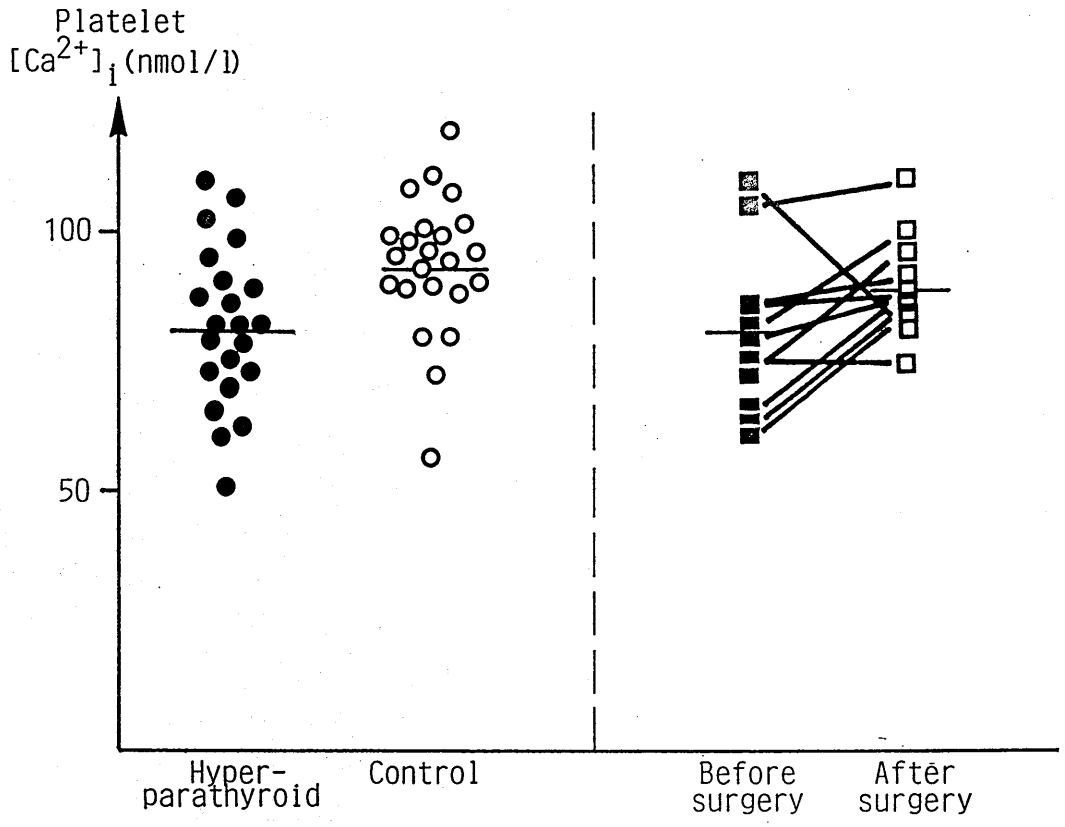


Figure 19

Left panel - scatter diagram of the $[Ca^{2+}]_i$ in hyperparathyroid patients (●) and matched controls (o). Horizontal lines represent median values. There were 22 estimations in the hyperparathyroid group, in one patient measurements were technically unsatisfactory (95% CI 0.1 to 20.1, $P<0.05$). Right panel - $[Ca^{2+}]_i$ for individual patients before (■) and after (□) parathyroidectomy. Horizontal lines represent median values ($n=11$, 95% CI -2.1 to 20.1, $P=0.068$).

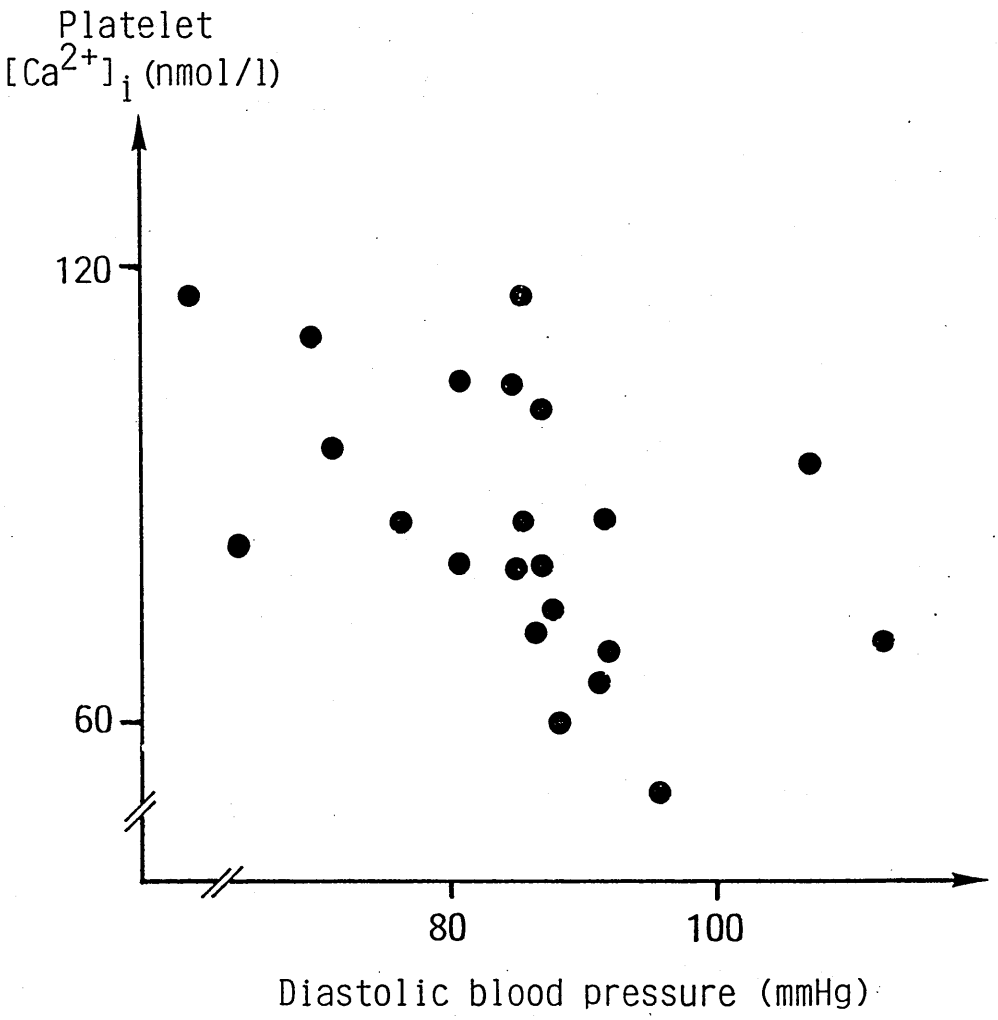


Figure 20

Relationship between platelet calcium $[Ca^{2+}]_i$ and diastolic blood pressure in the hyperparathyroid group ($n = 22$, $r=-0.46$, $P<0.05$).

