

## Abnormal Cellular Calcium Regulation in Essential Hypertension

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**Summary:** The plasticity of cellular  $\text{Ca}^{2+}$  control and the events regulated by  $[\text{Ca}^{2+}]_i$  and other messengers make it difficult to assign causative or consequential roles to deranged platelet  $\text{Ca}^{2+}$ -linked processes in the pathophysiology of essential hypertension. Our studies in human platelets support an underlying membrane pathology as being causative since observed derangements including partial membrane depolarization and enhanced calcium influx, enhanced hormone responsiveness and coupling to adenylate cyclase, increased phosphoinositide metab-

olism, as well as stimulated  $\text{Ca}^{2+}$ -ATPase extrusion activity are membrane associated systems. Modification of phosphoinositide metabolism may be a key factor accounting for the multifaceted membrane abnormalities and eventually contribute to the elevated cytosolic  $[\text{Ca}^{2+}]_i$  concentration in essential hypertension. Whether these membrane abnormalities can also be found in human smooth muscle cells has yet to be determined.  
**Key Words:** Hypertension—Enhanced calcium influx—Enhanced hormone sensitivity.

Changes in cytosolic free calcium regulate the extent of myosin phosphorylation (1) and it is the subsequent alteration in contractile protein activity within vascular smooth muscle cells that finally determines the state of arteriolar tone and peripheral vasoconstriction (2). While elevated peripheral arteriolar tone is the hemodynamic hallmark of essential hypertension, there is no direct proof that arterial intracellular free calcium concentration is elevated in hypertensive animal or man. There is pharmacological evidence to support that in both experimental and clinical hypertension arteriolar constriction is more sensitive to calcium and that excess calcium influx-dependent vasoconstriction can be relieved by administration of calcium antagonists (3,4). Because of the difficulties involved in clinically investigating calcium metabolism in vascular smooth muscle, recent methodological developments have tended toward the investigation of calcium influx and extrusion mechanisms as well as subcellular calcium concentrations in different circulating blood cells (5,6) which are more easily accessible. Derangements in these cells may reflect

cellular abnormalities occurring in the smooth muscle cell. The present paper reviews evidence for increased calcium influx and decreased calcium extrusion in platelets from patients with essential hypertension. These observed calcium homeostatic abnormalities could explain an elevated intracellular free calcium concentration and they are suggestive of a platelet membrane calcium handling defect in patients with essential hypertension. This may account for their increased hormone sensitivity of the platelet as well as the increased risk for thromboembolic complications in hypertensive patients.

### ELEVATED INTRACELLULAR FREE CALCIUM CONCENTRATION IN ESSENTIAL HYPERTENSION

Intracellular free calcium concentration  $[\text{Ca}^{2+}]_i$  can be assessed with sensitive and nonmembrane disruptive fluorescent dye techniques (5,6) which have been used primarily in platelets and lymphocytes. Because of interference of hemoglobin with

the fluorescent dyes, this method cannot be used in erythrocytes and in these cells free calcium concentrations have been measured with ion selective electrodes (7).

As shown in Fig. 1, Quin2-assessed  $[Ca^{2+}]_i$  in platelets is elevated in patients with elevated blood pressure and there is a direct correlation between calcium concentration and the height of both systolic and diastolic blood pressure (8,9). Elevated concentrations of cytosolic calcium in circulating cells from hypertensive subjects have been confirmed for both platelets (10,11) and erythrocytes (7), while comparative analyses in lymphocytes have not yet revealed differences in free calcium concentration (10,13). A direct relationship between  $[Ca^{2+}]_i$  and the height of blood pressure has also been confirmed by Ashley et al. (13).

Intracellular free calcium in both platelets and erythrocytes has been shown to decrease with antihypertensive treatment, irrespective of the drug used (9,14). These data are suggestive of a parallel reduction in excess calcium influx dependent vasoconstriction and normalization of cytosolic free calcium concentration (15), although this relationship is apparently not directly or temporally correlated.

There are two ways of interpreting the relationship between elevated free calcium concentration and circulating blood cells and blood pressure. Firstly, the activation of platelets and erythrocytes could occur as a direct response to mechanical effects of elevated blood pressure per se. Removal of these increased shear forces by any form of blood-pressure lowering might be expected to deactivate and normalize cell function. One potential argument against such a simple interpretation is provided by the fact that platelets drawn from patients with essential hypertension during antihypertensive therapy and with a normal blood pressure have a normal  $[Ca^{2+}]_i$ ; however, they still display an enhanced  $[Ca^{2+}]_i$  response and functional sensitivity to activating hormones (16,17). An alternative explanation may be that circulating cells integrate hormonal, ionic, and pharmacological influences

that control or interfere with cardiovascular regulation. For example, different sets of membrane receptors may account for the difference observed between lymphocytes and platelets or erythrocytes. We briefly discuss possible mechanisms involved in the elevation of cytosolic free calcium concentrations.

