

# Effects of Nifedipine on Cation Transport and Na - K-ATPase Activity in Erythrocytes and Electrolyte Homeostasis in Rats

Najma Shaheen and Tabassum Mahboob\*

Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan

**Abstract:** Calcium channel antagonists have been reported to reduce blood pressure in those individuals at risk of cardiac and cerebrovascular events. There is no specific study available regarding the role of electrolyte alterations in blood pressure lowering effects of nifedipine. The present study was designed to investigate the role of electrolyte homeostasis, changes in ouabain-sensitive Na<sup>+</sup> K<sup>+</sup> adenosine triphosphatase (ATPase) activity, and net sodium efflux and potassium influx across blood cell membranes. Rats were divided into two experimental groups. Nifedipine (20mg/kg body weight) was administered by gastric tube to the test group. Control group received same volume of deionized water. The intra-erythrocyte sodium, serum sodium, potassium, calcium and sodium, calcium content of heart and kidney tissues were decreased significantly. Whereas, intra-erythrocyte potassium was slightly decreased or remained normal in nifedipine treated rats as compared to normal healthy rats. The Na - K - ATPase activity, serum magnesium, potassium and magnesium content in heart and kidney tissues were increased significantly. Results confirmed that nifedipine represses ion channels, transporters and calcium-binding proteins in tissues. Erythrocyte studies indicate that nifedipine blocks the entrance of calcium into the cells but also stimulate Na - K - ATPase activity, resulting in reduction of intracellular sodium concentration, thus suggesting direct nifedipine-induced blood pressure reduction.

**Keywords:** Nifedipine, electrolyte homeostasis, Na-K-ATPase, cation transport.

## INTRODUCTION

Nifedipine, a 1, 4 dihydropyridine is originally categorized as a cardioactive agent. It is widely used in the management of systemic hypertension, angina pectoris and supraventricular arrhythmias [1, 2]. Nifedipine a calcium entry blocker are primarily vasodilators, lowering blood pressure by decreasing peripheral vascular resistance and prevents the progression of carotid atherosclerosis at the level of the small arterioles which can be followed by an autonomic counter-regulation especially in drugs with a rapid onset of action [3, 4]. In experiments nifedipine have been shown to increase renal blood flow and glomerular filtration rate and to augment urine flow and electrolyte excretion [5, 6] by dilating afferent arterioles preferentially. Previous investigators have reported for some reasons that are not yet clear that nifedipine have an advantage in the treatment of hypertension that are apparently related to specific action on calcium entry into vascular smooth muscle, endocrine function, and renal hemodynamics. The renin-angiotensin system seems to be activated to a somewhat lesser degree by calcium channel blocking agents than it is by non-specific vasodilators [7]. Current evidence suggests that dihydropyridine calcium channel blockers exert a renoprotective effect in insulin-dependent diabetes mellitus (IDDM) patients with overt nephropathy [8].

Despite the advent of major new development of angiotensin converting enzyme inhibitors, diuretics and beta-adrenergic blocking agents. These all therapeutic classes represent a new approach to antihypertensive therapy and a new tool for examining the underlying mechanisms of hypertension. Among the classes of calcium channel blockers, dihydropyridine derivatives are widely used because of their potent vasodilating activity and weak cardiodepressant action [9].

In chronic experimental animals the studies on the electrolyte content of heart and kidney tissues are however, still lacking. Therefore, the aim of this study was to examine the disturbances of serum and red cell electrolytes in association with membrane Na - K - ATPase activity as well as its influence on metabolic parameters in tissues.

## MATERIALS AND METHODS

### Animals and Adaptation Phase

Male Wistar Albino rats (180-250g b.w. n=16) were purchased from the animal house of Agha Khan University Hospital, Karachi. Animals were individually caged in a quite temperature controlled room (23 ± 4°C), Rats had free access to water and standard rat diet. After the period of habituation experiments were started on these rats. Rats were divided into two experimental groups. Control and test (8 animals in each group). In test group nifedipine (20mg/kg/day) was given by gastric tube.

\*Address corresponding to this author at the Department of Biochemistry, University of Karachi, Karachi-75270, Pakistan; E-mail: tab60@hotmail.com

## Sample Collection

After 3 days treatment animals were decapitated and blood was sampled from the head wound in the lithium heparin coated tubes. A portion of blood was used to collect serum. Heart and kidneys were excised, trimmed of connective tissues, rinsed with deionized water to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues were then kept in freezer until analysis. Preparation of RBC membrane fractions was begun within 30 minutes of blood collection.

