Relationship of Vascular Sodium-Potassium Pump Activity to Intracellular Sodium in Hypertensive Rats

TOMMY A. BROCK, PH.D., JEFFREY B. SMITH, PH.D., AND HENRY W. OVERBECK, M.D., PH.D.

SUMMARY The activity of the sodium-potassium (Na\(^+\)-K\(^+\)) pump in arterial tissue from rats with chronic DOCA-salt or one-kidney Grollman renal hypertension was assayed in vitro as the rate of ouabain-sensitive \(\text{Rb}^+\) uptake. Estimates of total cell Na\(^+\) and tissue K\(^-\) were made by a lithium-substitution method on the same segment of arterial tissue. In both freshly excised tail arteries and aortas, and in aortas after overnight cold storage at 4° C and a 3-hour equilibration at 37° C in Krebs-Henseleit, cell Na\(^+\) content did not differ significantly between control and hypertensive groups. However, ouabain-sensitive \(\text{Rb}^+\) uptake was increased in arteries from DOCA-salt and renal hypertensive rats as compared to controls. In overnight-stored and 3-hour equilibrated aortic tissue, we then used low Na\(^+\) medium to reduce, or monensin, a Na\(^+\) ionophore, to increase total cell Na\(^+\), to study the relationship between cell Na\(^+\) and Na\(^+\)-K\(^+\) pump activity. We observed a sigmoid relationship between total cell Na\(^+\) and ouabain-sensitive \(\text{Rb}^+\) uptake in tissue from all groups of rats. However, in tissue from DOCA-salt and renal hypertensive rats, the relationship between cell Na\(^+\) and pump activity was shifted, indicating a greater pump activity for each level of total cell Na\(^+\) and greater maximal pumping. These data suggest that increases observed in pump activity in vitro arterial tissue from hypertensive rats may not be solely attributable to elevated cell Na\(^+\) content and may also involve increases in number of active sarcolemmal pump molecules per unit tissue weight or in their turnover rate.

(Hypertension 4 (suppl II): II-43-II-48, 1982)

KEY WORDS • Na\(^+\)-K\(^+\) ATPase • aorta • tail artery • monensin • DOCA-salt hypertension • Grollman hypertension

THE presence of an electrogenic Na\(^+\)-K\(^+\) pump in the sarcolemma of vascular smooth muscle is well documented.\(^1\)\(^-\)\(^3\) Stimulation of this Na\(^+\)-K\(^+\) pump results in hyperpolarization of the cell membrane, muscle relaxation, and depression of contractile responses to vasoconstrictor drugs.\(^4\)\(^-\)\(^10\) As measured by different in vitro techniques, increased active transport of Na\(^+\) and K\(^-\) has been observed in arterial smooth muscle from spontaneously hypertensive rats,\(^11\)\(^-\)\(^12\) from Dahl salt-sensitive hypertensive rats,\(^13\)\(^-\)\(^15\) and from DOCA-salt hypertensive rats.\(^11\)\(^-\)\(^12\)\(^-\)\(^18\) It has been suggested that these increases in vascular Na\(^+\)-K\(^+\) pump activity in hypertension result from elevations in the concentration of intracellular Na\(^+\), associated with increased passive permeability of the cell membrane to Na\(^+\).\(^19\)\(^-\)\(^22\) Overbeck et al.\(^23\)\(^-\)\(^26\) however, suggested that additional factors may be involved. They found that the lower levels of Na\(^+\)-K\(^+\) pump activity in tissues from normotensive control animals could not be explained by insufficient amounts of total cell Na\(^+\).

There is little information regarding the dependence of the Na\(^+\)-K\(^+\) pump on internal Na\(^+\) in vascular smooth muscle. Therefore, in this study we investigated the relationship between total cell Na\(^+\) and pump activity. To alter cell Na\(^+\), we used a medium with low Na\(^+\) concentration, different excision and incubation techniques, and monensin, a Na\(^+\) ionophore. It has been previously shown that monensin increases the entry of Na\(^+\) into Swiss 3T3 cells, thereby stimulating Na\(^+\)-K\(^+\) pump activity.\(^27\) We studied arterial tissue from rats in the chronic, uncomplicated stages of one-kidney DOCA-salt and one-kidney, one-figure-8 (Grollman) hypertension. We describe procedures for assaying Na\(^+\)-K\(^+\) pump activity and measuring total cell Na\(^+\) and tissue K\(^-\) on the same segment of arterial tissue.
Methods

