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ANTIHYPERTENSIVE AND METABOLIC EFFECTS OF VERAPAMIL: ROLE OF NA-K-ATPASE AND ELECTROLYTES HOMEOSTASIS IN MALE AND FEMALE RATS

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ABSTRACT

Calcium channel antagonists have been shown to be effective in the management of coronary vascular diseases. Although initially restricted to the treatment of angiopectoris could prevent cardiovascular complication in patients with diabetes. In view of a possible role of electrolytes in the therapeutic effects of verapamil, the present study concern effects of verapamil on serum, red cell, tissue electrolytes and Na-K-ATPase activity in both male and female rats. Verapamil (30 mg/kg body weight) was administered intraperitoneally to the test group. Control group received same volume of deionize water. A slight decreased Na-K-ATPase activity was observed in both sexes. Verapamil treatment decreases serum sodium and magnesium levels in both male and female rats. However serum potassium was slightly increased in both sexes. Verapamil treatment in red cells decreases sodium and increases potassium content in both sexes. Verapamil administration decreases sodium and calcium content in heart, liver and kidney tissues in both sexes, whereas an increased content of potassium and magnesium was observed in these tissues except liver in which the magnesium content was slightly decreased in both male and female rats. The results showed that the changes in electrolyte levels are more pronounced in female than in male rats. The results reported in the present study suggests that the drug has been shown to inhibit aldosterone biosynthesis to a variable degree. Although the antihypertensive effect of verapamil is considered to be mainly due to its vasodilation or by inhibition of vasoconstriction. It is suggested that verapamil lower blood pressure by correcting a pathophysiological derangements of electrolytes present in hypertension. The cellular mechanism involved is the altered serum, intracellular, tissue electrolytes and ATPase activity.

INTRODUCTION

Verapamil is a calcium channel blocker used clinically in the treatment of coronary vascular disease, supraventricular arrhythimias, peripheral vascular disease, hypertension and other non-cardiovascular conditions (Noll and Luscher, 1998; Braunwald, 1982; Halperin and Cubeddu, 1986; Allert and Adams, 1987).

It is a phenylalkylamine derivative which antagonises calcium influx through the slow channels of vascular smooth muscle and cardiac cell membranes. By reducing intracellular free calcium concentrations, causes coronary and peripheral vasodilation and electrical activity in the atrioventricular and sinoatrial nodes (Koffi *et al*., 1999; Katz *et al*., 1985; McTavish and Sorkin, 1989; Kulkarni *et al*., 2001).

Although the antihypertensive effect of verapamil is considered to be mainly due to its generalized systemic vasodilation resulting in a marked reduction in systemic vascular resistance and, consequently blood pressure. The chronic treatment with different antihypertensive agents directly modulates adrenocortical aldosterone synthesis in spontaneously hypertensive rats *in vivo*

via different mechanisms (Otsuka *et al*., 2000). Another important direct effect of calcium channel blocker on renal function is the increase of sodium and H_2O excretion by a tubular action that occurs in the absence of hemodynamic changes. The mechanism of the tubular effects of calcium channel antagonists is not understood at present (Osswald *et al*., 1990). In micromolar concentrations it has been shown that verapamil suppressed Ca^{2+} entry and simultaneously elevate the agonist induced plasma membrane depolarization due to Na^+ inward current via these channels (Cheglakov and Avdonin, 2000). Thus the verapamil seem to modulate the platelet receptor-operated channels suppressing Ca^{2+} entry and elevating Na^+ currents via these channels. Apart from lowering of blood pressure verapamil has also have other theoretically beneficial effects i-e. renal protection and weak antiplatelet activity (Opie and Schall, 2002). The purpose of the present study is to investigate the gender and antihypertensive effects in response to verapamil on serum electrolytes, Na-K-ATPase activity, and electrolyte content of heart, liver and kidney tissues.

MATERIALS AND METHODS

Animals and Diet:

Wistar albino rats of both sexes (190-210 g b.w.) were taken for the study. Animals were individually caged in a quite temperature controlled room (23 ± 4°C). Rats had free access to water and standard rat diet.

Drug Administration:

Rats were divided into four experimental groups; male control, male test, female control, female test (6 animals in each group). Control male and female rats were given water throughout the experiment. Test animals were injected with verapamil (30 mg/kg; i.p.). Control animals received an equal volume of deionize water through the same route.

