Arch. Pharm. Res. Vol. 16, No. 4, pp. 305-311, 1993

Altered Cardiac Na^+,K^+-ATPase Activity in Prehypertensive Spontaneously Hypertensive Rat

Shin-Woong Lee[1], Jeung-Soo Lee[1] and Earl T. Wallick[2]

[1]*College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea and* [2]*Department of Pharmacology and Cell Biophysics, University of Cincinnati, College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267-0575, U.S.A*

(Received October 18, 1993)

Na^+,K^+-ATPase activity, Na^+-dependent phosphorylation, and [^{3}H]ouabain binding in sarcolemma prepared from 4 week old spontaneously hypertensive rat(SHR) ventricles were compared to the same parameters in sarcolemma from age matched normotensive Wistar-Kyoto (WKY) rat ventricles to examine whether the reduced myocardial Na^+-pump activity in SHR is an inherited enzymatic defect or a second phenomenon due to sustained hypertension. The total body weights, ventricular weights, and blood pressures were the same for SHR and WKY. No significant differences in sarcolemmal protein content and protein recovery were noted between the two groups. Sarcolemma isolated from SHR ventricles showed significantly less Na^+,K^+-ATPase activity and number of phosphorylation sites when compared to sarcolemma from the WKY ventricles. Equilibrium binding of [^{3}H]ouabain and the turnover number of myocardial Na^+,K^+-ATPase, however, were the same for both groups. These results indicate that the low affinity (α, or α_1) isoform for ouabain is reduced in SHR compared to WKY but that the high affinity (α+, or α_2) α isoform is the same in ventricles of SHR and WKY. The reduced amount of isoform of the Na^+,K^+-ATPase in prehypertensive SHR ventricles may play some role in the development of hypertension.

Key words: Hypertension, Na^+,K^+-ATPase, Cardiac sarcolemma, [^{3}H]Ouabain binding, Spontaneously hypertensive rat(SHR)

INTRODUCTION

Although abnormal ion transport across the vascular smooth muscle membrane has been assumed to play some role in the development of hypertension (Haddy and Pamnani, 1987; Jones, 1973; 1974), the precise mechanism of the development of hypertension is by no means certain. Several investigators (Blaustein, 1980; Haddy, 1974; 1975; Overbeck *et al.*,1976) have suggested that there is an association between inhibition of the Na^+-pump and hypertension. This hypothesis relies heavily upon the proposed "major role" of the Na^+-pump in regulation of vascular smooth muscle tone and an as yet unidentified humoral factor(s) (Blaustein, 1980; Haddy *et al.*, 1987; Haddy and Overbeck, 1976; Huang *et al.*, 1978; Kaloyanides *et al.*, 1978; Kelly, 1987). This hypothesis has evolved from studies of surgically and chemically induced models of hypertension.

Correspondence to: Shin-Woong Lee, College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea

The spontaneously hypertensive rat (SHR) has been used as an animal model of essential hypertension in humans. This genetic model may have a completely different etiology from the experimentally induced models. Studies of membranes prepared from hearts of SHR (Lee *et al.*, 1983; Sower *et al.*, 1983) generally reported a reduction in Na^+,K^+-ATPase activity compared to normotensive WKY, although at least one study (David-Dufilho *et al.*, 1984) indicated an increased Na^+,K^+-ATPase activity in membranes isolated from the young SHR.

Our previous study(Lee *et al.*, 1983) indicated that an enriched sarcolemmal preparation from the hearts of adult SHR had, compared to WKY, a reduced number of [^{3}H]ouabain binding sites, decreased Na^+,K^+-ATPase activity, and Na^+-dependent phosphorylation. The IC_{50} for inhibition of the enzyme activity was much higher than the K_D for [^{3}H]ouabain binding, suggesting the presence of at least two forms of the Na^+,K^+-ATPase. In addition to the question of multiple forms, an unresolved problem arising from our previous study was whether the reduced Na^+-pump acti-

vity in the hearts of SHR is associated with the development of hypertension itself in these animals or is a consequence of inherited pathological features that later result in a reduced pump activity. The purpose of the present study was to determine whether there are differences in Na^+,K^+-ATPase activity, Na^+-dependent phosphorylation and the interaction of ouabain with Na^+,K^+-ATPase of sarcolemma prepared from ventricular myocardium of 4 week-old SHR and litter matched control normotensive WKY rats.