Animals

We induced DOCA-salt hypertension in uninephrectomized, male Sprague-Dawley rats (125-175 g) by implanting strips of silicone rubber impregnated with deoxytocorticosterone acetate (150 mg/kg). Uninephrectomized rats served as normotensive controls. Both groups were given a standard diet (Na+ 0.39%, K+ 0.96%), and 1% NaCl:0.2% KCl solution to drink ad libitum. To prepare one-kidney, one figure-8 (Grollman) renal hypertensive animals we tied a figure-8 ligature (0 silk) around the left kidney of male Sprague-Dawley rats (75-100 g) and then 1 week later removed the contralateral kidney. Sham-constricted uninephrectomized rats served as normotensive controls. Both groups of rats received the standard diet and tap water ad libitum. In all rats, systolic blood pressure (SBP) was monitored weekly by indirect tail cuff plethysmography (Natsume, Peninsula Laboratories) in conscious restrained animals. Arterial tissue was excised from these animals approximately 6 weeks after the surgical procedures and approximately 4 weeks after the development of significant hypertension (SBP > 150 mm Hg).

Tissue **Rb** Uptake, Na+ and K+ Measurements

The activity of the vascular Na+-K+ pump was assayed by measuring ouabain-sensitive **Rb**+ uptake in: 1) thoracic aortas that were stored overnight at 4° C and then allowed to restore ionic gradients; and 2) in freshly excised tail arteries and aortas. Aortas that were stored overnight were excised from unanesthetized, decapitated rats, while fresh tail arteries and aortas to be processed immediately were excised from rats anesthetized with sodium pentobarbital, 50 mg/kg body weight. In brief, the excised vessels were rapidly dissected free from loose connective tissue in 4° C Krebs-Henseleit (K-H) solution (mM): NaCl, 118; NaHCO3, 27; KCl, 4.8; KH2PO4, 1.0; MgSO4•7H2O, 1.2; CaCl2•2H2O, 1.25; and glucose, 11.1; pH 7.4. Each vessel was opened longitudinally and cut transversely into segments. Aortic segments to be stored overnight at 4° C were transferred to cold K-H solution prior to measuring **Rb**+ uptake. The following day, these segments were equilibrated for 3 hours at 37° C in continuously aerated (95% O2:5% CO2) K-H solution prior to measuring **Rb**+ uptake. In the freshly excised arterial tissue **Rb**+ uptake was measured immediately (less than 5 minutes) after excision.

For measurements of **Rb**+ uptake, tissue segments were preincubated in 2.0 ml of aerated K-H solution at 37° C for 5 minutes. **RbCl** (New England Nuclear; approximately 150-175 cpm • nmole-1) was then added, and tissue **Rb**+ uptake was allowed to occur for 15 minutes. Immediately following this incubation period each segment was rapidly rinsed (10 seconds) in a 4° C buffered salt solution in which LiCl was substituted for NaCl (mM): LiCl, 120; CaCl2, 2.0; MgCl2, 1.0; KCl, 5.0; and HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-Tris buffer, 20; pH 7.6. The segment was then incubated in a separate tube containing this same buffer for 40 minutes. This procedure allowed the measurement of cellular Na+ by methods developed by Friedman and coworkers.22 23 Tissues were gently blotted, transferred to acid-washed tubes, and weighed. Tissues were then dried (100° C, 48 hours) and reweighed. To each sample, 1 ml of 0.75 N HNO3 was added, and the radioactivity present in a 0.9 ml extract was determined by Cerenkov radiation in a liquid scintillation counter (Packard B-2450). The Na+ and K+ contents of this same extract were determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 372). The rate of ouabain-sensitive **Rb**+ uptake was calculated as the difference in the rates of **Rb**+ uptake by aortic tissue from DOCA-salt, one-kidney Grollman and control rats.

Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM). Data were analyzed by unpaired Student's t test where appropriate. A p value ≤ 0.05 was considered to be significant.
Results

Systolic blood pressures were significantly elevated ($p < 0.05$) in both groups of hypertensive animals at 2 weeks following surgery, and at 6 weeks the values were (mm Hg, mean ± SEM): control, 135 ± 5, vs DOCA-salt, 190 ± 4; control, 131 ± 4, vs one-kidney Grollman, 203 ± 6. Serum creatinine in the same rats remained within the normal range in all groups of animals (mg%): control, 0.7 ± 0.1; DOCA, 0.8 ± 0.2; one-kidney Grollman, 0.6 ± 0.1. Serum $K^+$ also did not differ between groups (mEq/liter): control, 3.6 ± 0.3; DOCA, 3.8 ± 0.1; one-kidney Grollman, 3.4 ± 0.2. All animals were apparently in good general health 6 weeks after the surgical procedures when arterial tissue was excised.