Sample Collection:

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

Serum Electrolyte Measurements:

Serum was analyzed for the estimation of sodium and potassium by flame photometry and magnesium by the method of Hallry and Skypeck (1964).

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 as describe earlier (Leblondel and Allain, 1988). Final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration. Neither electrolytes were detectable in the final wash. Washed erythrocytes were than used for the estimation of intraerythrocyte sodium and potassium (Tabassum *et al*., 1996).

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 $MgSO_4.H_2O$ and 1 mmol/L EDTA. 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 (Dryer and Tammes, 1957). 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 (liver, kidney and heart) 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 cool to room temperature and analyzed for sodium potassium and calcium by flame photometry and magnesium content by the method of Hallry and Skypeck (1964).

Statistical Analysis:

Results are presented as mean ± SD. Two Way ANOVA was used to analyzed the effect of sex, treatment and interaction on Na-K-ATPase activity and electrolytes. Post-hoc analysis was done by using Newman Keul's test.

RESULTS

Table-1 shows the changes in the serum concentration of sodium, potassium and magnesium after acute verapamil treatment. Two way ANOVA showed a significant treatment effect on serum sodium and magnesium ($P<0.01$, $P<0.05$). Sex effect was significant ($P<0.01$) for serum potassium. Interactions between sex and treatment were non-significant. Acute treatment with verapamil decreased sodium in both sexes but less significantly in male ($P<0.05$) than in female ($P<0.01$) rats. A non-significant increase and decrease in potassium and magnesium was observed in serum concentration of verapamil injected male and female rats. The decrease in serum sodium and increase in potassium concentration was more in female than male rats ($P<0.05$).

Table-2 shows the effect of acute verapamil administration on Na-K-ATPase activity and red cell electrolytes in male and female rats. Two way ANOVA showed a significant ($P<0.01$)

treatment effect on red cell sodium and potassium. Sex effect was significant for ($P<0.05$) for red cell sodium content. Interaction between sex and treatment were non-significant. Acute verapamil administration decreased sodium ($P<0.05$) and increased potassium ($P<0.01$) in both sexes. A non-significant decrease on Na-K-ATPase activity was observed in male and female rats.

Table-1
Effects of acute intraperitoneal administration of verapamil (30 mg/kg) on serum electrolytes in male and female rats

	Male		Female		Two way ANOVA (df 1, 20)		
	Control	**Test**	**Control**	**Test**	**Sex**	**Treat-ment**	**Inter-action**
Sodium (mEq/L)	139.5 ±0.51	134.16* ±1.57	138.83 ±1.38	130.16^{+**} ±0.15	F=3.88 n.s	F=34.99 p<0.01	F=1.98 n.s
Potassium (mEq/L)	6.62 ±0.15	7.55 ±0.10	6.02 ±0.31	6.71^{+} ±0.12	F=11.44 p<0.01	F=0.868 n.s	F=0.318 n.s
Magnesium (mEq/L)	2.07 ±0.02	1.66 ±0.13	1.86 ±0.200	1.42 ±0.094	F=1.23 n.s	F=4.41 p<0.05	F=0.0084 n.s

Values are means ± S.D. (in µmol/g wet weight n = 6)
Significant difference by Newman Keuls test from respective controls
**p<0.01, *p<0.05, from similarly treated male rats + p<0.05; following two way ANOVA

Table-2
Effects of acute intraperitoneal administration of verapamil (30 mg/kg) in red cell sodium, potassium and Na-K-ATPase activity in male and female rats

	Male		Female		Two way ANOVA (df 1, 20)		
	Control	**Test**	**Control**	**Test**	**Sex**	**Treat-ment**	**Inter-action**
RBC sodium (mmol/L)	3.65 ±0.30	2.73* ±0.11	3.03 ±0.24	2.11* ±0.053	F=6.24 p<0.05	F=13.63 p<0.01	F=0.004 n.s
RBC potassium (mmol/L)	120.53 ±0.44	132.54** ±0.96	118.20 ±3.17	130.83** ±1.26	F=1.06 n.s	F=39.66 p<0.01	F=0.270 n.s
Na-K-ATPase activity (nmol/mg protein/hr)	197.02 ±24.04	190.33 ±26.70	194.65 ±20.37	183.41 ±15.68	F=0.035 n.s	F=0.1308 n.s	F=0.08 n.s