MATERIALS AND METHODS

Materials

[γ-^{32}P]ATP (25-45 Ci/mmol) and [^{3}H]ouabain (19.5 Ci/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. Adenosine triphosphate (disodium salt, vanadium free) was obtained from Boehringer-Mannheim, Mannheim, Germany. Ethyleneglycol-bis-(β-aminoethylether)-N,N,N',N'-tetraacetic acid(EGTA), L-histidine, nicotinamide adenine dinucleotide (reduced form, NADH), ouabain octahydrate, phosphoenolpyruvate tricyclohexylammonium salt, pyruvate kinase/lactic dehydrogenase (suspension), Tris(hydroxymethyl)aminomethane, and Tris adenosine triphosphate (Tris ATP) were obtained from Sigma Chemical Co., St. Louis MO, U.S.A. All other reagents were of reagent grade.

Animals

Four week old SHR and litter matched control normotensive WKY of either sex were received from Life Science, Seoul, Korea. On the day preceding the studies, blood pressure was measured by the tail-cuff method (Friedman and Freed, 1949).

Preparation of ventricular sarcolemma

The rats were killed by a blow on the head, and hearts were rapidly removed. After the atria, connective tissue and major vessels were removed, the remaining ventricles were weighed individually and collectively and ventricular tissue from WKY and SHR groups of 15 rats was pooled. Ventricular sarcolemma was prepared identically for both groups of rats by a modification of the method described by Van Alstyne *et al.* (1980). The pooled ventricular tissue was minced and homogenized using a polytron PT-20; twice for 5 sec at one-half maximum speed in 5 volumes of a solution containing 10 mM $NaHCO_3$, 5 mM NaN_3, and 0.25 M sucrose at a pH of 7.0. Aliquots of the homogenates were analyzed for enzyme activities. Microsomes were prepared by differential centrifugation. Ventricular membranes highly enriched in sarcolemma were isolated by discontinuous sucrose gradient centrifugation of the microsomal suspension, and the final sarcolemma fraction was suspended in 10 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose. Protein content was estimated by the method of Lowry *et al.* (1951), employing bovine serum albumin as a standard.

Measurement of Na^+,K^+-ATPase activity

Na^+,K^+-ATPase activity was determined by the spectrophotometric coupled-enzyme assay (Schwartz *et al.*, 1969) as previously described. ATPase activity was measured in a reaction medium containing 5 mM Na_2 ATP, 5 mM $MgCl_2$, 100 mM NaCl, 10 mM KCl, 1 mM phosphoenolpyruvate, 0.4 mM NADH, 25 µl of a combined pyruvate kinase/lactic dehydrogenase suspension, and 25 mM L-histidine, pH 7.4. After equilibration of the medium at 37°C, 5-10 µg (protein) of sarcolemma, or 20-30 µg of homogenate, which had been previously diluted 20-30 times in 10 mM Tris-HCl (pH 7.4) containing 0.25 M sucrose, was added to a final volume of 2.5 ml to initiate the ATPase reaction. Activity was measured by monitoring the decrease in absorbance at 340 nm due to oxidation of NADH. In the absence of inhibitors, this determination is a measure of total ATPase activity. Mitochondrial ATPase activity was estimated by measuring the difference between total ATPase activity and the activity remaining after the addition of 5 mM NaN_3. Na^+,K^+-ATPase activity was determined in the same cuvette by measuring the difference between the azide-insensitive ATPase activity and the activity remaining 30 min after the addition of 1 mM ouabain. This concentration of ouabain was required to fully inhibit rat ventricular Na^+,K^+-ATPase as determined from concentration-inhibition curves. A residual Mg^{2+}-ATPase activity was measured after the further addition of EGTA to 0.1 mM, which also represented a basal ATPase activity for the determination of Ca^{2+}-ATPase activity. After addition of 0.2 mM $CaCl_2$ to the cuvette, the ATPase activity expressed minus the basal Mg^{2+}-ATPase activity represented the Ca^{2+}-ATPase activity that was fully inhibited by 1 mM EGTA.