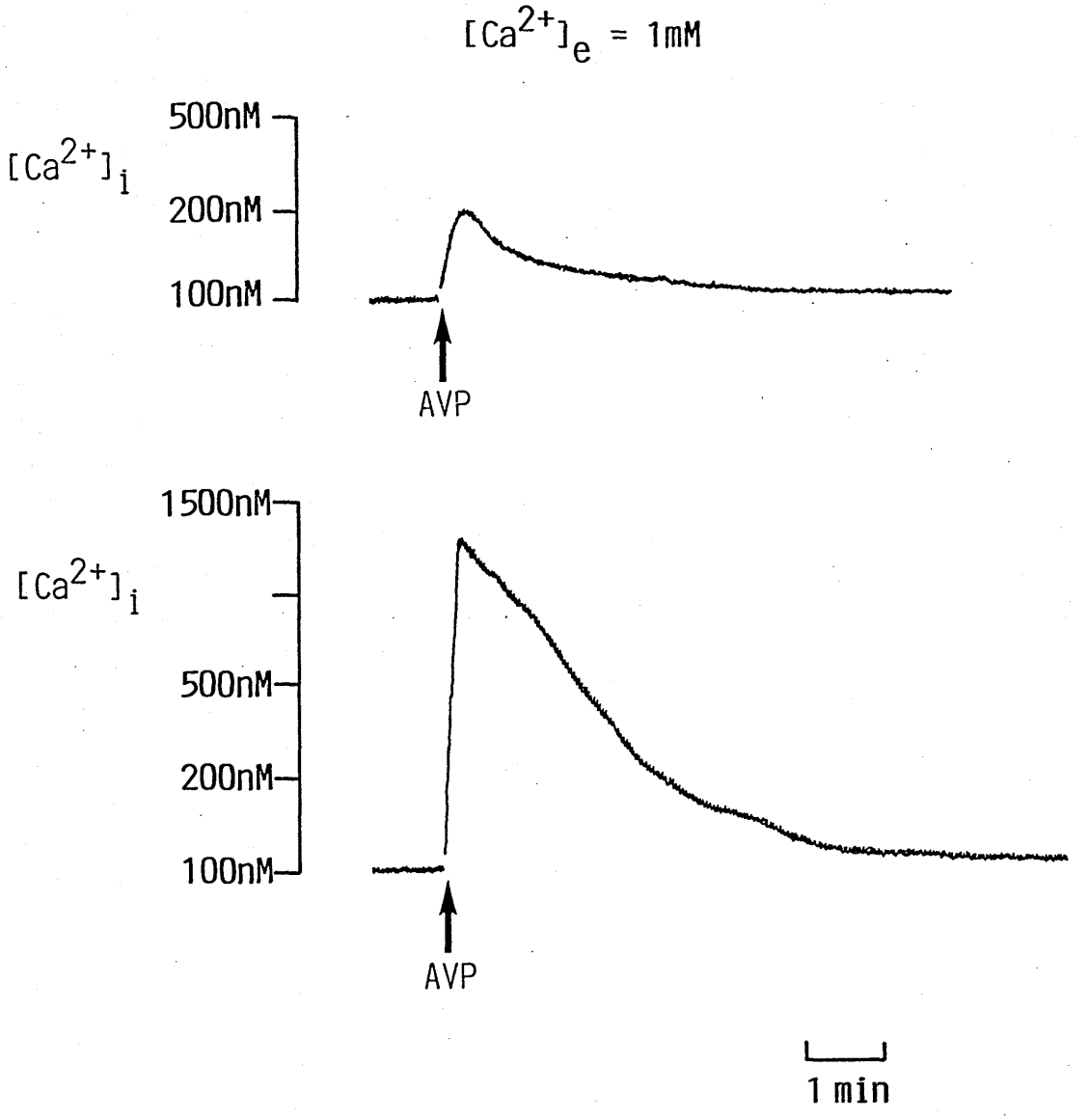


Figure 21

Effect of AVP on $[Ca^{2+}]_i$ in hyperparathyroid patients (upper panel) and matched controls (lower panel). AVP 10^{-6} mol/l was added as indicated. Extracellular calcium was 1mmol/l. The appropriate calibration scales for $[Ca^{2+}]_i$ are shown on the left side of each fluorescence trace. Gaps in the recording show periods of addition.