Values are means ± S.D. (in µmol/g wet weight n = 6)
Significant difference by Newman Keuls test from respective controls
** p<0.01, *p<0.05

Table-3 shows the effect of acute intraperitoneal administration of verapamil on electrolyte contents in the heart tissues. Two way ANOVA showed a significant sex effect on sodium and magnesium contents (P<0.01) and on potassium and calcium (P<0.05) contents. Treatment effect was significant for sodium, potassium and calcium (P<0.01) contents. Interaction between sex and treatment were significant for sodium content (P<0.01) only. Acute administration of verapamil decreased (P<0.01) sodium content and increased (P<0.05) potassium content in female rats only. Calcium content decreased significantly in males (P<0.01) but not in female rats. Mean values of calcium were smaller (P<0.05) in control female than observed for control males. A non-significant increase in magnesium content was observed in heart tissues of verapamil injected male and female rats.

Table-3
Effects of acute intraperitoneal administration of verapamil (30 mg/kg)
on heart electrolyte content in male and female rats

	Male		**Female**		**Two way ANOVA (df 1, 20)**		
	Control	**Test**	**Control**	**Test**	**Sex**	**Treat-ment**	**Inter-action**
Sodium (µmol/g)	40.24 ±0.13	38.62 ±0.008	39.51 ±0.008	34.45** ±1.00	F=19.28 p<0.01	F=35.84 p<0.01	F=9.46 p<0.01
Potassium (µmol/g)	72.03 ±0.13	73.48 ±0.25	70.50 ±0.35	72.75* ±0.856	F=4.53 p<0.05	F=12.08 p<0.01	F=0.57 n.s
Calcium (µmol/g)	2.3 ±0.0032	1.73** ±0.10	2.09^{+} ±0.019	1.66 ±0.031	F=5.88 p<0.05	F=71.70 p<0.01	F=1.19 n.s
Magnesium (µmol/g)	6.97 ±0.03	7.08 ±0.057	6.81 ±0.05	6.93 ±0.061	F=5.24 p<0.01	F=2.95 n.s	F=0.004 n.s

Values are means ± S.D. (in µmol/g wet weight, n = 6)
Significant difference by Newman Keuls test from respective controls
**p<0.01, *p<0.05. From similarly treated male rats + p<0.05; following two way ANOVA

Table-4 shows the effect of acute verapamil treatment on kidney electrolytes in male and female rats. Treatment effect was found to be significant (P<0.01) for all the electrolytes examined after verapamil administration. Sex effect was also significant for all electrolytes except calcium. Potassium and magnesium (P<0.01) contents were increased in both male and female rats after the treatment, whereas calcium and sodium content was decreased (P<0.01) in both sexes. The mean values of potassium and magnesium in control females were significantly (P<0.01) smaller than in control male rats and the sex difference persisted in the drug treated group; suggesting a comparable effect of drug treatment. After verapamil administration increases in potassium, magnesium (P<0.01) and decreases in sodium (P<0.01) content are more pronounced in female than in male rats.

Table-5 shows the effect of acute verapamil administration on electrolyte contents in liver tissues. Two way ANOVA showed a significant treatment and sex (P<0.01) effect on all the electrolytes examined. Calcium content of liver in control females (P<0.05) was smaller than control males and this sex difference persisted in verapamil treated group. Calcium content was decreased (P<0.01) in both male and female rats. The increases of potassium were significant (P<0.05) for female rats only, however sodium content decreased more significantly (P<0.05) for

female rats. Acute verapamil administration decreased magnesium content in female (P<0.01) than in male (P<0.05) rats. Mean values of potassium and sodium in control female was significantly (P<0.01) smaller than in control male rats and sex difference persisted in the drug treated group. After verapamil treatment increases in potassium (P<0.01) and decreases in sodium (P<0.01) are more pronounced in female than in male rats.

