Membrane Handling of Calcium in Essential Hypertension

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The relationship between cytosolic free Ca^{++} and increase in blood pressure may hold the key to unravel the causes and origin of essential hypertension. In 20, essential hypertensive patients and 25 normotensive controls, total serum calcium, serum ionized calcium and free erythrocyte intracellular Ca^{++}, were measured.

For calcium loading, Ionophore A23187 was used. Intracelluar Ca^{++} was measured by ion selective electrodes, which showed a highly significant increase in hypertensive patients as compared to normotensive controls. [1.00±0.11 versus 0.72±0.06mmol/L]. While total serum calcium showed no significant difference, serum ionized Ca^{++} was significantly decreased in hypertensives as compared to controls. [1.028±0.11 versus 1.22±0.13mmol/L].

The present results confirm an association between elevated erythrocyte Ca^{++} and essential hypertension. If generalized, the defect could lead to raised intracellular Ca^{++} in smooth muscle causing increased vascular tone and arterial hypertension.

Key words: Cytosolic Ca^{++}, altered calcium metabolism, essential hypertension

Essential hypertension is a disorder characterized by increased peripheral resistance. Two processes may contribute either singly or jointly to this disturbance: increased vasoconstrictor tone of the resistance vessels and increased thickening of the vessel wall[1].

Previously, essential hypertension was related to a primary abnormality in Na+ metabolism[2]. Studies, however, revealed a number of cell membrane abnormalities. Agonist induced hydrolysis of membrane phosphatidyl inositol 4, 5, bisphosphate generates 1,2 diacylglycerol which via activation of protein-kinase-C enhances Na+-H+ antiport. This causes elevation of intracellular pH which via inositol 1,4,5 triphosphate releases calcium from intracellular stores and causes Ca^{++} influx via the Ca^{++} channels[3]. This rise in calcium causes elevation of the enzyme myosin light chain kinase which couples excitation to contraction leading to vasoconstruction[4]

Other cell membrane abnormalities include: an increase in plasma membrane permeability to Na+ which via the Na+-Ca++ exchanger raises intracellular Ca++; a decrease in calcium binding to the inner side of the plasma membrane; a reduced efficiency of the Mg++ dependent Ca++-ATPase pump (5), all of which are responsible for raising cytosolic Ca++.

Since Ca++ is the major determinant of the peripheral vascular tone, changes in intracellular Ca++ may be involved in the pathoaetiology of essential hypertension. Altered Ca++ metabolism resulting in increased basal cytosolic Ca++ has been reported in many cell types from hypertensive patients as well as spontaneously hypertensive rats[6].

The easy accessibility of erythrocytes and alterations in cellular regulation of ions has made them the primary choice to study the role of ions at cellular level in essential hypertension. Results are then extrapolated to smooth muscle in resistance vessels of humans[7]

Objectives:

The newer concepts of altered cell membrane behavior in essential hypertension was the basic drive to design this study with the objectives of..

1. To establish the correlation of calcium channel studies as a baseline effective physioaetiological mechanism of essential hypertension.
2. To correlate the different possibilities of Ca^{++}channel transports across the smooth muscle cell membrane in essential hypertension by a model design study of erythrocytes.
3. To broaden the spectrum for future therapeutic use of Ca^{++}-channel blockers on multiple modes of ionic Ca movement in essential hypertension.

Patients and Methods:

The control sample consisting of 25 normotensive individuals both male and female with ages between 23-62 years were obtained from the Bio Chemistry Laboratories of Punjab University and Fatima Jinnah Medical College. Lahore Inclusion criteria for the control sample was the blood pressure reading of <= 135/85 mmHg on three different occasions. The history performa and clinical examination performa was evaluated for the exclusion of any systematic or metabolic disorder[8].

The hypertensive group consisted of 20 patients both male and female with ages between 25-60 years. They were identified by screening the different patients attending the hypertensive clinic and outpatient department of Sir Ganga Ram Hospital Lahore. Inclusion criteria was patients with mild to moderate hypertension (Mild BP= <160/100 mm Hg, Moderate=<180/110 mm Hg[9].

Patients were labeled as hypertensives when their mean B.P. of three readings on three separate occasions fell in the above mentioned range. Secondary hypertension was excluded by history performa, clinical evaluation performa and laboratory investigation on blood for urea

and creatinine, urine for glucose and albumin and abdominal ultrasound for kidneys. No person was on antihypertensive medication at the time of study. If drugs had been used, a washout of two weeks was given prior to examination. Women were denied contraceptive medication.

Methodology:

Calcium influx and outflux.

Principle:

The compound Calcium Ionophore III A23187 in the presence of Ca^{++} ions evokes a rapid Ca^{++} influx without changing the fluxes of Na+ and K+. This high cation permeability is stopped by washing cells in bovine serum albumin. Inosine was then used to study the extrusion magnitude of Ca^{++}. MgCl2 was used to stop the reaction at various intervals. This technique was first used by Sarkadi et.al[10] and was modified by Wehling et.al[11].

1. *Calcium Loading of RBC by ionophore A23187:*
   8.0 ml of freshly drawn heparanized blood was washed three times in isotonic saline and packed RBC were mixed with solution containing calcium chloride and Ionophore A23187. After 2 minutes, cells were washed three times with 0.5 % bovine serum albumin chilled at 0 C and centrifuged in a refrigerated centrifuge.
2. *Calcium extrusion from Calcium loaded RBCs:*
   Immediately after the last wash out of ionophore RBCs were suspended in inosine. Reaction was stopped at various intervals by addition of ice cold $MgCl_2$ and subsequent three washing of each sample was done three times in $MgCl_2$. After centrifugation RBC pellets were immediately frozen at –18 C.
3. *Electrolyte Determination:*
   Cells were haemolyzed upon bringing them to room temperature and RBC intracellular calcium was measured by ion-selective electrodes. Serum ionized calcium was also measured by ion selective electrodes. Serum total Calcium was measured by Spinreact Ca-determination Kit.