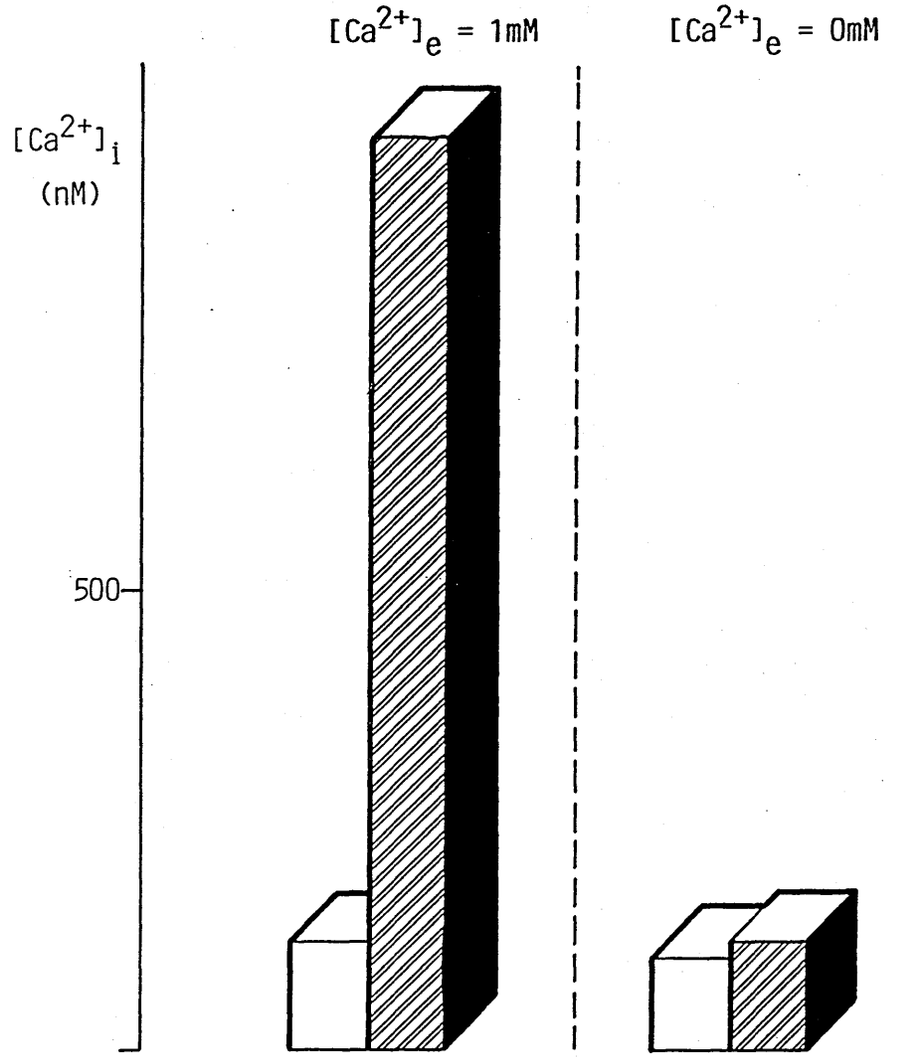


Figure 22

Comparison between the AVP-induced increase in $[Ca^{2+}]_i$ in patients with primary hyperparathyroidism (□) and matched control subjects (▨).

Left panel - in the presence of 1mmol/l external Ca^{2+} (95% CI 761 to 1551, P<0.001). Right panel - in the absence of external Ca^{2+} (95% CI 12 to 44, P=0.002).

parathyroid and control groups respectively (95% CI 12 to 44, P=0.002; Figure 22).

3.2.5. Renin-angiotensin system, catecholamines and atrial natriuretic peptide; effects of parathyroidectomy

Plasma concentrations of active renin, angiotensin II, aldosterone, adrenaline, noradrenaline and atrial natriuretic peptide were not different between the groups (Table 7, Figures 23, 24 and 25). A weak negative correlation was observed between plasma concentrations of active renin and atrial natriuretic peptide in the hyperparathyroid group ($r=-0.4$; $0.05 < P < 0.1$; Figure 26). After parathyroidectomy there was a small but significant increase in plasma concentration of aldosterone. Otherwise plasma concentrations of active renin, angiotensin II, catecholamines or atrial natriuretic peptide were similar before and after surgery (Table 8).

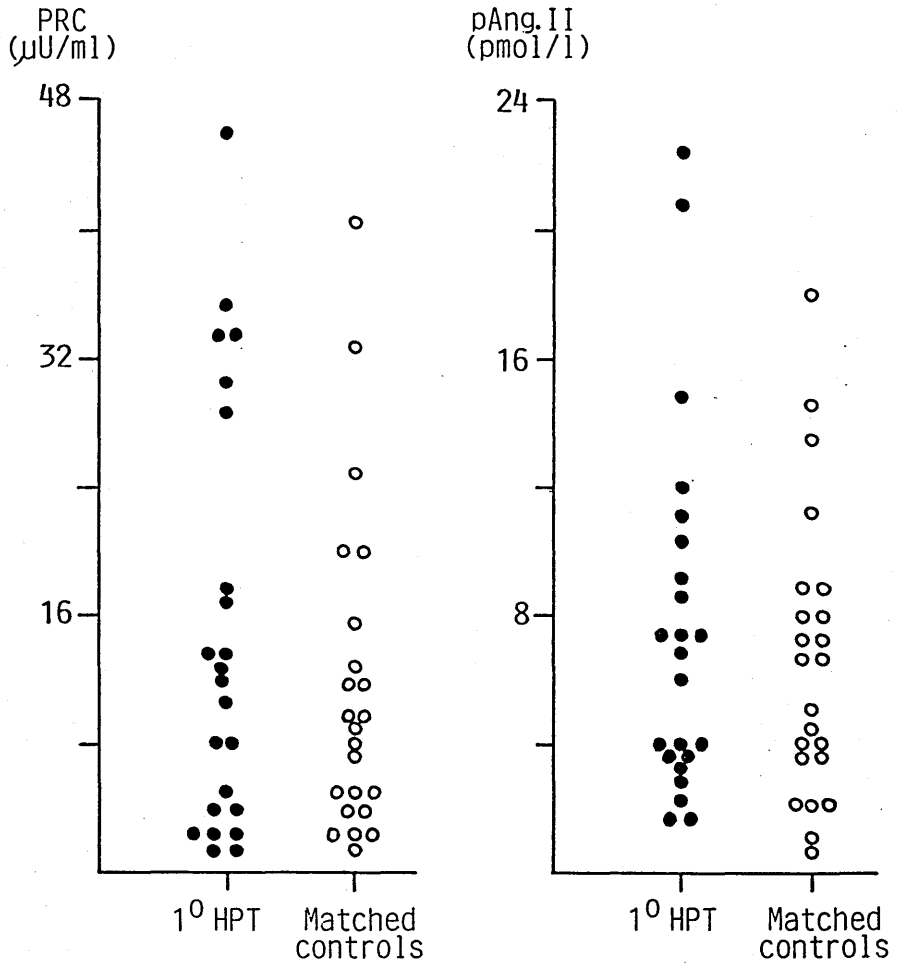


Figure 23

Scatter diagram of plasma renin concentrations (PRC) and plasma concentrations of angiotensin II (pAngII) in hyperparathyroid patients (●) and matched control subjects (o).

