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Platelet Membrane and Calcium Control Abnormalities in Essential Hypertension

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The mechanisms whereby intracellular calcium concentration is controlled are briefly reviewed. With the current knowledge of both calcium homeostasis and the function and properties of cellular Ca^{2+}-target proteins/signal transduction systems, a dysfunction of cellular calcium metabolism is considered in relation to the pathogenesis of hypertension. Although the enhanced peripheral vascular resistance characteristic of hypertension is ultimately a function of Ca^{2+} availability for smooth muscle cell contraction, the platelet possesses many parallel biochemical and physiologic properties. Therefore, we have utilized the platelet as the cell model for investigating the role of Ca^{2+} in hypertension disorders. An overview of Ca^{2+}-linked platelet processes altered in essential hypertension is presented, and an attempt is made to integrate these multiple aberrations in a fundamental membrane lesion. Am J Hypertens 1:42-46, 1988

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Calcium is a fundamental regulator of cellular function. An understanding of the general principles of cellular Ca^{2+} regulation is crucial to the pathophysiology of hypertension and its effective treatment, because the heart, adrenal glomerulosa, neural synapses, juxtaglomerular apparatus, platelets, and smooth muscle cells use Ca^{2+} as a positive intracellular messenger.

Intracellular calcium concentrations ($[Ca^{2+}]_i$) are regulated by a complex array of transport mechanisms at various membrane and intracellular locations. The entry of Ca^{2+} from the extracellular space is mediated by voltage-sensitive Ca^{2+} channels and other putative mechanisms including release of Ca^{2+} bound to membrane surfaces, entry through receptor-mediated channels, or nondefined passive leak across the membrane. Ca^{2+} is also released from internal endoplasmic reticulum and from mitochondria. In resting cells, $[Ca^{2+}]_i$ is maintained at 10^{-7} M, that is, considerably lower than millimolar extracellular free Ca^{2+}. In stimulated cells, $[Ca^{2+}]_i$ does not generally exceed 10^{-5} M. Therefore, under both conditions, internal calcium levels are necessarily maintained by the action of active transport mechanisms that remove Ca^{2+} from the cytosol, including the plasma membrane ATP-dependent Ca^{2+} pump, the plasma membrane Na^+-Ca^{2+} exchanger, the endoplasmic reticulum ATP-dependent Ca^{2+} pump, and the mitochondria Ca^{2+} pump. The net result of the operation of all these Ca^{2+}-translocating mechanisms is the imbalance of Ca^{2+} concentrations within the cells, which are therefore primed for Ca^{2+}-signaling events to permit a rapid and large increase incytosolic Ca^{2+}.[1-3]

ESSENTIAL HYPERTENSION, PLATELETS, AND CALCIUM

In the pathophysiology of essential hypertension (EHT), several factors have been proposed, including enhanced sympathetic nervous system activity, reduced renin-angiotensin-aldosterone axis endocrine control, dietary salt, and genetic factor(s). Direct clinical corollaries for an integrative contributory role for Ca^{2+} in the pathophysiology of EHT are that patients with EHT exhibit

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excess calcium-influx-dependent vasoconstriction and that blood pressure in these patients is normalized following therapy with calcium antagonists.[4-6] A key characteristic of EHT is elevated peripheral vascular resistance, which is ultimately mediated by enhanced vascular contractility. An altered state of vascular reactivity can result from alterations in either cellular calcium metabolism or the sensitivity of response elements to the actions of Ca^{2+}.[7-10] Investigations in search of support for the hypothesis that perturbation of calcium metabolism is a fundamental lesion in EHT have been carried out on a wide variety of tissue and cell types including myocardium, smooth muscle, erythrocytes, adipocytes, hepatocytes, synaptosomes, and platelets in both human and animal models of hypertension.[7-10]

A specific role for platelets in the pathophysiology of hypertension should be considered, because these cells are mediators of thrombotic complications, vectors for vascular tone, and promoters of atherosclerosis. There are many similarities between platelets and smooth muscle cells. Both platelets and smooth muscle cells have an adenylate cyclase system that can be activated by adrenaline via α-2 adrenoceptors and inhibited by prostaglandins with attendant changes in calcium.[11] Calcium can be selectively stimulated via angiotensin II receptors,[12] and calcium entry can be blocked by slow calcium channel inhibitors.[13] There are similar calcium-dependent contractile systems and similar pools for regulation of intracellular calcium (the dense tubular system in platelets and the sarcoplasmic reticulum in smooth muscle cells).[1,2] There are comparable calcium-dependent physiologic functions: shape change, aggregation and secretion in platelets, and contraction in smooth muscle cells. Hormone-receptor activation leads to parallel alterations in the clinical setting of EHT: increased sensitivity, shape change and aggregation of platelets, and increased vascular resistance of resistance vessels. These corollaries, together with easy clinical accessibility and preparative cellular homogeneity of platelets, focused our investigations on the human platelet as a model reflecting events occurring in smooth muscle cells.