Figure 1 illustrates $^{86}Rb^+$ uptake by aortic tissue that had been stored overnight at 4° C and allowed to equilibrate for 3 hours the next day. As indicated, both the ouabain-insensitive and the ouabain-sensitive components of $^{86}Rb^+$ uptake were increased in aortic tissue from DOCA-salt and one-kidney Grollman hypertensive rats, as compared to their respective controls ($p < 0.05$). The results in figures 2 and 3 demonstrate that cell $Na^+$ was not different in the hypertensive and control groups. Tissue $K^+$ also was not significantly different in any of the groups (control vs DOCA-salt, 82 ± 7, vs 80 ± 6 mmoles • kg dry wt$^{-1}$; control vs one-kidney Grollman, 82 ± 6 vs 87 ± 6 mmoles • kg dry wt$^{-1}$).

Figure 2 shows the rate of ouabain-sensitive $^{86}Rb^+$ uptakes in aortic tissue from DOCA-salt and control

---

**Figure 2.** Top: Mean ± SEM of in vitro ouabain-sensitive $^{86}Rb^+$ uptake by freshly excised or stored aortas from control and DOCA-salt rats. Tissue was stored overnight at 4° C and allowed to equilibrate the following day for 3 hours prior to $^{86}Rb^+$ uptake studies. The concentration of monensin was 10 μg/ml. Bottom: Mean ± SEM of total cell $Na^+$ in the same tissue. Cellular $Na^+$ was measured using a lithium-substitution method. The numbers in parentheses represent the number of animals in each group. *$p \leq 0.05$.

**Figure 3.** Top: Mean ± SEM of in vitro ouabain-sensitive $^{86}Rb^+$ uptake by aortic tissue from one-kidney Grollman hypertensive rats under different incubation conditions. Tissue was stored overnight at 4° C and allowed to equilibrate the following day for 3 hours prior to $^{86}Rb^+$ uptake studies. The tissue was then placed in buffer containing either 150 mM Na$^+$ or 20 mM Na$^+$. The concentration of monensin was 10 μg/ml. Bottom: Mean ± SEM of total cell $Na^+$ in the same tissues. Data are expressed as previously described. *$p \leq 0.05$. 
rats under incubation conditions that produced different levels of total cell Na\(^+\). In freshly excised tissue, Na\(^+\)-K\(^+\) pump activity was highest. Equilibrating stored tissues for 3 hours in K-H markedly reduced pump activity. Monensin stimulated ouabain-sensitive \(^{86}\)Rb\(^+\) uptake in equilibrated tissues, but not to the level found in fresh tissue. These changes in ouabain-sensitive \(^{86}\)Rb\(^+\) uptake reflected changes in total cell Na\(^+\) (fig. 2, \(p < 0.05\)). It should be noted, however, that ouabain-sensitive \(^{86}\)Rb\(^+\) uptake was always higher in hypertensive tissue for any given condition, although total cell Na\(^+\) did not differ between groups. In freshly excised tissue, aortic K\(^+\) content was significantly lower in the DOCA-salt group (DOCA vs control: 83 ± 10 vs 120 ± 5 mmoles • kg dry wt\(^{-1}\), \(p < 0.05\)). Unchanged cell Na\(^+\) and decreased K\(^+\) content of tail arteries from DOCA-hypertensive rats have previously been found.\(^{26}\)

We also assayed pump activity in freshly excised tail arteries from DOCA-salt and control rats. As in aortic tissue, ouabain-sensitive \(^{86}\)Rb\(^+\) uptake was higher in tail arteries from DOCA-salt hypertensive rats (control, \(n = 7\) vs DOCA-salt, \(n = 7\): 18.8 ± 2.6 vs 27.3 ± 3.0 nmoles • 15 min\(^{-1}\) • mg dry wt\(^{-1}\), \(p < 0.05\)). Again, total cell Na\(^+\) did not differ between groups (control vs DOCA-salt: 61.6 ± 3.2 vs 60.2 ± 3.7 mmoles • kg dry wt\(^{-1}\)). No differences were detected in ouabain-insensitive \(^{86}\)Rb\(^+\) uptake.