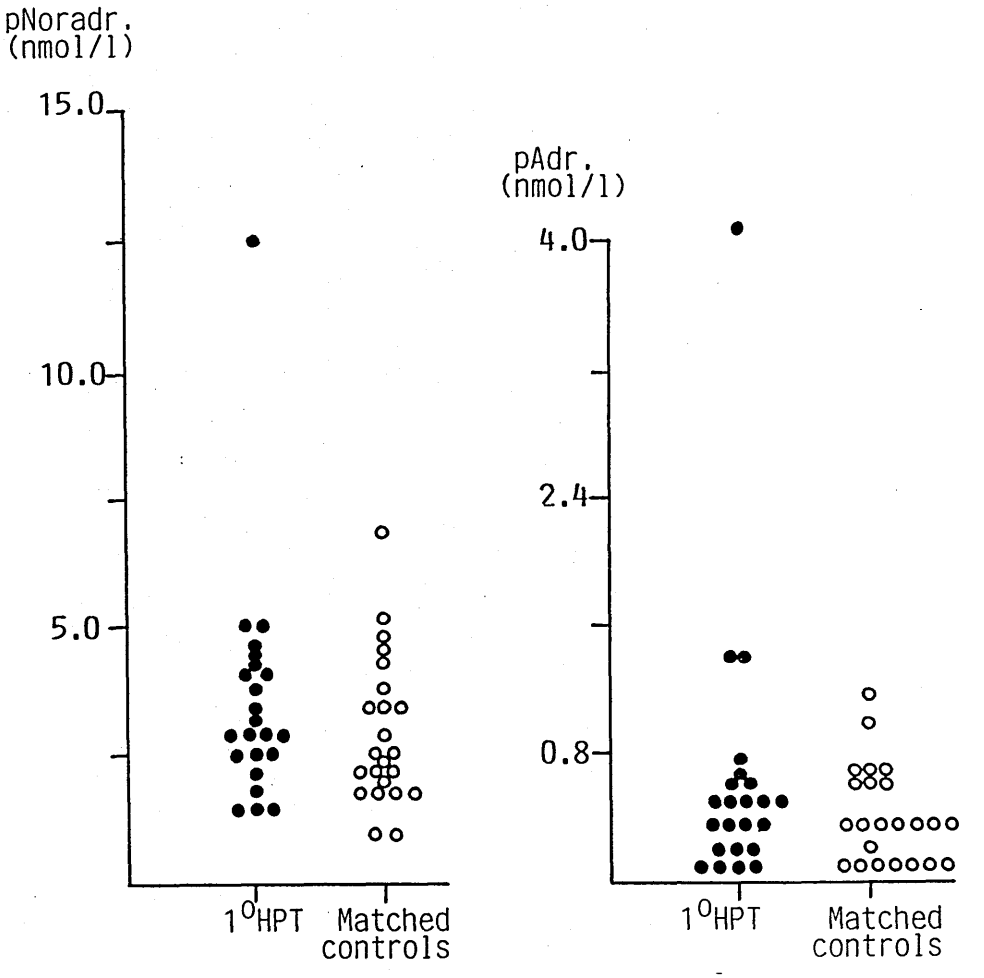


Figure 24

Scatter diagram of plasma concentrations of noradrenaline (pNoradr.) and adrenaline (pAdr.) in hyperparathyroid patients (●) and matched control subjects (o).

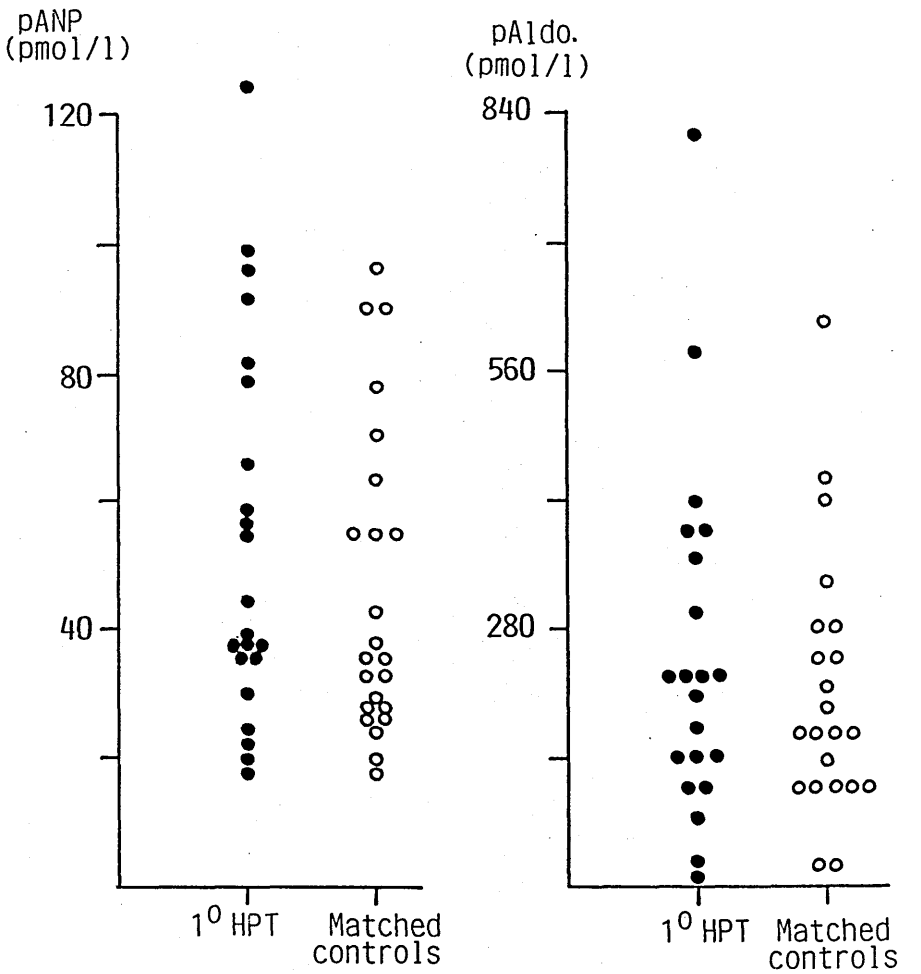


Figure 25

Scatter diagram of plasma concentrations of atrial natriuretic peptide (pANP) and aldosterone (pAldo) in hyperparathyroid patients (●) and matched control subjects (o).