Table-4
Effects of acute intraperitoneal administration of verapamil (30 mg/kg)
on electrolyte content of kidney tissues in male and female rats

	Male		Female		Two way ANOVA (df 1, 20)		
	Control	**Test**	**Control**	**Test**	**Sex**	**Treat-ment**	**Inter-action**
Sodium (µmol/g)	41.51 ±0.19	38.66^{**} ±0.196	40.10 ±0.045	36.96^{++**} ±0.38	F=35.15 p<0.01	F=131.03 p<0.01	F=0.29 n.s
Potassium (µmol/g)	69.61 ±0.62	72.25^{**} ±0.516	65.1^{++} ±0.049	67.18^{++**} ±0.16	F=111.08 p<0.01	F=26.74 p<0.01	F=0.45 n.s
Calcium (µmol/g)	2.90 ±0.06	2.15^{**} ±0.10	2.87 ±0.05	2.06^{**} ±0.018	F=0.797 n.s	F=117.35 p<0.01	F=0.183 n.s
Magnesium (µmol/g)	6.42 ±0.010	6.90^{**} ±0.0027	5.86^{++} ±0.15	6.32^{++**} ±0.012	F=43.48 p<0.01	F=28.86 p<0.01	F=0.017 n.s

Values are means ± S.D. (in µmol/g wet weight n = 6)
Significant difference by Newman Keuls test from respective controls
**p<0.01, *p<0.05. From similarly treated male rats + p<0.05; ++ p<0.01 following two way ANOVA

Table-5
Effects of acute intraperitoneal administration of verapamil (30 mg/kg)
on liver electrolytes content in male and female rats

	Male		Female		Two way ANOVA (df 1, 20)		
	Control	**Test**	**Control**	**Test**	**Sex**	**Treat-ment**	**Inter-action**
Sodium (µmol/g)	26.00 ±0.06	24.81 ±0.07	23.33^{++} ±0.84	21.40^{++*} ±0.5	F=31.96 p<0.01	F=8.43 p<0.01	F=0.46 n.s
Potassium (µmol/g)	82.60 ±0.37	83.53 ±0.18	79.15^{++} ±0.11	80.58^{++*} ±0.62	F=59.39 p<0.01	F=8.1 p<0.01	F=0.36 n.s
Calcium (µmol/g)	0.98 ±0.005	0.93** ±0.004	0.95^{+} ±0.004	0.88^{**} ±0.012	F=18.82 p<0.01	F=44.97 p<0.01	F=0.34 n.s
Magnesium (µmol/g)	7.52 ±0.10	7.00^{*} ±0.01	7.30 ±0.04	6.69^{**} ±0.163	F=5.60 p<0.01	F=25.80 p<0.01	F=0.174 n.s.

Values are means ± S.D. (in µmol/g wet weight n = 6)
Significant difference by Newman Keuls test from respective controls
**p<0.01, *p<0.05. From similarly treated male rats + p<0.05; ++ p<0.01 following two way ANOVA

DISCUSSION

Several epidemiological, experimental and clinical studies have shown that calcium channel antagonists produces antihypertensive effects in humans and animals (Pucas *et al.,* 2000; Jesmin *et al.,* 2002; Lopez *et al.,* 1997). Although the role of sodium, potassium and magnesium in the blood pressure regulation is well established. However, none of these studies showed the role of electrolytes (sodium, potassium and magnesium) and Na-K-ATPase in the blood pressure lowering effect of verapamil. Their potential effects on electrolyte homeostasis are unpredictable.

Effect of verapamil on serum electrolytes:

A marked decrease in serum sodium concentration observed during the present study (Table-1) which may be due (a) a change in glomerular filteration rate and/or renal blood flow (Zanchetti and Leonetti, 1985; Berkhin and Gurevich, 1980), (b) interference with aldosterone secretion and/or aldosterone action on the distil tubules (Fakunding and Catt, 1980; Eiskjaer *et al*., 1989; Otsuka *et al*., 2000; Blanchouin-Emeric *et al*., 1988). (c) interference with adrenergic sodium handling (Zanchetti *et al*., 1985), (d) inhibition of transepithelial Na^+ transport by blocking epithelial Na^+ channel (ENaC) (Segal *et al*., 2002), (e) involvement of $Na^{+4}Ca^{2+}$ countertransport system (MacLaughlin *et al*., 1985), (f) the mechanism of the tubular effect of verapamil remains a controversial area (Ossawald *et al*., 1990). Therefore it can be postulated that due to intracellular calcium depletion, verapamil and high extracellular potassium concentration acting through different mechanisms are effective stimuli for kallikrein (Bompart, 1995). As kinins are generated in the kidney by the action of the enzyme kallikrein on the protein substrate kininogen. Many studies in intact animals have indicated that kinins are natriuretic (Gill *et al*., 1965; Willis *et al*., 1969; Tomita *et al.,* 1985) as a result of which hyponatremia observed during the present study.