It is the purpose of this paper to present a summary of present findings with respect to cellular Ca^{2+} handling in platelets from patients with EHT in search of support for the hypothesis[7-10] that a generalized membrane defect is the common denominator underlying multiple Ca^{2+}-associated abnormalities in hypertension. Our own specific observations are summarized in Table 1.

CYTOSOLIC FREE CALCIUM CONCENTRATIONS

Intracellular calcium content has been demonstrated to be elevated in platelets, erythrocytes, and lymphocytes from patients with EHT.[7-10,14-16] A correlation between platelet $[Ca^{2+}]_i$ and blood pressure has also been established.[14] Normalization of platelet $[Ca^{2+}]_i$ occurs following antihypertensive therapy.[17] Whatever the underlying mechanism, it is not entirely corrected by antihypertensive therapy, because platelets from treated EHT patients still exhibit an amplified, stimulated $[Ca^{2+}]_i$ response relative to control subjects.[17] These data imply an intrinsic cellular defect and possibly disturbed mechanisms, including membrane Ca^{2+} binding, Ca^{2+} influx, hormone-receptor transduction coupling, univalent cation transport, and Ca^{2+} efflux/sequestration.[7-10]

TABLE 1. PLATELET Ca^{2+}-LINKED ABNORMALITIES IN ESSENTIAL HYPERTENSION

System	Directional Alteration	Consequence
Cytosolic free calcium	Increased[11,14,17]	Cell activation promoted
Membrane potential	Decreased[21]	Ca^{2+} influx increased
(Ca^{2+}-calmodulin)-dependent ATPase	Capacity increased but activation potential decreased[24]	Compensatory but inefficient stimulated Ca^{2+} extrusion
Hormone (receptor) sensitivity	Increased[43]	Potentiation of stimulation
Phosphoinositide metabolism	Phosphoinositide compositional equilibrium shifted toward polyphosphoinositide formation[37]	Potential for internal Ca^{2+} release increased; modification of membrane-associated processes
Adenylate cyclase	Activity ratio (stimulated/basal) increased	Compensatory

A brief summary of our findings with respect to EHT and disorders of platelet systems that involve Ca^{2+}. The directional nature of alterations is indicated, and other than proposed compensatory mechanisms, all modifications are considered to facilitate platelet shape change, aggregation, and secretion. Similar abnormalities may contribute to enhanced smooth muscle contraction in EHT.

MEMBRANE Ca^{2+} BINDING AND Ca^{2+} INFLUX

Calcium binds to various components of the cell membrane, including anionic phospholipids and proteins. Altered calcium handling is suggested by decreased Ca^{2+} binding to the inner and outer surface of plasma membranes of erythrocytes, adipocytes, and hepatocytes,[7-10] perhaps due to a reduction in the number of Ca^{2+}-binding sites (as opposed to an affinity alteration).[18] Defective calcium binding has been proposed to favor depolarization with consequent activation of potential operated calcium channels.[8] While studies of

the function of potential dependent Ca^{2+} channels in hypertension are scarce, available data for synaptosomes and platelets of SHR[19,20] and platelets of EHT[21] indicate partial depolarization of plasma membranes. Such an abnormality could give rise to an increased basal Ca^{2+} influx and hence increased $[Ca^{2+}]_i$.