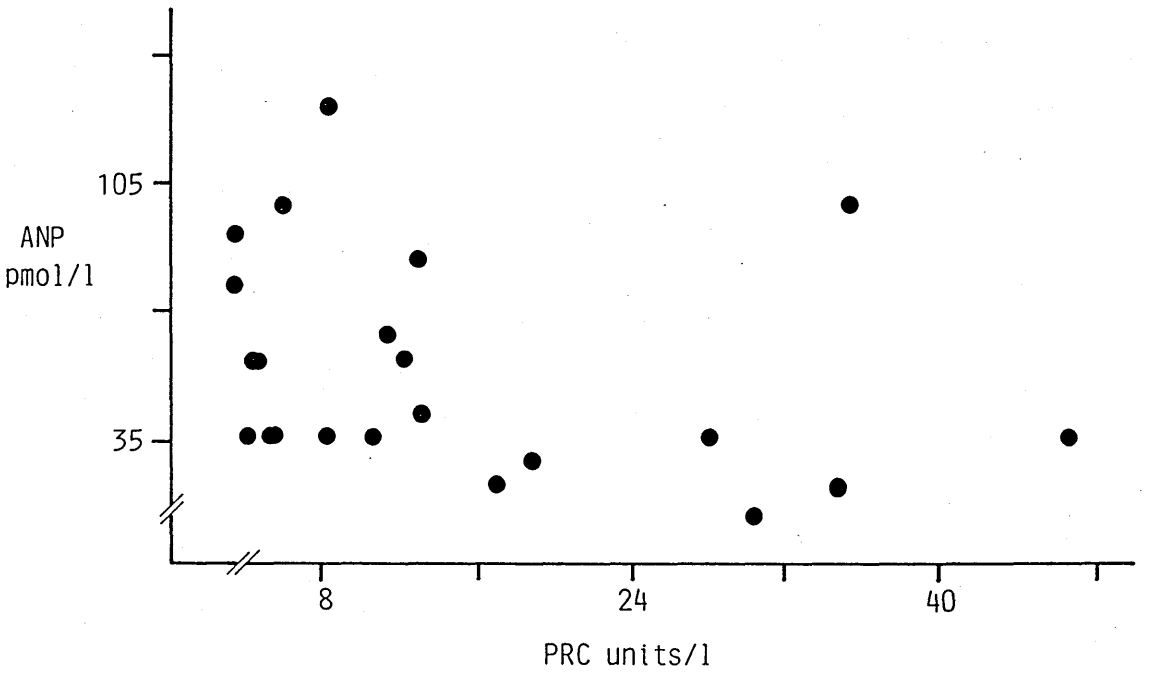


Figure 26

Relationship between atrial natriuretic peptide (ANP) and plasma renin concentration (PRC) in the hyperparathyroid group (n = 22, r=-0.4, 0.05<P<0.1).

CHAPTER 4 - DISCUSSION

Section 1 - Extracellular calcium, parathyroid hormone and plasma catecholamines in essential hypertension

4.1.1. Extracellular calcium

As found in a previous study from our laboratory (12), serum concentrations of total and ionized calcium did not differ between untreated hypertensive patients and matched control subjects. These results do not confirm the original observation by McCarron (6), but are consistent with subsequent studies (5,8,18,19). Tillman and Semple (12) reviewed possible confounding factors in ionized calcium measurements such as posture, ambulation, blood pH and diurnal variation. In contrast to some earlier studies, sampling conditions were strictly standarized with the blood collected anaerobically without stasis or forearm exercise and at the same time of the day. Patients had blood sampled via an indwelling cannula rather than by the direct venepuncture (6,8,18,19), a procedure that may be less likely to provoke hyperventilation. Mild respiratory alkalosis from overbreathing reduces serum concentration of calcium ions. It is noteworthy that earlier studies did not consider effects of treatment with some patients on thiazide diuretics which increase serum concentrations of ionized calcium (312,313).

4.1.2. Enhanced parathyroid gland activity secondary to hypercalciuria

Increased urinary calcium excretion, accompanied by enhanced parathyroid gland activity, has been reported in

hypertensive subjects (5,11,12). Strazzullo et al (5) have argued that this hypercalciuria is related to a defect in renal tubular calcium handling in essential hypertension, but reported similar levels of serum total and ionized calcium concentrations in patients and controls. It appeared that the enhanced urinary calcium output in essential hypertension was not dependent on an increased filtered load of calcium, but likely to be accounted for by a primary renal defect.

In the current study urinary calcium excretion was not measured but, as in other studies (5,11,20), plasma PTH concentrations were slightly increased in patients with essential hypertension. Parathyroid overactivity may be a compensatory response to a proximal tubular calcium leak to increase calcium reabsorption in the distal part of the nephron (5). The process of calcium reabsorption is dependent on passive entry of calcium from the tubular lumen into the cell interior down its electromechanical gradient and active extrusion at the contraluminal surface (314). Although calcium ATP-ase activity has not been measured in renal tissue, a reduced rate of calcium efflux has been found in vascular smooth muscle from SHR (315) and in red blood cells of hypertensive subjects (316). Such a defect may reflect a widespread abnormality of transmembrane calcium transport in hypertension that has been proposed by several groups of investigators (62,76,317,318). A similar defect in renal tubular cells could be the mechanism that determines the hypercalciuria.

The possible effect of increased PTH levels on blood pressure has also been considered. The development of

hypertension in rats with genetic or DOCA-induced hypertension is attenuated by parathyroidectomy (319). Grobbee et al (20) have reported increased plasma concentrations of intact (1-84) parathyroid hormone in young subjects with mildly raised blood pressures and a significant relationship between the two variables. The hypothesis that enhanced parathyroid gland activity is implicated in the development of essential hypertension has not been disproved.

4.1.3. The interrelationship of plasma catecholamines and parathyroid gland activity

The sympathetic nervous system may affect parathyroid gland activity (22). Grobbee et al observed increased concentrations of noradrenaline, adrenaline, and dopamine in a group of young subjects with mild hypertension (23) selected from the same population of patients described in reference (20). Plasma concentrations of noradrenaline but not adrenaline were significantly higher in my group of patients with essential hypertension than in normotensive controls, but patients and controls were slightly older than in the previous study. Extrapolations from venous noradrenaline levels to activity of the sympathetic system should be done cautiously (223,344), but it does seem that this small group of patients with essential hypertension had slightly increased plasma concentrations of noradrenaline and parathyroid hormone.

Direct application of PTH to cardiac myocytes increases calcium uptake (157) but its action on vascular smooth muscle cells is less well defined. Nickols (167) demonstrated that PTH relaxes aortic strips and that cAMP may be involved

in the mechanism of PTH action in vascular tissue. In acute experiments it is clear that PTH acts as a vasodilator and lowers arterial pressure (158-160). Saglikes et al (159) have shown that the pressor effects produced by bolus injections of noradrenaline and angiotensin II were reduced by PTH. The possibility that chronic effects are different cannot be ruled out.

Section 2 - Intracellular calcium in essential hypertension

4.2.1. The importance of adequate matching for age and body weight

In man, most studies on free calcium concentrations in cells have been carried out in platelets, cells that share with smooth muscle a calcium-dependent-contraction-coupling mechanism (106). Compared with some studies in primary hypertension the differences in free calcium concentration observed were quite small. The results were similar to Lenz et al (118) who showed overlap between values in normotensive and hypertensive subjects, and only a small difference between the groups. In contrast to earlier studies (105,109,116,119), in the present series patients were well matched for age and body weight. The weak correlation between weight and $[Ca^{2+}]i$ was not significant but weight reduction in obese hypertensives seems to reduce platelet free calcium concentrations (320). It is unlikely that predominance of men in the hypertensive group affected the results; there was no difference in $[Ca^{2+}]i$ between men and women in the control group and the absence of sex difference has been confirmed by

others (105,109). Degree of hypertension may be another relevant factor, although there were no obvious differences in pressure levels between current study and previous reports and indeed many of the hypertensives had evidence of left ventricular hypertrophy.