In aortic tissue from the rats with one-kidney Grollman hypertension, total cell Na\(^+\) was lowered by incubation of the segments in the low Na\(^+\) medium (fig. 3). Again a decrease in total cell Na\(^+\) was accompanied by a reduction in ouabain-sensitive \(^{86}\)Rb\(^+\) uptake. However, \(^{86}\)Rb\(^+\) uptake remained greater in the renal hypertensive group. In the presence of monensin, ouabain-sensitive \(^{86}\)Rb\(^+\) uptake remained greater in tissue from the renal hypertensive group. The observed differences in \(^{86}\)Rb\(^+\) uptake could not be attributed to differences in total cell Na\(^+\).

Figure 4 represents a plot of ouabain-sensitive \(^{86}\)Rb\(^+\) uptake as a function of total cell Na\(^+\) content in aortic tissue from control and DOCA-salt rats. This figure represents a composite of all data collected in freshly excised aortas and in aortas stored overnight at 4° C and allowed to equilibrate for 3 hours. Data from control normotensive animals drinking water or saline were pooled. Number of animals is indicated in the parentheses.

Discussion

The functional status of the Na\(^+\)-K\(^+\) pump in vascular smooth muscle in hypertension is not well understood, in part because the relationship between pump activity and intracellular Na\(^+\) is not clear. We designed the present study to examine the effect of induced changes in total cell Na\(^+\) on pump activity in arterial tissue from rats with chronic DOCA-salt and one-kidney, one figure-8 (Grollman) hypertension. The most significant finding of this study is that Na\(^+\)-K\(^+\) pump activity, as assessed by ouabain-sensitive \(^{86}\)Rb\(^+\) uptake, is always increased in arterial tissue from hypertensive as compared to normotensive rats under conditions that altered cell Na\(^+\). These differences in pump activity cannot be attributed to differences in total cell Na\(^+\).

Our results in freshly excised arterial tissue from rats with chronic DOCA-salt hypertension differ from the decreases in in vitro vascular pump activity previously reported in fresh tissue.\(^{22}\) These observed decreases in pump activity in DOCA-salt (presumably volume-expanded) hypertension have been attributed to the effects of a circulating digitalis-like substance.\(^{20, 31}\) Technical differences related to assay conditions, to duration or severity of the hypertension, or to tissue K\(^+\) levels may explain the discrepancies.

Under the usual in vitro conditions of excess K\(^+\) and ATP, the activity of the Na\(^+\)-K\(^+\) pump in cells is limited by the relatively low concentration of intracellular Na\(^+\).\(^{23}\) In the present study, we observed a similar dependence of the pump on total cell Na\(^+\) in aortic tissue from both control and hypertensive rats. In freshly excised aortas with high levels of intracellular Na\(^+\), ouabain-sensitive \(^{86}\)Rb\(^+\) uptake was extremely high, while in tissue allowed to equilibrate
with K-H solution in order to lower total cell Na\(^+\), "Rb\(^+\) uptake was much less (fig. 2). Incubation of aortic tissue in low Na medium reduced total cell Na\(^+\) and "Rb\(^+\) uptake to their lowest levels, whereas addition of monensin, a Na\(^+\) ionophore, increased both total cell Na\(^+\) and "Rb\(^+\) uptake in equilibrated tissues (figs. 2 and 3), as has previously been found in Swiss 3T3 cells. These observations suggest that normally the low level of Na\(^+\) inside the vascular smooth muscle cell limits the activity of the Na\(^+\)-K\(^+\) pump and that elevating total cell Na\(^+\) increases pump activity.

A sigmoid relationship between cell Na\(^+\) and pump activity is seen in erythrocytes. There appears to be a similar sigmoid relationship between total cell Na\(^+\) and pump activity in aortic tissue from control, DOCA-salt, and Grollman hypertensive rats (fig. 4). This type of relationship between pump activity and internal Na\(^+\) in vascular smooth muscle was also seen by Jones. As compared to the controls, the curve for tissue from the DOCA-salt hypertensive rats was shifted, as was also observed by Jones. These data suggest that, for any level of total cell Na\(^+\), Na\(^+\)-K\(^+\) pump activity is higher in arterial tissue from the DOCA-salt hypertensive rats.