Results:

Erythrocytes intracellular Ca^{++} was found to be significantly ($p < 0.001$), higher in essential hypertensive group with value of 1.00 +- 0.011 mmol/L as compared to the control value of 0.72 +- 0.06 mmol/L. Serum ionized Ca^{++} fraction was significantly ($P < 0.001$) lower, 1.028 +- 0.11 mmol/L in the hypertensive group as compared to the control value of 1.22 +- 0.013 mm/L. However the total serum calcium did not show any significant difference between the two groups; 2.12+- 0.24 mmol/L in hypertensives and 2.25 +- 0.26 mmol/L in controls.

Table 1: Intracellular free Ca^{++} ,serum ionized Ca^{++} and total serum calcium and blood pressure in control and patient group.

Parameter	Control (N=25)	Hypertensives (N=20)	P Value
Stystolic BP (mmHg)	124 ± 6.52	157 ± 9.08	P<0.001*
Diastolic BP (mmHg)	79 ± 4.38	99 ± 5.27	P<0.001*
Mean BP	94 ± 4.76	118 ± 6.49	P<0.001*
RBC Ca^{2+} (mmol/l)	0.72 ± 0.06	1.00 ± 0.11	P<0.001*
Serum ionized Ca^{2+} (mmol/l)	1.22 ± 0.13	1.028 ± 0.11	P<0.001*
Total serum calcium (mmol/l)	2.25 ± 0.26	2.12 ± 0.24	NS

Data is expressed as mean ±SD, *Highly significant statistically, NS: Non-significant statistically

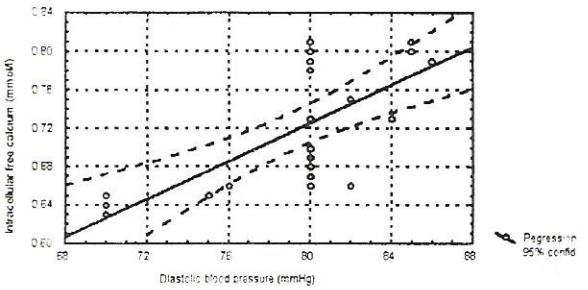

Fig.1. Relationship between diastolic blood pressure and intracellular calcium control group, r=0.68833 and p=0.00143. Both values are statistically significant (correlation is linear and direct).

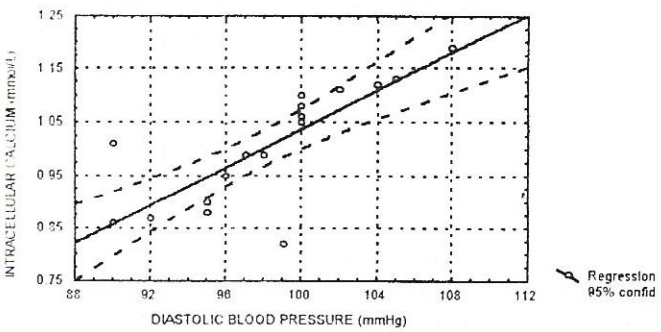

Fig.2. Relationship between diastolic blood pressure and intracellular calcium in hypertensive group, r=0.79977 and p=0.000023 both statistically significant (correlation is linear and direct).

Discussion:

Since Ca^{++} ions seem to directly participate in the control of erythrocyte membrane structure and deformability and because cell Ca^{++} metabolism has been repeatedly proposed to be modified in hypertension, the intracellular calcium ions concentration $(Ca^{++})^i$ was investigated in red blood cells from hypertensive and normotensive subjects.

Our results show an increase in the level of intracellular Ca^{++} and a decrease in the serum Ca^{++} in essential hypertension patients. The total serum Ca^{++} content, remained unchanged. This study is in accordance with a number of similar findings[12,13,14]. However a few workers have found different results[15,16] in whom problems in intracellular Ca^{++} measurements was a common cause. Also cell membrane behavior in vitro is modified by different techniques used for cell separation and cation content study.

The goal of this paper is to show the possibility of a new approach to the understanding of the major pathogenic basis of essential hypertension. Membranopathy relates to the presence of wide spread abnormalities in ion transport function of cell plasma membrane resulting in insufficient membrane control over intracellular calcium[17]. Essential hypertension is a polygenic disorder. Perturbed intracellular calcium homeostasis could arise from a number of abnormalities that may be intrinsic to the cell[18] There are several possible explanations for altered handling of calcium in essential hypertension: loss of a stabilizing effect of bound calcium on membrane potential as a result of decreased calcium binding leading to depolarization and hyperexcitability; increased calcium-channel function related to a decrease in extra cellular ionized calcium and reduced extrusion pathways for calcium from the cell leading to increased intracellular calcium[19]. Increase in the myoplasmic calcium concentration causes the Ca^{++} to bind to calmodulin. This calcium-calmodulin complex activates myosin-light chain kinase which phosphorylates myosin light chain and activates myosin's ATPase. The phosphorylated myosin cyclically binds to actin filaments producing shortening[20].

Since calcium is very finely regulated by calcium regulatory hormones i.e. parathyroid hormone, calcitonin, and 1-25-dihydroxy vitamin D, it is suggested that further research needs to be directed at the actions of these hormones on cellular ion handling and to establish whether alterations of these hormones contribute to the constellation of ionic changes within the cell.

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