4.2.2. The relationship between platelet $[Ca^{2+}]i$ and blood pressure

A very close correlation between cytosolic free calcium concentration and diastolic blood pressure (r=0.9) was shown by Erne et al (105). Subsequent studies have shown weaker correlations (116,118,321) or lack of correlation (119). There was no correlation between $[Ca^{2+}]i$ and blood pressure or left ventricular mass in my series of patients. The current study included only patients with mild to moderate essential hypertension (WHO grade I and II). The first report (105) which showed the closest correlation between $[Ca^{2+}]i$ and blood pressure, was also the only one which included some hypertensive emergencies. This subset of patients seems to have been too small to have affected the overall results, but increased platelet turnover and thrombocytopenia is frequent in malignant hypertension (120). It is not known if intracellular free calcium in platelets is related to the age of the cell.

Pritchard et al (321) demonstrated a positive correlation between $[Ca^{2+}]i$ and mean arterial pressure in a group of 18 subjects who were either normotensive or had mild to moderate essential hypertension. The majority of patients studied by Pritchard et al (321) were on long-term blood pressure lowering treatment and the treatment had only been

withdrawn for a minimum of two weeks before sampling. The same practice of grouping together results of patients with untreated essential hypertension, and patients in whom the treatment had been discontinued for a limited period of time, has been used by other investigators (105,109,116,119). It must be doubtful if withdrawal of treatment for only two weeks is sufficient to allow the full reemergence of high blood pressure. I have included only newly diagnosed, untreated patients and this may contribute to differences in the results.

In common with other studies (105,109,321), I found significant positive correlation between changes in $[Ca^{2+}]i$ and reductions in systolic pressure during treatment. The number of subjects studied, however, was small and it was necessary to combine patients treated with different classes of drugs to obtain this result. It has been shown before that $[Ca^{2+}]i$ lowering effects are independent of the class of antihypertensive drugs used. Similar results have been obtained for calcium-channel blockers (105,109), beta-adreno-receptor blockers (105), thiazide diuretics (105) and angiotensin converting enzyme inhibitors (109). In both studies platelet free calcium decreased in parallel with blood pressure and the drugs examined appeared equipotent in this respect (105,109). Despite one report (146), which showed a lack of correlation between changes in $[Ca^{2+}]i$ and changes of blood pressure during treatment, most studies are consistent with the hypothesis that changes in platelet $[Ca^{2+}]i$ are related to changes in blood pressure.

4.2.3. Methodological aspects

Various groups have used different methods to isolate platelets and the possibility that this could influence the measurements of cell calcium should be considered. I prepared platelets by centrifugation instead of gel filtration because preliminary experiments showed that intracellular calcium assay is only stable for two hours. After this period values tend to increase, perhaps due to leakage of the dye. Nevertheless, differences in $[Ca^{2+}]i$ have been demonstrated previously using platelets prepared by centrifugation (116). It seems unlikely that methodological reasons could explain the lack of difference in platelet calcium levels between normotensive and hypertensive subjects.

4.2.4. Mechanisms involved in $[Ca^{2+}]i$ reponse to arginine vasopressin (AVP)

Interest in calcium in platelets has extended to studies of mechanisms that control free calcium levels. It has been postulated that Ca^{2+} enters the cell through two separate types of excitable channels: receptor-operated and voltage-dependent calcium channels (as described in 1.2.2. and Figure 1). In platelets there is some evidence suggesting the presence of voltage-dependent channels (56), although this has been questioned recently (324). Zschauer et al have demonstrated channels with the expected properties of receptor-operated calcium channels in thrombin-activated cells (325). The Ca^{2+} leak which causes the passive inward diffusion of Ca^{2+} in relaxed vascular smooth muscle has not yet been identified in platelets.

After platelet activation the increase in $[Ca^{2+}]i$ is

mediated by calcium influx and release of calcium from intracellular stores (56,95,119,322). A large amount of calcium is bound to or stored in the dense tubular system, plasma membrane and mitochondria. AVP, like other agonists, induces the release of calcium from the dense tubular system and this is probably mediated by hydrolysis of phosphatidyl inositols (56,322). The relative contributions to increases of $[Ca^{2+}]i$ from internal stores compared with influx vary between different cell types and agonists. Responses of $[Ca^{2+}]i$ to AVP have been studied previously, but only in platelets from normal subjects (322). The receptor for AVP on platelets is of the V_1 type, and similar to the receptor present on vascular smooth muscle (323).

The magnitude of the $[Ca^{2+}]i$ increase in response to AVP in the presence of 1 mmol/l extracellular calcium was similar to that observed by Hallam et al (322). In the absence of extracellular calcium the increase was much smaller, which suggests that the increase in $[Ca^{2+}]i$ after AVP is largely dependent on calcium influx from the extracellular space. The difference between platelets from hypertensive and control subjects was also in this extracellular component, although the sensitivity of the assay may have limited ability to detect differences in the much smaller changes recorded in the presence of a calcium chelator. It has been mentioned previously (1.3.2.) that the extra calcium buffering introduced by Quin 2 alters the pattern of evoked changes in $[Ca^{2+}]i$ (94,98,322). This may lead to an underestimation of the contribution from intracellular stores after agonist treatment (96,322). However, the intracellular calcium

concentration has been reported to be independent of the intracellular Quin 2 concentration when measured in the range 0.5-5 mmol/l (94). Previous studies which employed identical loading conditions found that the mean concentrations of Quin 2 in platelets were 1-4 mmol/l and 1-2.8 mmol/l, respectively, with no differences between cells from patients and controls (105,109).

It is possible that Ca^{2+} influx is important in replenishing intracellular stores that have been discharged by inositol trisphosphate thereby maintaining the initial calcium response, perhaps by Ca^{2+} activation of non-selective cation channels in plasma membrane (326). The evidence presented makes it unlikely that platelet calcium abnormalities in hypertension are caused by overactivity of the receptor linked phosphatidyl inositol system and its link with calcium release or entry into the cells. The results with AVP tend to be opposite to the findings of a previous study which used thrombin as an agonist (119). It is not clear if this difference reflects receptor or post-receptor mechanisms. Basal plasma AVP concentrations in patients with essential hypertension have been variously reported to be either slightly higher (327) or lower (328) than in normotensive control subjects. Inaba et al (329) found an increased density of AVP receptors on platelets from hypertensive patients. Intrinsic differences in AVP receptor density and affinity or downregulation in response to high circulating levels of vasopressin cannot be excluded. It might also be relevant that Bukoski et al (143) demonstrated depressed $[Ca^{2+}]i$ transients in response to noradrenaline in

mesenteric resistance vessels of the SHR as compared to WKY. There have been no comparable studies in human resistance vessels.

Intracellular calcium homeostasis is maintained by the enzyme Ca^{2+}-ATPase which is activated by calmodulin (65,66). Enzyme activity in platelets from hypertensive subjects seems to be altered, differences have been described in both total enzyme capacity and the calmodulin stimulated component. The second postulated calcium efflux system, Na^+/Ca^{2+} exchanger, is probably not present in platelets because inhibition of Na^+/K^+ ATPase with ouabain does not cause changes in basal or stimulated $[Ca^{2+}]i$ (119).

