Potassium and Activity of the Sodium Hydrogen Exchanger Isoform 1 in Vascular Smooth Muscle of Hypertensive Rats

Paulene A. Quinn and Leong L. Ng

Low potassium intake is inversely associated with blood pressure. In vitro, the proliferation of vascular smooth muscle cells (VSMCs) shows an inverse correlation with $[K^+]$. In hypertension, many studies have established that the ubiquitous Na^+/H^+ exchanger isoform 1 (NHE-1) exhibits increased activity and is permissive for cell proliferation. Changes in extracellular $[K^+]$ lead to altered intracellular Na^+ content, which could affect NHE activity and NHE-1 protein expression. We therefore investigated the effects of altering extracellular $[K^+]$ on NHE activity and NHE-1 expression in cultured VSMCs of both the spontaneously hypertensive rat (SHR) and its normotensive Wistar-Kyoto counterpart (WKY). Culture of SHR VSMCs for 48 hours in media containing 2, 4, 6, and 8 mmol · L^{-1} $[K^+]$ led to activation of NHE-1 in the low $[K^+]$ media (NHE-1 activity at $[K^+]$ 2, 4, 6, and 8 mmol · L^{-1} were 34.3 ± 1.7, 29.5 ± 1.1, 27.7 ± 1.4, and 26.1 ± 2.1 mmol · L^{-1} min^{-1}, $P < .006$ by analysis of variance [ANOVA]). This was not associated with any significant changes in intracellular pH. By contrast, WKY VSMCs did not exhibit any significant activation of NHE-1 in low $[K^+]$ media (NHE-1 activity at $[K^+]$ 2, 4, 6, and 8 mmol · L^{-1} were 24.3 ± 2.9, 22.3 ± 1.7, 19.0 ± 1.8, and 18.6 ± 1.6 mmol · l^{-1} min^{-1}, P = not significant [NS] by ANOVA). Culture of SHR or WKY VSMCs in low $[K^+]$ media did not alter NHE-1 protein expression, suggesting the enhancement of activity in SHR cells was due to an increased turnover number of NHE-1. This response of NHE-1 in SHR VSMCs to K^+ depletion indicated a direct effect on these cells and could potentially enhance the contractile or proliferative phenotype of these cells in vivo.

PREVIOUS EPIDEMIOLOGIC studies have suggested that the prevalence of hypertension may vary inversely with the dietary intake of K^+.[1] Furthermore, dietary depletion of K^+ leads to higher blood pressure levels and an increased salt sensitivity,[2] whereas K^+ supplementation may lower blood pressure.[3] Stroke-associated mortality in humans is also lower in those with a higher intake of K^+,[4] and this protective effect is greater than could be accounted for by the blood pressure lowering effect of K^+. Similar findings have been reported in hypertensive rats, in which a high potassium intake protects against stroke mortality, independent of an effect on blood pressure.[5] The mechanism for the lowering of blood pressure or stroke mortality is not known, but suggestions for possible targets of K^+ include the kidney tubular epithelium and the blood vessel wall.

Depletion of K^+ leads to kidney hypertrophy, and this is demonstrated in vitro by increased kidney epithelial cell proliferation when exposed to low $[K^+]$ media.[6] This mechanism is dependent on the presence of Na^+, consistent with the view that a transient increase in intracellular Na^+ ($[Na^+]_i$) from increased influx is necessary.[6] This effect of K^+ is also manifest with vascular smooth muscle cells (VSMCs) in culture, in which there is an inverse correlation between VSMC proliferation and $[K^+]$ of the growth medium, and a direct correlation with $[Na^+]_i$.[7] Although part of the effect of low $[K^+]$ media on $[Na^+]_i$ is mediated by a reduced Na^+,K^+-adenosine triphosphate (ATP)ase activity leading to lowered Na^+ efflux, prolonged incubations in low $[K^+]$ media lead to a compensatory upregulation of Na^+,K^+-ATPase activity and increased synthesis of pump units.[8] An additional mechanism may be altered Na^+ influx, and the majority of this influx in many cell types (including VSMCs) is mediated by the Na^+/H^+ exchanger (NHE).

The NHE is an ubiquitous membrane transporter that utilizes the inwardly directed Na^+ gradient (generated by the Na^+,K^+-ATPase) to expel H^+ from the cell.[9] Its many functions include regulation of cell pH and volume[9] and a permissive role in the regulation of cell proliferation.[10] NHE activity is crucial for cell cycle progression through an effect on ribonucleotide reductase, a critical enzyme for DNA synthesis,[10] and VSMC proliferation has been demonstrated to be affected by NHE activity.[11] Since the cloning of the first isoform of NHE (NHE-1) by Sardet et al,[12] other members of this family of exchangers have been described that have a more restricted localization to epithelia,[13] and which may affect trans-epithelial Na^+ absorption. Several studies have consistently demonstrated an elevated NHE activity in leukocytes, platelets, red blood cells, VSMCs, and striated muscle cells in hypertensive humans and spontaneously hypertensive rats (SHR),[14-18] and this abnormality may be associated with the increased VSMC proliferation.[16] This phenomenon is also likely to reflect changes in NHE-1 rather than the other epithelial isoforms. Using NHE-1 isoform-specific antibodies, we have previously demonstrated that in VSMC cultures from SHR, the increased cell proliferation and NHE activity were associated with no change in expression of NHE-1 protein, indicating that transport was mediated by an increased turnover number for NHE-1.[19] These studies were performed using media containing 5 mmol · L^{-1} K^+, and the effects of altered K^+ on NHE-1 activity or expression are currently unknown, although reduced $[K^+]$ enhances VSMC proliferation.[7] However, in low $[K^+]$ media, the increase in $[Na^+]_i$ may reduce the driving force for H^+ expulsion by the NHE, and this may lead to compensatory changes in NHE activity and NHE-1 expression. Such an adaptation has been shown to occur with the Na^+,K^+-ATPase[8] when cells are incubated in low $[K^+]$ media, but has never been documented

From the Department of Medicine and Therapeutics, University of Leicester, Leicester, UK.