Friedman et al. have demonstrated that there is a slightly increased exchange of Li\(^+\) for cell Na\(^+\) and K\(^+\) in tail artery from DOCA-salt hypertensive rats. Since we used a Li\(^+\) substitution method to estimate cell Na\(^+\), increased Na\(^+\)-Li\(^+\) exchange might account for a portion of the shift of the DOCA-curve. Jones did not use the Li\(^+\) substitution method to estimate cellular Na\(^+\), but found a similar shift in the curve relating K\(^+\)-stimulated Na\(^+\) efflux and cell Na\(^+\) in aortic tissue from DOCA-salt hypertensive rats.

There is strong evidence that the vascular smooth muscle membrane is more permeable to Na\(^+\) in animals with certain forms of hypertension. This "leakiness" of the plasma membrane would be expected to supply the pump with more of its rate-limiting substrate, Na\(^+\). The net result would be an increase in the active transport of Na\(^+\) out of the cell, thus preventing Na\(^+\) accumulation. Therefore, the increased pump activity seen in hypertensive arteries may be the major factor in maintaining cell Na\(^+\) at normal levels in our preparations.

In contrast, under in vitro conditions where total cell Na\(^+\) is not rate-limiting for maximal pump activity, e.g., freshly dissected tissue with excess intracellular Na\(^+\), increased entry of Na\(^+\) into the cell would not be expected to increase pump activity because the Na\(^+\) sites on the pump molecule would be saturated. Thus, our data, and those of others, suggest that factors additional to the level of intracellular sodium may be involved in elevating pump activity in arteries from hypertensive rats. Jones found that maximal K\(^+\)-stimulated Na\(^+\) efflux is increased in aortic tissue from DOCA-salt hypertensive rats. In the present study, freshly excised, Na\(^+\)-loaded aortic tissue from DOCA-hypertensive rats exhibited a similar increase in maximal pump activity. The amount of total cell Na\(^+\) in freshly-excised aortic tissue from normal animals has been shown by Overbeck et al. to be sufficient to maximally stimulate the Na\(^+\)-K\(^+\) pump; however, maximal pumping by Na\(^+\)-loaded normotensive control arteries remains lower than that of hypertensive (Dahl saltsensitive and Goldblatt) arteries.

Monensin increased membrane permeability to Na\(^+\) in aortic tissue from both normotensive and hypertensive rats. Yet, even in the presence of monensin, sodium pump activity for a given level of total cell Na\(^+\) in normotensive tissues remained lower than that in corresponding tissue from DOCA-salt or Grollman hypertensive rats (fig. 2 and 3), suggesting that differences in pump activity may not simply reflect differences in membrane permeability.

Thus, we and others have interpreted the elevated pump activity in arteries from hypertensive rats studied in vitro as evidence for an increase in the number of active pump molecules per unit tissue weight or in their turnover rate. Overbeck et al. have suggested that a circulating, digitalis-like pump inhibitor may induce new pump molecules in vivo. Observations of increased pump activity in hypertensive vascular tissue studied in vitro remains compatible with this hypothesis, because there is in vivo evidence in renal hypertensive rats for decreased pump activity suggesting inhibition. In this regard, when vascular tissue is removed and studied in vitro, the inhibitor may rapidly dissociate from rat tissue and the effects of the added active pump molecules are then seen as increased pump activity. Involvement of endogenous substances that stimulate the pump, such as prostaglandins, or induced synthesis of pump molecules by mineralocorticoids, merit consideration as additional factors that may increase vascular pump activity in hypertension. Further studies are needed to clarify these possibilities.

References


27. Smith JB, Rozengurt E: Serum stimulates the Na\(^+\)-K\(^+\) pump in quiescent fibroblasts by increasing Na\(^+\) entry. Proc Natl Acad Sci 75: 5560, 1978


31. De Wardener HE, MacGregor GA: Dahl's hypothesis that a saluretic substance may be responsible for a sustained rise in arterial pressure: Its possible role in essential hypertension. Kidney Internat 18(1), 1980


Relationship of vascular sodium-potassium pump activity to intracellular sodium in hypertensive rats.
T A Brock, J B Smith and H W Overbeck

Hypertension. 1982;4:43-48
doi: 10.1161/01.HYP.4.3_Pt_2.43

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/4/3_Pt_2/43

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/