4.2.5. The relationship between abnormal platelet function and possible abnormalities in stimulus-response coupling pathways in essential hypertension

Platelets have receptors for many different agonists including adrenaline, noradrenaline, serotonin, vasopressin and thrombin (331). Depending on the type of receptor, stimulation either triggers changes in cAMP or promotes formation of inositol trisphosphate (IP_3) and diacylglycerol. Increased platelet calcium and diacylglycerol concentrations precipitate shape change, secretion and aggregation (331,332).

Aggregatory responses of platelets from hypertensive patients have been studied extensively and sometimes with conflicting results. Three out of eight studies found an increased aggregatory response to adenosine diphosphate (ADP) and one to noradrenaline (333-336). From a review by Nyrop

and Zweifler (337), it appeared that severity of hypertension was an important variable. Differences in aggregation were no longer apparent when mean arterial pressure was reduced below 120 mmHg with antihypertensive drug treatment (334,335). Abnormalities of platelet aggregation in hypertension may be a consequence of the vascular changes. I did not examine platelet aggregation but recent experiments from our laboratory (data not shown) suggest that changes in platelet calcium run parallel to changes in aggregatory responses. Since an increase in free calcium concentration is a necessary part of the process of activation, the higher $[Ca^{2+}]i$ concentrations in some studies may even reflect changes that occurred after blood sampling as a result of an enhanced tendency to activation and aggregation. The validity of the platelet as a model for the vascular smooth muscle cell is not yet firmly established.

Section 3 - Hypertension and primary hyperparathyroidism - an association?

4.3.1. Hyperparathyroidism as a long-term complication of the urinary calcium leak in essential hypertension

Increased parathyroid gland function is probably one of the most frequent endocrine disorders associated with hypertension. Primary hyperparathyroidism is said to be eight to ten times more frequent in patients with essential hypertension than in the general population (212,217). In addition, it has been reported that thiazide diuretics cause overt hypercalcaemia in a subgroup of hypertensive patients (213), a hypercalcaemic effect that may be related to the

level of endogenous parathyroid hormone activity (313). McCarron et al (11) have even postulated that hyperparathyroidism might be a consequence of the renal calcium leak in essential hypertension.

4.3.2. The prevalence of hypertension in primary hyperparathyroidism

In previous studies the prevalence of hypertension in primary hyperparathyroidism has been variously estimated between 10 and 70% (34,43,45,212,213). These figures must be viewed cautiously because the studies were retrospective and used different diagnostic criteria for hypertension. In the present small series of patients who were examined prospectively I found that five out of 23 patients (22%) had high blood pressure. This is similar to a recent study from Newcastle (195) where eight out of 24 patients were hypertensive. I used the Hawksley random zero sphygmomanometer which eliminates some of the observer bias that may affect measurements with the ordinary mercury instrument.

4.3.3. Does surgical cure of hyperparathyroidism also cure hypertension?

As in several other studies, parathyroid surgery did not lower blood pressure (44,195,222,225,226). This finding must be interpreted in the context of the knowledge that correction of a secondary cause of hypertension often lowers blood pressure but does not always normalize it (338). In the present series, four out of 23 patients had a family history of high blood pressure in at least one first degree relative.

Richards et al (222) found that three of six patients with high blood pressure and hyperparathyroidism had a positive family history of hypertension. The number of patients studied may be too small for definitive comments, but it appears that a subgroup of patients with high blood pressure and hyperparathyroidism may have coincidental essential hypertension.

In a meta-analysis Daniels and Goodman (34) have found that blood pressure returned to normal in 28% of patients. This subgroup may represent true hyperparathyroid-induced hypertension.

Section 4 - Why is $[Ca^{2+}]i$ lower in patients with primary hyperparathyroidism than in matched control subjects?

4.4.1. Possible role of parathyroid hormone and cyclic AMP

PTH enhances entry of calcium into many tissues (157) and might be expected to have a direct vasoconstrictor action on vascular smooth muscle cells. However, PTH itself is a vasodilator, as discussed in detail in 1.5.3. (156,159-166). The relaxant effect of PTH in vascular smooth muscle is primarily mediated by cAMP which stimulates phosphorylation of myosin light-chain kinase and lowers $[Ca^{2+}]i$ levels (167,169). Elevated serum concentrations of parathyroid hormone in hypertensive patients might tend to buffer the increased peripheral vascular resistance caused directly by hypercalcaemia. Chronic excess of PTH could also result in lowering of $[Ca^{2+}]i$ in some tissues and the platelet may be an example of this.

4.4.2. Membrane-stabilizing effect of high extracellular calcium concentration

Another explanation for the cell calcium findings might be the "membrane-stabilizing effect" of high extracellular calcium concentrations (51,62,72,74). Increasing calcium concentration from 1.6 to 4.1 mmol/l potentiates the contraction of helical strips of the rat tail artery to noradrenaline (72). Jones and Hart (74) have shown that supraphysiological concentrations of calcium ions (2.5 and 5.0 mmol/l) cause a reduction in the rate of potassium turnover in the rat aorta and the same phenomenon has also been described in lymphocytes (73). Calcium probably decreases membrane permeability to monovalent ions, alters membrane potential and thereby changes membrane permeability to calcium or release of membrane-bound ion into the cytosol. Such a process might lead to reductions in $[Ca^{2+}]i$ in platelets exposed to high extracellular calcium concentrations and may also reduce calcium influx in response to vasopressin.

It seems unlikely that the difference in $[Ca^{2+}]i$ concentrations between patients with primary hyperparathyroidism and matched control subjects was due to increased cellular calcium in subjects with essential hypertension for two reasons. Firstly, platelet calcium concentrations in the hyperparathyroid group tended to be lower than those in normotensive controls. Secondly, platelet $[Ca^{2+}]i$ concentrations were similar in patients with essential hypertension and control subjects (Study I).

Section 5 - Renin-angiotensin-aldosterone system and catecholamines in primary hyperparathyroidism

4.5.1. Renin and angiotensin II

Brinton et al (194) proposed that high plasma renin activity could be an important factor in the hypertension of hyperparathyroidism. I found similar plasma concentrations of renin and angiotensin II in patients with primary hyperparathyroidism and control subjects and no changes after surgical treatment. Similar results were obtained by Salahudeen et al (195) and Ganguly et al (221) who also showed that the responsiveness of renin to stimulation was depressed in hyperparathyroid patients. These results have not been supported by Richards et al (222) who studied eight patients before and after parathyroid surgery and did record a small fall in plasma renin activity.