Table-1 shows that the serum potassium is slightly increased in both male and female rats after the administration of verapamil. The results are consistent with some previous reports (Lopez *et al*., 1997; Lehtonen and Gordin, 1984).

These changes in potassium may be due to the fact that calcium channel antagonists can inhibit aldosterone secretion in the adrenals (Shamiss *et al*., 1993). Although the plasma renin activity did not change as reported earlier (Chellingsworth and Kendall, 1988; Shamiss *et al*., 1993; Anavekar and Doyle, 1986; Roy *et al*., 1983). The present finding suggest that the rise in the concentration of potassium may be due to the alteration in potassium transport produced by verapamil which does not appear to be dependent on adrenal function or peripheral sympathetic activity. Impaired Ca^{2+} entry into cells may alter K^+ transport (Sugarman and Kahn, 1986).

A decrease in the treatment effect on the serum concentration of magnesium was observed after administration of verapamil (Table-1). The results are in consistent with (Rubio-Luengo *et al*., 1995; Dietz *et al*., 1983). It is proposed that verapamil stimulate the Mg^{2+}-carrying transport system and magnesium diffusion in the cell so that Mg^{2+} influx is enhanced. This in turn causes suppression of the slow inward Ca^{2+} current and fast inward Na^+ current resulting into decreased transport of Ca^{2+} and Na^+ (Singh, 1987). The other possibility is that calmodulin-stimulated plasma membrane (Ca^{2+} + Mg^{2+})-ATPase is inhibited by verapamil (Raess and Gersten, 1987; Kim and Raess, 1988).

Effect of verapamil on Na-K-ATPase and red cell Na^+ and K^+:

Table-2 shows that verapamil treatment decreased sodium and increased potassium in red blood cells in both male and female rats. It has been demonstrated that K^+ transport across the

animals erythrocyte is altered by verapamil (Deepak, 1990). These results may be a consequence of maintainous of Na-K-ATPase activity in the erythrocyte membrane. Our results are in agreement with those published previously (Deepak, 1990; Raess and Gersten, 1987). It can be postulated that the vasodilative action of verapamil may be attributed to decrease cytosolic calcium as a result of maintainous of Na-K-ATPase activity. As all ATPase tended to be decreased in hypertension (Vincenzi *et al*., 1986).

Effect of verapamil on tissue electrolytes:

In the present study, it was observed that sodium and calcium content of heart, kidney and liver were decreased as shown in Table-3, 4 and 5. Whereas K^+ and Mg^{2+} content was increased after verapamil administration as shown in Table-3 and 4. Previously in has been shown that verapamil-like group has a predominant effect on the heart. The widely accepted mechanisms of there action is an inhibition of Ca^{2+} influx-via voltage-activated slow channels into smooth and cardiac muscles (Zakhari, 1986).

The results are also in accordance with those previously published that verapamil reduce the influx of Ca^{2+} through cell membrane passage ways in excitable tissues (Allert and Adams, 1987). The decreased Ca^{2+} and sodium content in kidney, liver and heart tissues may be due to the inhibition of cellular Ca^{2+} uptake through voltage-dependent Ca^{2+} channels as a result of binding to receptors-associated with channels (Papaioannou *et al.,* 1987). The aortic cell possess channels that are functionally similar to those found intact vascular tissues (Pucas, 2000).

The Na-K-ATPase produces the electrochemical concentration gradient for Na^+, which is the driving force for Ca^{2+} removal from the cytosol via the Na^+/Ca^{2+}exchange (Ravens and Himmel, 1999). Our results can be explained that as a result of the increment of this gradient by decreased intracellular Na^+ concentration leads to cellular Ca^{2+} unload resulting in improved contractile function.

Therefore the decrease in tissue sodium also decrease the Ca^{2+} influx through Na^+-Ca^{2+} exchange mechanism and thus cause a decreased intracellular Ca^{2+} content. The decreased intracellular calcium results in a vasorelaxant action and thus lowering the blood pressure.

Sex difference in the effect of verapamil on Na-K-ATPase and electrolyte levels of serum and tissues:

The sex difference in the effects on electrolyte levels may be due to difference in the rate of absorption of the drug in two sexes (Aarons *et al*., 1989). Difference in the activity of Na-K-ATPase (Lasker *et al*., 1985) in male and female may be also involved. The results show that electrolyte changes in serum and in tissues after verapamil administration are more pronounced in females than in males (White, 2001).

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