## Intraerythrocyte Sodium and Potassium Estimations

Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room temperature by suspension in the magnesium chloride solution (112 mmol/L), centrifugation at 450 x g at 4°C for 5 minutes and aspiration of the supernatant [10]. Final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration. Neither electrolytes were detectable in the final wash. Washed erythrocytes were then lysed and used for the estimation of intraerythrocyte sodium and potassium [11].

## Erythrocyte Membrane Preparation

The packed red cells extracted by centrifugation at 4°C, 450 x g for 15 minutes were resuspended and diluted in 25 volumes of 0.011 mol/L. Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged for 30 minutes at 12,000 rpm at 4°C and the membrane pellet was resuspended in 30 ml of 0.011 mol/L. Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein/ml of Tris buffer. The membrane suspension was stored at -80°C until the assay was performed.

## Erythrocyte Na- K- ATPase Activity Measurement

ATPase activity was measured in a final volume of 1 ml as follows: Membrane (400µg) were preincubated for 10 minutes at 37°C in a mixture containing 92 mmol/L Tris-HCl (pH 7.4), 100 mmol/L NaCl, 20 mmol/L KCl, 5 mmol/L MgSO<sub>4</sub>.H<sub>2</sub>O and 1 mmol/L EDTA [12]. Assays were performed with or without 1 mmol/L Ouabain, a specific inhibitor of Na-K-ATPase. After incubation with 4 mmol/L ATP (Vanadate free, Sigma) at 37°C for 10 minutes, the reaction was

stopped by adding of ice cold trichloroacetic acid to a final concentration of 5%. After centrifugation at 4°C, 5500 g for 10 minutes. The amount of inorganic phosphate in the supernatant was determined [13]. Na-K-ATPase activity was calculated as the difference between inorganic phosphate released during the 10 minute incubation with and without ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released/milligram protein/hour.

All assays were performed in duplicate, and blanks for substrate, membrane and incubation time were included to compensate for endogenous phosphate and non-enzyme related breakdown of ATP. Under these experimental conditions, the coefficient of variation was 7.5%.

## Tissue Digestion and Electrolyte Measurements

Frozen tissues (heart and kidneys) were digested for 3 hours at room temperature and then at 70°C for another 3 hour in 20 ml deionized water followed by 10 ml of concentrated nitric acid and perchloric acid (equal volume). The samples were initially heated very gently. After foaming subsided temperature was increased to produce steady boiling. The excess acids were boiled off to near dryness. The digest then cooled to room temperature and analyzed for sodium, potassium, calcium and magnesium content [14, 15].

## Electrolytes Estimation

Concentration of sodium, potassium and calcium in serum, heart and kidney were analyzed by flame photometer (corning 410). Serum magnesium was estimated by the method described earlier [16]. Red cell sodium and potassium were estimated by the method of Fortes Meyer and Starkey [10].

## Statistical Analysis

Results are presented as mean  $\pm$  S.E.M Significant difference from control and test values was evaluated by student's t-test.

## RESULTS

Effects of chronic nifedipine treatment on serum electrolytes, Na-K-ATPase activity, red cell sodium and potassium in male rats:

Table-1 shows the changes in the serum concentration of sodium, potassium, calcium, red cell electrolytes and on Na-K-ATPase activity after chronic

nifedipine treatment. A significant ( $P < 0.01$ ) decrease in serum concentration of sodium, potassium, calcium and red cell sodium level was observed in chronic nifedipine treated rats as compared to control. Whereas, membrane Na-K-ATPase activity and serum magnesium were increased significantly ( $P < 0.01$ ). No significant decrease was observed in red cell potassium level as compared to control rats.

**Table 1: Effects of Chronic Administration of Nifedipine (20 mg/kg) on Serum Electrolytes, Na - K - ATPase Activity, Red Cell Sodium and Potassium in Male Rats**

	Control	Test
Sodium(mEq/L)	139.37 $\pm$ 0.37	135.43** $\pm$ 0.92
Potassium(mEq/L)	6.15 $\pm$ 0.15	5.37** $\pm$ 0.11
Calcium(mEq/L)	5.30 $\pm$ 0.07	4.85** $\pm$ 0.12
Magnesium(mEq/L)	1.88 $\pm$ 0.10	2.88** $\pm$ 1.08
Na - K - ATPase activity (nmol/mg protein/hr)	187.88 $\pm$ 14.52	121.96** $\pm$ 2.60
Red cell Sodium (mmol/L)	4.85 $\pm$ 0.16	3.37** $\pm$ 0.18
Red cell Potassium (mmol/L)	119.53 $\pm$ 1.23	116.03 $\pm$ 1.44

Values are mean $\pm$ S.E. M  
\*\* $P < 0.01$

### Effects of Chronic Nifedipine Treatment on Heart Electrolytes in Male Rats

Table-2 shows the effect of chronic nifedipine administration on electrolyte contents in the heart tissues. The sodium and calcium content were decreased significantly ( $P < 0.01$ ,  $P < 0.05$ ) respectively in chronic nifedipine treated rats as compared to controls. Whereas, magnesium content was increased significantly ( $P < 0.05$ ) in chronic nifedipine treated group. No significant increase was observed in potassium content as compared to control rats.