Sodium-dependent phosphorylation

Steady state sodium dependent phosphorylation was carried out at 0 °C by a filtration method described previously (Wallick *et al.*, 1978). The reaction medium, in a final volume of 0.5 ml, contained 2 mM $MgCl_2$, 30 mM histidine (pH 7.4), 100 mM NaCl, and 50 µg of protein. The reaction was initiated by the addition, with a digital pipette, of [γ-^{32}P]ATP (200 µM) and terminated after 15 seconds by the addition of 3 ml of an ice cold wash solution containing 10% trichloroacetic acid, 0.6 mM Na_2ATP, and 0.6 mM H_3PO_4. The solution was filtered by suction through 0.45 µm Gelman filters, which were washed three times with 5 ml of the cold wash solution and placed in

8 ml of scintillation fluid for counting. Background phosphorylation was measured using the same conditions as above, except that the reaction medium contained a 25-fold excess (5 mM) of unlabeled ATP, and was subtracted from the total ^{32}P-incorporation into membrane fraction.

Measurement of [^{3}H]ouabain binding

Binding of [^{3}H]ouabain was carried out by a filtration method as previously described (Wallick and Schwartz, 1974). Binding medium contained 100 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$, 5 mM Na_2ATP, 25 mM histidine, pH 7.4, 0.4 mM NADH, and 1 mM phosphoenolpyruvate. These conditions were selected so that they would be the same as those used for the measurement of ATPase activity. After incubation of sarcolemmal fraction (20 μg/ml), microsomal fraction (100 μg/ml) or homogenate (200 μg/ml) for 90 min at 37 ℃ with 200 nM [^{3}H]ouabain, the reaction mixture was rapidly filtered on 0.45 μm Gelman filters. Preliminary experiments showed that a 90 min incubation was sufficient to reach equilibrium. The filters were then washed three times with 5 ml of ice cold 100 mM KCl and incubated for 10 hours in scintillation fluid prior to determination of radioactivity. Nonspecific ouabain binding observed in the presence of 5.5 mM unlabeled ouabain was subtracted from values observed in its absence to calculate specific [^{3}H]ouabain binding.

Statistics

Statistical analysis was performed with Student's t test, and the level for significance was taken as a probability less than 5% ($p<0.05$).

RESULTS

Description of experimental groups

Normotensive WKY rats and SHR were matched according to strain and age (4 weeks). The WKY was paired with the SHR and studied concomitantly. Mean systolic blood pressure, body weight, and ventricular weight measured either the day of sacrifice or the previous day are given in Table I. The mean systolic blood pressure of SHR was the same as for WKY. Mean body weights and ventricular weights were also not significantly different between normotensive and hypertensive rats. Therefore, the heart weight to body weight ratio was the same in two groups.

Table I. Blood pressure, body and ventricular weights and ratios in normotensive WKY rats and prehypertensive SHR

	WKY	SHR
Blood pressure (mmHg)	118 ± 2	120 ± 2
Body weight (g)	129 ± 8	121 ± 7
Ventricular weight (g)	0.45± 0.03	0.44± 0.02
Ventricular/body weight ratio (mg/g)	3.49 ± 0.12	3.63± 0.13

Values represent the mean± SEM obtained from 45 rats. Determinations of blood pressure were made the day previous to sacrifice. There were no statistical differences.

Table II. Protein yield and recovery during preparation of ventricular sarcolemma from WKY and SHR

	WKY	SHR
Homogenate (mg/g wet wt.)	56.2 ± 2.7	55.3 ± 2.5
Microsome (mg/g wet wt.)	4.96 ± 0.28	4.77 ± 0.26
Recovery from homogenate (%)	8.82 ± 0.46	8.63 ± 0.44
Sarcolemma (mg/g wet wt.)	0.077± 0.003	0.075± 0.004
Recovery from homogenate (%)	0.137± 0.068	0.136± 0.071

Values are the mean± SEM of three different preparations of WKY and SHR. Each preparation was obtained from the pooled ventricles from WKY and SHR groups of 15 rats.

Protein yield and recovery

The yield of protein in milligrams of protein per gram of wet weight of ventricle in the homogenate, microsome, and sarcolemma was not significantly different between WKY and SHR ventricles. The yield of protein expressed in terms of milligrams of protein per ventricle and the recovery of protein (mg/g wet weight) in the microsomal and sarcolemmal fractions from the homogenate were the same for both the WKY and SHR (Table II).