Plasma concentrations of aldosterone were similar in the hyperparathyroid group and control subjects and this again confirms earlier experience (195,221). The small but significant increase in plasma aldosterone concentration after surgery may have resulted from release of a tonic inhibitory effect of hypercalcaemia (47,222). Sowers and Barrett (47) studied rats with hypercalcaemia of malignancy and showed diminished aldosterone responses to angiotensin II and potassium. An effect of calcium on the metabolic clearance of aldosterone has not been excluded (222). There was no convincing evidence that renin, angiotensin II or aldosterone were responsible for the high blood pressure in hyperparathyroidism.

4.5.2. Catecholamines and hyperparathyroidism

Release of catecholamines is calcium-dependent (22). Although Vlachakis et al (220) reported higher concentrations of catecholamines and metabolites together with increased vascular reactivity to noradrenaline in patients with hyperparathyroidism, these findings were not replicated in later studies (221,222). I found that plasma concentrations of adrenaline and noradrenaline were similar in patients with primary hyperparathyroidism and control subjects, and not affected by surgery. One patient (No 13, Table 5) had elevated plasma concentrations of noradrenaline and adrenaline at 12.4 nmol/l and 4.1 nmol/l respectively, the normal range for our laboratory being below 5 nmol/l for noradrenaline and 1 nmol/l for adrenaline. The possibility of phaeochromocytoma was raised but repeated measurements of plasma and urinary catecholamines have not confirmed this diagnosis. No reason for the isolated elevation of plasma levels of catecholamines was established.

4.5.3. The relationship between plasma renin concentration and atrial natriuretic peptide

Atrial natriuretic peptide (ANP) is released from the heart in response to increases in atrial distension (339). This mechanical stimulus is linked to hormone secretion by activation of second messengers, namely intracellular calcium and phosphoinositol system (340,341). Plasma ANP concentrations appear to be increased in some patients with essential hypertension (342) but measurements have not been reported in patients with primary hyperparathyroidism. I found that plasma ANP concentrations were similar in the

hyperparathyroid group to levels in normotensive control subjects and controls matched for blood pressure.

Richards et al (343) showed that plasma ANP concentrations increased after sodium loading and were inversely correlated with plasma concentrations of renin. Although patients in the study were on free diet, there was a weak inverse relationship between plasma concentrations of renin and ANP in the hyperparathyroid group.

Section 6 - Left ventricular hypertrophy in primary hyperparathyroidism

It has been reported that mortality from cardiovascular causes is increased in patients with primary hyperparathyroidism (211). Increased blood pressure could explain the relationship but mortality could also be related to left ventricular hypertrophy which has been described in an earlier series of patients with hyperparathyroidism (302). The excess of left ventricular hypertrophy in my patients may be related to direct actions of parathyroid hormone and/or extracellular hypercalcaemia on cardiac myocytes. An effect of differences in duration of hypertension between the two groups cannot be excluded. There was a tendency of left ventricular mass index to fall six to twelve months after parathyroid surgery. The absence of any correlation between left ventricular mass index and systolic or diastolic pressure in the hyperparathyroid group compared to the blood pressure matched control group was perhaps significant. This raises questions about the role of factors other than blood pressure in the development of left ventricular hypertrophy

of hyperparathyroidism. Parathyroid hormone and calcium are both candidates.

Left ventricular hypertrophy may be associated with an increased prevalence of complex ventricular arrhythmias and sudden cardiac death (269,273,274) but any link between primary hyperparathyroidism and these effects is speculative.

CHAPTER 5 - FINAL CONCLUSIONS

Section 1 - Study I

The first study examined platelet free calcium in essential hypertension and the interrelationships between intracellular calcium, blood pressure, left ventricular mass, levels of renin, angiotensin II and catecholamines. The following conclusions were drawn:

- Resting platelet free calcium concentrations were similar in 30 patients with essential hypertension and 30 well-matched control subjects. Close matching for age and body weight may be relevant to this result.

- Vasopressin caused a transient increase in platelet free calcium concentration, an increase that was significantly lower in the hypertensive group than the control subjects. In the absence of external calcium, vasopressin caused much smaller changes in cytosolic calcium, and there was then no difference between the responses of the two groups. Intracellular calcium increases after AVP seem to depend mainly on influx.

- In a subgroup of patients, in whom platelet calcium concentrations were measured after treatment with verapamil or atenolol, the changes in systolic pressure were correlated with changes in cytosolic free calcium concentrations. These parallel changes of $[Ca^{2+}]i$ and blood pressure lend some support to the hypothesis that changes in the platelet reflect changes in vascular

smooth muscle, but the platelet as a model for the vascular smooth muscle cell has not yet been validated.

- In untreated patients with essential hypertension platelet free calcium was not correlated with blood pressure, left ventricular mass, activity of the renin-angiotensin system or plasma concentrations of catecholamines.

Section 2 - Study II

The second study examined the relationships between cellular calcium metabolism, blood pressure and left ventricular mass in a group of patients with primary hyperparathyroidism. Other determinants of blood pressure such as the renin-angiotensin system, catecholamines and atrial natriuretic peptide were also measured. In the patients who had surgical treatment by parathyroidectomy the same variables were measured again six to twelve months after surgery. The following conclusions were drawn:

- Patients with relatively mild primary hyperparathyroidism had significantly elevated blood pressures compared with age-matched controls and 22% of patients were hypertensive (WHO grades I and II). Parathyroid surgery had no blood pressure-lowering effects and the relationship between the two conditions may be more complex than previously suspected.

- Despite chronic extracellular hypercalcaemia, intracellular free calcium concentrations and calcium transients in response to vasopressin were lower in

patients with hyperparathyroidism than in controls matched for age, sex and blood pressure. There was an inverse relationship between platelet calcium and diastolic blood pressure in the hyperparathyroid group and free cytosolic calcium concentrations tended to increase after parathyroidectomy. It appears that high external calcium and/or parathyroid hormone may lower intracellular calcium in platelets, perhaps by an effect of calcium on membrane stability or by cAMP-dependent mechanisms activated by parathyroid hormone.

- Left ventricular mass index was increased in the hyperparathyroid group as compared to control subjects matched for age, sex and blood pressure. Parathyroidectomy was associated with a small reduction of this index. There was therefore slightly more left ventricular hypertrophy than might be expected from blood pressure alone. This may reflect a direct action of calcium or PTH on cardiac myocytes.

- Plasma concentrations of the components of the renin-angiotensin system, catecholamines and atrial natriuretic peptide were similar in hyperparathyroid patients and in two control groups. Parathyroidectomy was followed by a small but significant increase in plasma aldosterone concentration, other variables remained unchanged.

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