**Table 2: Effects of Chronic Administration of Nifedipine (20 mg/kg) on Electrolytes Content of Heart Tissue in Male Rats**

	Control	Test
Sodium( $\mu$ mol/g)	39.44 $\pm$ 0.24	38.18** $\pm$ 0.29
Potassium( $\mu$ mol/g)	69.86 $\pm$ 0.48	70.94 $\pm$ 0.26
Calcium( $\mu$ mol/g)	1.11 $\pm$ 0.05	0.84* $\pm$ 0.05
Magnesium( $\mu$ mol/g)	6.88 $\pm$ 0.03	7.06* $\pm$ 0.04

Values are mean $\pm$ S.E. M  
\*\* $P < 0.01$ , \* $P < 0.05$

### Effects of Chronic Nifedipine Treatment on Kidney Electrolytes in Male Rats

Table-3 shows the effect of chronic nifedipine treatment on electrolyte contents in the kidney tissues. The sodium and calcium content were decreased significantly ( $P < 0.01$ ) in chronic nifedipine treated rats as compared to controls. Whereas, potassium content was increased significantly ( $P < 0.01$ ) in chronic nifedipine treated group. No significant increase was observed in magnesium content as compared to control rats.

**Table3: Effects of Chronic Administration of Nifedipine(20 mg/kg) on Electrolytes Content of Kidney Tissue in Male Rats**

	Control	Test
Sodium( $\mu$ mol/g)	40.78 $\pm$ 0.22	38.88** $\pm$ 0.23
Potassium( $\mu$ mol/g)	63.65 $\pm$ 0.33	67.16** $\pm$ 0.41
Calcium( $\mu$ mol/g)	2.97 $\pm$ 0.09	2.10** $\pm$ 0.06
Magnesium( $\mu$ mol/g)	6.71 $\pm$ 0.10	6.78 $\pm$ 0.04

Values are mean $\pm$ S.E.M  
\*\* $P < 0.01$ .

### DISCUSSION

The present study indicates that serum sodium, potassium, and calcium levels were decreased in nifedipine treated rats. The hypocalcaemia observed during this study may contribute in the development of hypokalemia. The action of angiotensin II and potassium on aldosterone secretion is blocked by nifedipine through its calcium antagonizing action. Drugs that block the renin-angiotensin aldosterone system not only provide blood pressure control but also provide vascular protection [17]. Several investigators have suggested that major physiological stimulation of aldosterone production by the adrenal zona glomerulosa cells, angiotensin II and potassium, are to a varying degree dependant on extracellular calcium influx to achieve maximum stimulation [18, 19].

Our study showed that in nifedipine treated rats there was decrease intracellular sodium and a non-significant decrease in potassium content together with increased Na- K - ATPase activity. Some of the previous investigations, despite finding a reduction in intracellular sodium fails to demonstrate a change in the Na -K - ATPase activity [20, 21]. Other investigators have suggested that diltiazem, nifedipine, verapamil and nimodipine have stimulatory effects [22, 23].

Plasma magnesium is known as the co-factor for Na - K - ATPase activity. It is possible that nifedipine induced increase in serum magnesium may increase Na- K - ATPase activity leading to a decreased intracellular sodium and calcium. Nifedipine had no effect on potassium influx mediated by the sodium – potassium pump. Very little information is available regarding the effects of calcium channel blockers on Na - K - ATPase and intracellular levels of sodium and potassium probably because of the difference in experimental conditions and dosage of drugs.

The decreased sodium and calcium content with increased potassium content in heart and kidney tissue may be due to the improve activity of Na - K - ATPase, Ca<sup>++</sup>- ATPase and Na<sup>+</sup> - Ca<sup>++</sup> exchange mechanism. It is well established that angiotensin II stimulate the sodium potassium pump of vascular smooth muscle cells [24]. Nifedipine represses ion channels and transporters in tissues [25]. as dihydropyridine have the greatest hypotensive efficacy without affecting organ blood flow [26].

These results emphasize the role of Na - K - ATPase, serum and intracellular electrolytes in the antihypertensive effects of nifedipine in rats. It can be concluded that decrease tissue sodium also decrease the calcium influx into the cells resulting in vasodilation and by stimulating the ouabain sensitive pump activities and decrease in aldosterone production may contribute in the antihypertensive mechanism of nifedipine.

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