ATPase activity

Table III presents the ATPase activity of ventricular homogenate and sarcolemma. There were no differences in total, azide sensitive, EGTA sensitive, and basal ATPase activities of homogenates from normotensive and hypertensive rats. Similarly in sarcolemma, these ATPase activities of SHR were not significantly different from those of WKY. In homogenate and sarcolemma prepared from SHR ventricles, the ouabain-sensitive Na$^+$,K$^+$-ATPase activity was decreased about 20% when compared to the corresponding membranes isolated from WKY ventricles.

Sodium-dependent phosphorylation

Phosphorylation experiments to determine the number of active sites of the enzyme present in the sarcolemmal preparations were carried out. Sodium-dependent phosphorylation of the microsomal fraction from SHR ventricles tended to be lower than those from WKY ventricles. The number of phosphorylation sites in sarcolemma prepared from SHR ventricles was 20% lower than in sarcolemma prepared from WKY ventricles (Table IV). The turnover number (the ratio of enz-

Table III. ATPase activities of cardiac preparations from WKY and SHR

Fraction	Activity (μmol Pi/mg/hr)	
	WKY	SHR
Homogenate		
Total ATPase	79.3± 6.8	75.7± 5.6
Azide-sensitive ATPase	55.3± 5.2	56.2± 4.5
Ouabain-sensitive ATPase	13.8± 0.8	10.9± 0.7*
EGTA-sensitive ATPase	2.4± 0.4	2.1± 0.6
Basal ATPase	7.8± 0.6	6.5± 0.7
Sarcolemma		
Total ATPase	180.2± 8.3	158.5± 7.6
Azide-sensitive ATPase	5.3± 2.1	4.7± 2.3
Ouabain-sensitive ATPase	125.8± 6.2	101.3± 7.1*
EGTA-sensitive ATPase	4.1± 2.2	5.3± 2.4
Basal ATPase	45.0± 3.7	47.2± 3.5

Aliquots of the initial homogenate (20~30 μg) previously diluted 20~30 times in 10 mM Tris-Cl, pH 7.4, containing 0.25 M sucrose or of the sarcolemmal fraction (~5 μg) were added to individual cuvettes containing the media (for spectrophotometric determination of ATPase activity, see METHODS) and equilibrated at 37℃ prior to adding inhibitors. Values are mean± SEM of three different preparations of WKY and SHR.
*Significantly different ($p<0.05$) from corresponding value of WKY

Table IV. Sodium-dependent phosphorylation of ventricular membrane fractions from WKY and SHR

Fraction	E-P (pmol Pi/mg)	
	WKY	SHR
Microsome	110± 9	88± 10
Sarcolemma	368± 15	302± 13**

Measurement of Na^+-dependent phosphorylation was carried out as described in Methods. Values represent the mean ± SEM of three different preparations of WKY and SHR.
**Significantly different ($p<0.025$) from corresponding value of WKY

Table V. [^{3}H]Ouabain binding of ventricular homogenate and membrane fractions from WKY and SHR

Fraction	[^{3}H]Ouabain binding (pmol/mg)	
	WKY	SHR
Homogenate	0.61± 0.02	0.58± 0.02
Microsome	1.75± 0.08	1.68± 0.07
Sarcolemma	5.92± 0.31	5.87± 0.25

[^{3}H]Ouabain equilibrium bindings were carried out in the presence of 200 nM [^{3}H]ouabain as described in Methods. Values represent the mean± SEM of three different preparations of WKY and SHR.

yme activity to number of phosphorylation sites) was the same in microsomal and sarcolemmal preparations from ventricles of WKY and SHR.

[^{3}H]Ouabain Binding

Ouabain inhibits the hydrolysis of ATP catalyzed by Na^+,K^+-ATPase by preventing phosphorylation of the active site. In rat ventricular myocardium, there are two different types of binding sites for ouabain. One site, characterized by a low affinity site, represents a large proportion of the total number of sites, as detected by sodium-dependent phosphorylation. A higher affinity site which represents a small portion of the total number of active sites can be detected by the [^{3}H] ouabain binding assay. In this experiment, [^{3}H]ouabain equilibrium binding to homogenates, microsomes, and sarcolemma prepared from SHR ventricles was compared to those prepared from WKY ventricles. As shown in Table V, the equilibrium binding of ouabain in the presence of 200 nM [^{3}H]ouabain was the same for the WKY and SHR.