#### ENHANCED CALCIUM INFLUX IN PLATELETS OF PATIENTS WITH ESSENTIAL HYPERTENSION

A role for calcium influx in human platelet function can be deduced from experiments demonstrating platelet activation by calcium agonists and blockade of this activation by calcium antagonist pretreatment (18). In addition, the platelet response to certain hormones is decreased in the absence of extracellular calcium. That elevated cytosolic calcium in platelets from patients with essential hypertension can only be shown in the presence of extracellular calcium is perhaps the most convincing evidence for an enhanced influx component (11). The presence of potential operated calcium channels is supported by an increase in  $^{45}Ca$  influx following membrane depolarization by KCl. Receptor operated mechanisms also involve slow calcium channel influx (19).

#### ENHANCED HORMONE SENSITIVITY AND PHOSPHOINOSITOL TURNOVER

Other platelet activators such as serotonin, vasopressin, and thrombin increase cytosolic free calcium via activation of the phosphatidylinositol turnover (Table 1) and release of calcium pools (20–22). Some comparative studies in platelets from normotensive and hypertensive individuals indicate that in the thrombin preactivated platelet adrenalin induces a greater increase in free calcium concentration in patients with essential hypertension, and that this is functionally reflected by a more sensitive contractile protein phosphorylation response as well as a more sensitive platelets shape change

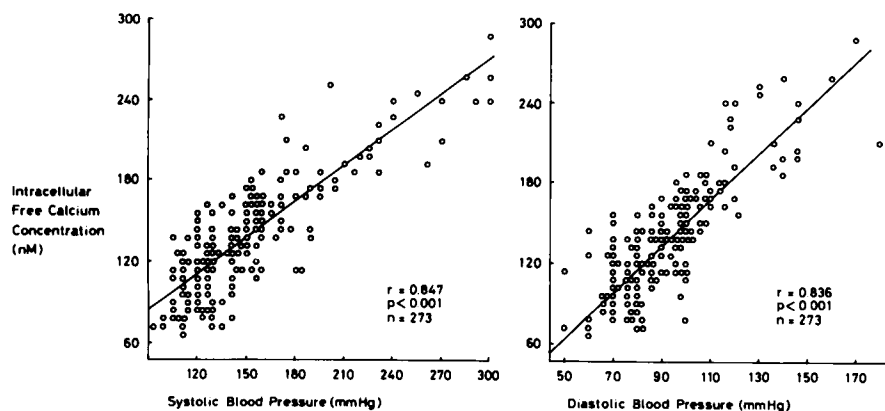


FIG. 1. Correlation between intracellular free calcium concentration in human platelets and blood pressure in normotensive and essential hypertensive patients.

**TABLE 1.** Platelet phosphoinositide metabolism in essential hypertension

	% <sup>32</sup> P-incorporation			
	PA	PI	PIP	PIP <sub>2</sub>
Normotensive subjects (n = 10)	7.1 ± 1.8	29.8 ± 4.7	32.4 ± 2.5	29.3 ± 3.3
Hypertensive patients (n = 9)	4.1 ± 1.1	5.9 ± 1.2	45.5 ± 2.3	43.3 ± 2.6
p value	NS	<0.001	<0.001	<0.01

Values represent mean ± SEM, where n = number of individuals; p values indicate significance of difference between subject groups; and NS, no significant difference.

response (16,17,23,24) (Fig. 2). These enhanced responses to hormonal activation are in accordance with a recently observed partial depolarization of platelets from patients with essential hypertension (25). It is possible that the widespread membrane defect accounts for increased calcium leakiness or passive calcium permeability in cells from hypertensive patients.

Platelet preactivation may also have direct implications for the observed reduced serotonin uptake reaction and altered uptake and efflux of noradrenaline in hypertensive patients (26–28), and may explain the high rate of thrombotic complications known in hypertensive patients (29).

#### DECREASED CALCIUM ATPase EXTRUSION ACTIVITY

Calcium ATPase activity in platelet membranes can be stimulated by calmodulin, and this is higher in patients with essential hypertension than in normotensive subjects (30,31). The absolute increase in calmodulin stimulated calcium ATPase activity was not different between normotensive and hypertensive subjects. However, the degree of calmodulin stimulation of calcium ATPase activity was reduced in patients with established essential hyper-