PROTEIN Ca^{2+} BINDING AND Ca^{2+} EFFLUX

Cytoplasmic divalent cation-binding substances have also been considered in the pathogenesis of hypertension,[7-10] but most of them (ie, glycerophosphates, nucleotides, and inorganic phosphates) are characterized by K_D values that are two to three orders of magnitude larger than the range of possible changes in $[Ca^{2+}]_i$. Rather, focus has been on the highly selective Ca^{2+}-binding proteins (K_D 10^{-6} to 10^{-8} M) such as calmodulin, which plays a central role in the Ca^{2+}-dependent regulation of eukaryotic cells.[22] In EHT, a modification of interaction between calmodulin and its target proteins has been proposed.[7,10] Of the many target proteins, the plasma membrane Ca^{2+}-ATPase is of major interest because of its critical role in maintaining Ca^{2+} homeostasis via the promotion of Ca^{2+} efflux.[23] The findings with respect to this efflux system in hypertension are somewhat varied, with reports of increased, decreased, or unaltered activities.[7,24-28] The regulation of Ca^{2+}-ATPase, however, is complex, and in addition to Ca^{2+} and calmodulin other factors such as membrane hydrophobicity, acidic phospholipids, polyphosphoinositides, and proteolysis can modulate Ca^{2+} efflux activity.[7-23]

PHOSPHOINOSITIDE METABOLISM

Phosphoinositides may influence not only the plasma membrane but also internal membranes. Stimulated Ca^{2+} release from large stores in the endoplasmic reticulum is believed to be mediated by inositol 1,4,5 trisphosphate, a product of hormone-activated phosphoinositide hydrolysis.[30-34] Phosphoinositide metabolism is also implicated in the regulation of Ca^{2+} influx/efflux via the plasma membrane, membrane fluidity, membrane Ca^{2+} binding, and membrane Ca^{2+}-ATPase and adenylate cyclase activities.[29-33] α_1-adrenoceptors are directly coupled to phosphoinositide turnover (as are vasopressin and angiotensin II receptors), while α_2-adrenoceptors are coupled to adenylate cyclase inhibition.[34] Postreceptor stimulus-coupling cascades are not mutually exclusive, and pivotal integrated cellular control is mediated via a triangle of second messengers, namely Ca^{2+}, cyclic AMP, and inositol 1,4,5 trisphosphate. Available evidence from studies on erythrocytes and platelets in animal and human models of hypertension point to the involvement of the phosphoinositides.[35-39] Similar investigations of smooth muscle cells, which exhibit many more blood pressure regulating hormone receptors than the platelets or erythrocytes, are necessary. The relationship between alterations in phosphoinositide metabolism and membrane-associated biochemical processes requires definition in order to assign causative or consequential roles in the pathogenesis of hypertension.

Ca^{2+} AND CYCLIC NUCLEOTIDES

Cyclic nucleotides are involved in the regulation of heart contractility, vascular smooth muscle tone, release and action of catecholamines, and control of renin secretion.[40] Most functions are regulated (either synergistically or antagonistically) by Ca^{2+} and cyclic AMP.[40] Accordingly, the finding of several anomalies in cyclic nucleotide metabolism in SHR and EHT is not surprising,[41-43] although many of the findings are qualitatively discrepant.

Of particular interest is adenylate cyclase, a membrane-bound enzyme that regulates the synthesis of cyclic AMP via stimulatory (N_s) or inhibitory (N_i) guanine nucleotide–binding proteins.[44] N_i is regulated by protein kinase C, a Ca^{2+}-activated, diacylglycerol-modulated enzyme.[29,45] Adenylate cyclase can also be activated by calmodulin and low concentrations of Ca^{2+} (> 0.8 μM) in many cells, including platelets and smooth muscle; higher Ca^{2+} concentrations are inhibitory.[46,47] Cyclic AMP in turn may influence $[Ca^{2+}]_i$ by promoting Ca^{2+} efflux/sequestration.[40,48] Thus, derangements in either phosphoinositide, Ca^{2+}, and/or cyclic AMP metabolism may be expected to have profound and manifold effects on cell function.

CONCLUDING REMARKS

In the past few years our studies on alterations in calcium and cyclic nucleotide metabolism in EHT have been confined to platelets. The complexities of both cellular Ca^{2+} control and coordinated second-messenger regulation of cellular function make it difficult to assign causative or consequential roles to deranged platelet Ca^{2+}-linked processes in the pathophysiology of essential hypertension. Nevertheless, our studies support an underlying membrane pathology as being causative because all the derangements described above—potential, Ca^{2+}-ATPase, hormone responsiveness, adenylate cyclase, and event cytosolic $[Ca^{2+}]_i$ concentrations—are membrane-associated systems. Modifications of phosphoinositide metabolism may be a key factor accounting for the multifaceted membrane abnormalities associated with EHT. The studies need to be extended to the smooth muscle cell itself to determine whether similar abnormalities are present in the vasculature and relevant to elevated peripheral vascular resistance in hypertension.

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