DISCUSSION

It is well established that the Na^+,K^+-ATPase is the enzymatic machinary of the Na^+-pump in most animal cells (Schwartz *et al.*, 1975; Wallick *et al.*, 1979). Alterations in Na^+-pump activity are associated with, and in general mediate, changes in intracellular ionic composition, particularly with respect of Na^+,K^+ and Ca^{2+}. Numerous studies have now led to the concept that inhibition of the Na^+,K^+-ATPase results in elevated intracellular Na^+ which in turn activates a Na^+-Ca^{2+} exchange, which results in an elevated intracellular Ca^{2+} concentration (Adams and Schwartz, 1980). This mechanism may play an important role in maintaining as well as altering the contractile tone of various types of muscles including myocardium and systemic vasculature (Ferratio and Page, 1978; Ganguli and Tobian, 1979; Marin *et al.*, 1991; Willems *et al.*, 1975). Thus, it seems likely that alterations in Na^+-pump activity may either participate in, or be a consequence of the development of the systemic hypertension. One way in which enzyme activity can be altered in hypertension is an up (or down) regulation of the number of Na^+-pump sites. In the various tissues obtained from various animal models of hypertension, both up and down (as well as no change) regulations of pump activity have been reported (Clough *et al.*, 1977; Gheyouche *et al.*, 1981; Gothberg *et al.*, 1980; Kaniike *et al.*, 1978; Pamnani *et al.*, 1978; Sakai and Inazu, 1991). These different results could be due to differences in models of hypertension, organs, membrane purity, or methods of expressing the experimental data. For example, measurement of rubidium uptake is strongly influenced by sodium loading (Akera *et al.*, 1981). It is also difficult to accurately measure differences bet-

ween Na^+,K^+-ATPase activity in the homogenate and microsomal fractions from normotensive and hypertensive rat hearts because the Na^+,K^+-ATPase activity in these fractions is low and because it comprises a small fraction of the total ATPase activity (Lee *et al.*, 1983; Whitmer *et al.*, 1986). In addition, the existence of multiple forms of Na^+,K^+-ATPase can complicate interpretation of the experimental data.

A number of previous reports have been demonstrated the existence of multiple forms of Na^+,K^+-ATPase(Adams *et al.*, 1982; Erdmann *et al.*, 1980; Hansen, 1976; Ng and Akera, 1987; Sweadner and Gilkeson, 1985). Based upon affinity for ouabain, we has detected two forms in sarcolemma isolated from rat heart with binding constants of ca. 0.3 μM and 50 μM (Adams *et al.*, 1982; Lee *et al.*, 1983; Whitmer *et al.*, 1986). In spite of abundant suggestive reports, details of relative amounts of the isoforms and their affinities for ouabain have been largely speculative. Shull *et al.*(1986) screened rat brain cDNA libraries with a cDNA insert corresponding to the mRNA for the sheep kidney Na^+, K^+-ATPase catalytic subunit. The catalytic cDNAs from the brain library were derived from three classes of messenger RNA. One brain cDNA, which was identical with the single form in rat kidney, encodes the $\alpha(\alpha_1)$ isoform. A second brain cDNA encodes the $\alpha+(\alpha_2)$ form of the enzyme and the third class of cDNA encodes the $\alpha_{III}(\alpha_3)$ isoform of the catalytic subunit.

In a previous study(Lee *et al.*, 1983), we compared Na^+,K^+-ATPase activity, phosphorylation, and [^{3}H] ouabain binding in sarcolemma prepared from adult SHR ventricles to the same parameters in sarcolemma from mature WKY rat ventricles, and found that number of available Na^+,K^+-ATPase active sites of both the and $\alpha+$ isoforms was lower in ventricles of SHR than in WKY. The reduction in the sites was not correlated with the degree of hypertrophy. This data supports the concept that there is an association between Na^+-pump inhibition and hypertension. It is not clear, however, whether the reduced number of sodium pump sites in SHR myocardium is a primary (possibly inherited defect) or a secondary phenomenon caused by the sustained hypertension. To resolve this question, Na^+,K^+-ATPase activity of prehypertensive SHR ventricles (4 weeks old) was compared to age matched normotensive WKY ventricles.