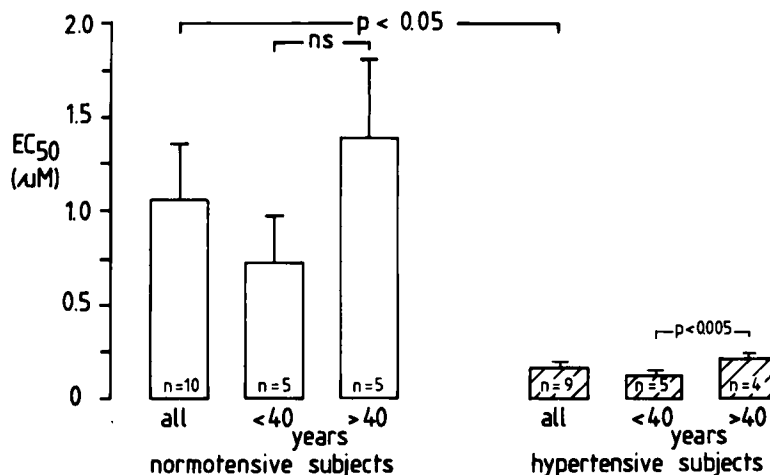
tension (30) (Fig. 3). This blunted calcium pump activity has also been demonstrated in other tissues such as erythrocytes of spontaneously hypertensive rats, cardiomyocytes and brain microsomes as well as in erythrocytes of hypertensive subjects (32). A decreased degree of calmodulin stimulation may contribute toward an elevated  $[Ca^{2+}]_i$  and the associated enhanced hormone sensitivity in platelets from essential hypertensive subjects. The elevated total calcium ATPase activity may represent an adaptive negative feedback mechanism to protect the cell against calcium overload.

#### MEMBRANE STRUCTURE AND FUNCTION ABNORMALITY IN HYPERTENSION

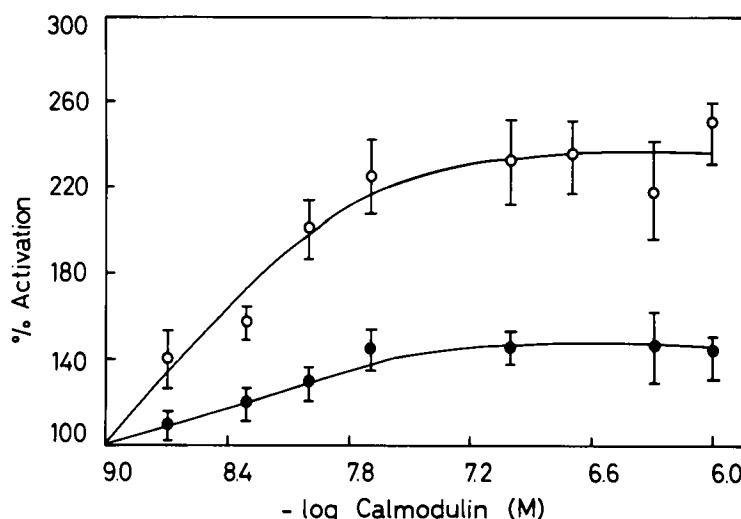
The observed enhanced calcium influx dependent mechanism and reduced calcium extrusion pump activity could both be the results of a common plasma membrane defect which has yet to be defined. The existence of such a membrane defect in hypertensive cells is supported by observations of enhanced membrane fluidity, altered concentration or reaction to calcium protein binding, and increased calcium permeability (34). It can be speculated that abnormal membrane function might be related to a different phospholipid composition or a defect in fatty acid metabolism (35). One or several genetic factors could contribute to, or potentially cause, an inherent membrane structural or functional defect of the plasma membrane.

#### CLINICAL EXPERIMENTAL CORRELATES

Both an altered calcium handling as evidenced by increased levels and other tissues (9,36)  $[Ca^{2+}]_i$  in platelets, and the excessive calcium influx dependent vasoconstriction in patients with essential hypertension, may be related to mechanisms involving excessive  $\alpha$ -1 and  $\alpha$ -2-adrenoceptor mediated as well as decreased  $\beta$ -2-adrenoceptor me-

**FIG. 2.** Platelet shape change response to adrenaline (EC<sub>50</sub> in  $\mu$ M) in normotensive and essential hypertensive subjects.

**FIG. 3.** Stimulation of platelet membrane  $\text{Ca}^{2+}$ -ATPase by calmodulin: comparison between 13 normotensive (o) and 14 essential hypertensive (o) subjects. Calmodulin-deficient membranes were assayed for  $\text{Ca}^{2+}$ -ATPase activity in the presence of varying concentrations of calmodulin. Stimulation by calmodulin is expressed relative to the activity obtained in the absence of calmodulin (taken as 100%). These values were derived from measurements of  $\text{Ca}^{2+}$ -ATPase activities (expressed as  $\text{P}_i$  released in nmol/mg membrane protein/min). Data are means  $\pm$  SEM.



diated responses (37). During antihypertensive therapy with calcium antagonists,  $\beta$ -blockers, the elevated free calcium concentration in platelets can be reduced in association with a normalization of excess calcium influx dependent vasoconstriction (15). Defects of calcium handling in platelets of essential hypertensive patients do have their hemodynamic corollary and, at least in part, explain mechanisms by which different drugs lower blood pressure.

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