The present data showed about 20% reduction of ventricular Na^+,K^+-ATPase activity in prehypertensive SHR. Because ventricular weights, the yield of protein in homogenates, and the recovery of sarcolemmal protein from the homogenates were not different in the two groups, this reduction cannot be accounted for by differences in protein yield or by artifacts of the isolation procedure. The reduction in Na^+,K^+-ATPase activity is due to a decrease in the amount of sodium pump sites per mg of protein since the intrinsic turnover number of enzyme was not changed in the two groups of animals.

Because only one band is observed on SDS polyacrylamide gels(Matsuda *et al.*, 1984) and because the low abundance of the higher affinity form coupled with the relative insensitivity to ouabain it has been difficult to characterize the multiple forms in rat heart. Assuming, however, that sodium dependent phosphorylation is a measure of the total number of sites of the both forms and that [^{3}H]ouabain binding detects only the higher affinity form it can be estimated that the ratio of high affinity form(now known to be $\alpha+$) to low affinity form (now known to be α) is 1 to 25 (Lee *et al.*, 1983). This estimation consists with the estimation by Young and Lingrel(1987) that the ratio of mRNA encoding $\alpha+$ to α in rat heart is 1 to 7. Other workers have reported the presence of ouabain sites in rat heart with much higher affinity (David-Dufilho *et al.*, 1984). The cause of this discrepancy is unknown but may lie in differences in methodologies or in the presence for some reason of an increased amount of the $\alpha_{III}(\alpha_3)$ isoform in these particular membrane preparations from rat heart. The majority of evidence suggests that the α and $\alpha+$ isoforms predominate in rat ventricle.

To assess whether the decrease in Na^+,K^+-ATPase activity in prehypertensive SHR ventricles is due to a decrease in α or $\alpha+$ or both, [^{3}H]ouabain binding and phosphorylation studies were carried out. [^{3}H] Ouabain binding experiments indicated that the number of binding sites at equilibrium for the higher affinity ($\alpha+$) site was the same for both SHR and WKY ventricles. Thus unlike the adult SHR where the ouabain site corresponding to the $\alpha+$ form was reduced 40-50% compared to WKY, there is no difference in immature SHR. The decrease in Na^+,K^+-ATPase activity in sarcolemma prepared from young prehypertensive SHR in the present study agrees well with the decrease in phosphorylation levels as compared to ventricular sarcolemma of normotensive WKY. These data indicate a reduction in the number of lower affinity ouabain binding sites (α isoform) in the prehypertensive SHR preparations compared to normotensive controls. Because the blood pressures were the same in two groups, this reduction cannot be accounted for by high blood pressure. Therefore, we conclude that the reduced number of α isoform of the Na^+,K^+-ATPase in the ventricles of SHR may be associated with inherited pathological features.

ACKNOWLEDGEMENT

This research was supported by the research grant from the Korea Science and Engineering Foundation (KOSEF 931-0700-028-1).

REFERENCES CITED

Adams, R. J. and Schwartz, A., Comparative mechanisms for contraction of cardiac and skeletal muscle. *Chest*, 78(Suppl), 123-139 (1980).

Adams, R. J., Schwartz, A., Grupp, G., Grupp, I., Lee, S.W., Wallick, E.T., Powell, T., Twist, A. and Gatheram, P., High affinity ouabain binding site and low dose positive inotropic effect in rat myocardium. *Nature*, 296, 167-169 (1982).

Akera, T., Yamamoto, S., Temma, K., Kim, D.-H. and Brody, T., Is ouabain-sensitive rubidium or potassium uptake a measure of sodium pump activity in isolated cardiac muscle? *Biochim. Biophys. Acta*, 640, 779-790 (1981).

Blaustein, M. P., Intracellular electrolytes and arterial hypertension, Thieme Stratton, Inc., New York, 151-157 (1980).

Clough, D. L., Pamnani, M. B., Overbeck, H. W. and Haddy, F. J., Decreased Na^+,K^+-ATPase in right ventricular myocardium of rats with one-kidney Goldblatt hypertension. *Physiologist*, 20, 18 (1977).

David-Dufilho, M., Devynck, M.-A., Beugras, J. P. and Meyer, P., Quantitative changes in cardiac Na^+,K^+-adenosine triphosphatase of spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, 6, 273-280 (1984).

Erdmann, E., Philipp, G. and Scholtz, H., Cardiac giycoside receptor, Na^+,K^+-ATPase activity and force of contraction in rat heart. *Biochem. Pharmacol.*, 29, 3219-3229 (1980).

Ferrario. C. M. and Page, I. H., Current views concerning cardiac output in the genesis of experimental hypertension. *Cir. Res.*, 43, 821-831 (1978).

Friedman, M. and Freed, S. C., Microphonic manometer for indirect determination of systolic blood pressure in the rat. *Proc. Soc. Exp. Biol. Med.*, 70, 670-672 (1949).

Ganguli, M. L. and Tobian, J., Cardiac output and peripheral resistance in strains of rats sensitive and resistant to NaCl hypertension. *Hypertension* 1, 3-7 (19 79).

Gheyouche, R., Uzan, A., Le Fur, G. and Corgier, M., Decrease in [^{3}H]ouabain binding sites in heart and brain from spontaneously hypertensive rats. *Experientia* 37, 492-493 (1981).

Gothberg, G., Jandhyala, B. and Folkow, B., Studies on the role of sodium potassiumactivated ATPase as determinant of vascular reactivity in Wistar-Kyoto and spontaneously hypertensive rats. *Clin. Science*, 59, 187S-189S (1980).

Haddy, F. J., Local control of vascular resistance as related to hypertension. *Arch. Intern. Med.*, 133, 916-931 (1974).

Haddy, F. J., Potassium and blood vessels. *Life Sci.*, 16, 1489-1498 (1975).

Haddy, F. J. and Overbeck, H. W., The role of humoral agents in volume expanded hypertension. *Life Sci.*, 19, 935-948 (1976).

Haddy, F. J., Pamnani, M. B. and Clough, D. L., Pathophysiological role of cation transport and natriuretic factors in hypertension. *Hypertension* 10, I101-107 (1987).

Haddy, F. J. and Pamnani, M. B., Pharmacologic agents for the in vivo detection of vascular sodium transport defects in hypertension. *Life Sci.*, 41(25), 2685-2696 (1987).

Hansen, O., Non-uniform populations of g-strophanthin binding sites (Na^+,K^+)-activated ATPase. *Biochim. Biophys. Acta*, 433, 388-392 (1976).

Huang, C.T., Cardona, P. and Michelakis, A.M., Existence of a new vasoactive factor in experimental hypertension. *Am. J. Physiol.*, 234, E25-E31 (1978).

Jones, A. W., Altered ion transport in vascular smooth muscle spontaneously hypertensive rats. Influence of aldosterone, norepinephrine, and angiotensin. *Cir. Res.*, 33, 563-572 (1973).

Jones, A. W., Altered ion transport in large and small arteries from spontaneously hypertensive rats and the influence of calcium. *Cir. Res.*, 34 and 35(Suppl I), I-117-I-122 (1974).

Kaloyanides, G. J., Balabanian, M. B. and Bowmann, R. L., Evidence that the brain participates in the humoral natriuretic mechanism of blood volume expansion in the dog. *J. Clin. Invest.*, 62, 1288-1295 (1978).

Kaniike, K., Sasagawa, S. and Asano, Y., Na^+,K^+-ATPase activities in brain and kidney microsome of strokeprone spontaneously hypertensive rats. *Jap. Heart J.*, 19, 595-596 (1978).

Kelly, R. A., Endogenous cardiac glycosidelike compounds. *Hypertension* 10, I 87-92 (1987).

Lee, S. W., Schwartz, A., Adams, R. J., Yamori, Y., Whitmer, K., Lane, L. K. and Wallick, E. T., Decrease in Na^+,K^+-ATPase activity and [^{3}H]ouabain binding sites in sarcolemma prepared from hearts of spontaneously hypertensive rats. *Hypertension* 5, 682-688 (1983).

Lowry, O. H., Rosebrough, H. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

Marin, J., Fernandez-Alfonso, M. S. and Sanchez-Ferrer, C. F., Sodium pump activity and contractile effect of ouabain in human placental veins. *Eur. J. Pharmacol.*, 201, 75-82 (1991).

Matsuda, T., Iwata, H. and Cooper, J. R., Specific inactivation of $\alpha(+)$ molecular form of (Na^+,K^+)-ATPase by pyrithiamin. *J. Biol. Chem.*, 259, 3858-3863 (1984).

Ng, Y. C. and Akera, T., Two classes of ouabain binding sites in ferret heart and two forms of Na^+,K^+-ATPase. *Amer. J. Physiol.*, 252, H1016-H1022 (1987).

Overbeck, H. W., Pamnani, M. B., Akera, T., Brody,

T. M. and Haddy, F. J., Depressed function of a ouabain-sensitive sodium-potassium pump in blood vessels from renal hypertensive dogs. *Circ. Res.,* 38, 48-52 (1976).

Pamnani, M. B., Clough, D. L. and Haddy, F. J., Altered activity of the sodium potassium pump in arteries of rat with steroid hypertension. *Clin. Sci. Molec. Med.,* 55, 41S-43S (1978).

Sakai, Y. and Inazu, M., Sodium pump activity and contraction of renal artery from spontaneously hypertensive rats. *Eur. J. Pharmacol.,* 200, 227-231 (1991).

Schwartz, A., Allen, J. C. and Harigaya, S., Possible involvement of cardiac Na^+,K^+-adenosine triphosphatase in the mechanism of action of cardiac glycosides. *J. Pharmacol. Exp. Ther.,* 168, 31-41 (1969).

Schwartz, A., Lindenmayer, G. E. and Allen, J. C., The sodium potassium adenosine triphosphatase: Pharmacological, Physiolgical, and biochemical aspects. *Pharmacol. Rev.,* 27, 3-134 (1975).

Shull, G. E., Greeb, J. and Lingrel, J. B., Molecular cloning of three distinct froms of the Na^+,K^+-ATPase α-subunit from rat brain. *Biochemistry* 25, 8125-8132 (1986).

Sowers, J. R., Beck, F., Stern, N. and Raghaven, S. R. V., Reduced sodium potassium dependent ATPase and its possible role in the development of hypertension in spontaneously hypertensive rats. *Clin. Exper. Hyper. Theory and Practice A5(1),* 71-86 (1983).

Sweadner, K. J., Enzymatic properties of separated isozymes of the Na^+,K^+-ATPase. Substrate affinities, kinetic cooperativity, and ion transport stoichiometry. *J. Biol. Chem.,* 260, 11508-11513 (1985).

Sweadner, K. J. and Gilkeson, R. C., Two isozymes of the Na^+,K^+-ATPase have distinct antigenic determinants. *J. Biol. Chem.,* 260, 9016-9022 (1985).

Van Alstyne, E., Burch R. M., Knickelbein, R. G., Hungerford, R. T., Gower, F. J., Webb, J. G., Poe, S. L. and Lindenmayer, G. E., Isolation of sealed vesicles highly enriched with sarcolemma markers from canine ventricle. *Biochim. Biophys. Acta.,* 602, 131-143 (1980).

Wallick, E. T., Anner, B. M., Ray, M. V. and Schwartz, A., Effect of temperture on phosphorylation and ouabain binding to N-ethylmaleimide treated Na^+, K^+-ATPase. *J. Biol. Chem.,* 253, 8778-8786 (1978).

Wallick, E. T. and Schwartz, A., Nature of the transport ATPase digitalis complex. X. Thermodynamics of the rate of ouabain binding. *J. Biol. Chem.,* 249, 5141-5147 (1974).

Wallick, E. T., Lane, L. K. and Schwartz, A., Biochemical mechanism of the sodium pump. *Ann. Rev. Physiol.,* 41, 397-411 (1979).

Whitmer, K. R., Lee, J. H., Martin, A. F., Lane, L. K., Lee, S. W., Schwartz, A., Overbeck, H. W. and Wallick, E. T., Myocardial Na^+,K^+-ATPase in one-kidney, one-clip hypertensive rats. *J. Mol. Cell. Cardiol.,* 18, 1085-1095 (1986).

Willems, W. J., Willems M. W. and Stekiel, W. J., The effect of tetrodotoxin(TTX) upon mesenteric venous membrane potential and diameter in spontaneously hypertensive rats(SHR)(abstr.). *Physiologist* 18, 451 (1975).

Young, R. M. and Lingrel, J. B., Tissue distribution of mRNAs encoding the isoforms and subunit of rat Na^+,K^+-ATPase. *Biochem. Biophys. Res. Commun.,* 145, 52-58 (1987).