Submitted July 26, 2000; accepted January 11, 2001.

Supported by the British Heart Foundation.

Address reprint requests to Leong L. Ng, MD, Department of Medicine and Therapeutics, Robert Kilpatrick Clinical Sciences Bldg, Leicester Royal Infirmary, Leicester LE2 7LX, UK.

0026-0495/01/5007-0012$35.00/0

doi:10.1053/meta.2001.24204

for NHE-1. A precedent for possible K^+ modulated changes in VSMC NHE-1 activity and expression lies in experiments performed on kidney brush border membrane vesicles from K^+-depleted rats.[20] In rats fed a K^+-deficient diet for up to 4 weeks, brush border membrane NHE activity was increased, as was basolateral membrane vesicle Na^+/HCO_3^- cotransport.[20] However, these studies on kidney membranes may reflect NHE-3 rather than NHE-1, and other humoral influences may also be operating in vivo. There are no studies on VSMC NHE-1 activity or expression in vitro reflecting a direct effect of K^+ on VSMC exchanger expression and function. We therefore investigated the effects on NHE-1 activity and expression of culturing SHR and Wistar-Kyoto (WKY) rat VSMCs in media of varying K^+ composition.

MATERIALS AND METHODS

Dulbecco's modified Eagle's medium (DMEM), Ham's F12 medium, and chick embryo extract were from ICN Flow (High Wycombe, Bucks, UK). Fetal calf serum (FCS) was from Advanced Protein Products, Birmingham, UK. Ham's F12 growth medium was buffered with 14 mmol · L^{-1} $NaHCO_3$ (pH 7.1 with 5% CO_2 in air) and contained 15% FCS, 0.5% (wt/vol) chick embryo extract, 2 mmol · L^{-1} glutamine and 10^5 IU penicillin L^{-1}, 100 mg streptomycin L^{-1}. Enhanced chemiluminescence kits were obtained from Amersham International, Amersham, UK. Protein A Sepharose CL4B was purchased from Pharmacia LKB Biotechnology, Uppsala, Sweden. 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF/AM) was from Cambridge Bioscience, Cambridge, UK. All other chemicals were from Sigma Chemical Co, Poole, UK. The NHE-1–specific inhibitor HOE-694 (3-methylsulfonyl-4-piperidinobenzoyl guanidine)[21] was obtained from Dr W. Scholz, Hoechst AG, Frankfurt, Germany.

Culture of Vascular Myocytes and Measurement of Na^+/H^+ Exchanger Activity

Vascular myocytes were obtained from thoracic aorta of 12 each of WKY and SHR, which were maintained at a colony established at the Biomedical Services Unit, Leicester University. Blood pressures of 12-week-old rats by the tail-cuff method confirmed values in SHR exceeding 170 mm Hg with values under 120 mm Hg in WKY. Vascular myocytes were obtained by enzymatic digestion with collagenase as described[17,19] and cultured in Ham's F12 growth medium. All experiments reported were performed on cultures between passage numbers 3 and 8. There were no significant changes in NHE-1 activity between these passages.

Measurements of NHE activity were performed on confluent vascular myocytes on cover slips.[17,19] Cells were seeded on cover slips and cultured for 1 to 2 days in media of varying K^+ composition. Ham's F12 media without K^+ was used to reconstitute these culture media, with added K^+ ranging from 2, 4, 6, and 8 mmol · L^{-1}. In preliminary experiments, we established that [K^+] below 1 mmol · L^{-1} was not tolerated by the vascular myocytes for periods more than 24 hours, and thus, we have chosen a value of 2 mmol · L^{-1} as the lowest concentration that could be sustained for longer periods. FCS was dialyzed in K^+-free HEPES-buffered saline for 2 days to deplete serum of K^+ before addition to the culture media.

Measurements of cytosolic pH (pH_i) were performed after loading cells with the fluorophore BCECF.[17,19] Intracellular pH and Na^+-dependent H^+ fluxes were measured in HEPES-buffered saline (HBSS) composed of (in mmol · L^{-1}) NaCl 130, KCl (varying from 2 to 8), $CaCl_2$ 1.8, $MgSO_4$ 1, glucose 5, HEPES 20, bovine serum albumin (BSA) 1 g · L^{-1}, pH 7.4, after clamping pH_i to 6.0.[17,19] Buffering capacity at pH_i 6.0 was also measured using an NH_4Cl pulse. Calibration was achieved with isotonic KCl buffers of different pH, containing nigericin and monensin (5 μmol · L^{-1} of each).

Western Blotting of VSMC Extracts for NHE-1 Protein

We have previously raised specific antibodies against rat NHE-1 using a glutathione-S-transferase containing the C-terminal cytoplasmic domain of rat NHE-1.[22] The protein A purified IgG fraction, G116, was used to detect rat NHE-1 protein in Western blots of VSMC extracts. VSMC from SHR and WKY were cultured for 48 hours in the growth media containing varying [K^+]. The VSMC cultures were rapidly snap frozen in liquid nitrogen and the cells then scraped into 0.5 mL of extraction buffer composed of (in mmol · L^{-1}) 50 Tris pH 7.4, NaCl 150, EDTA 5, phenylmethylsulphonyl fluoride 1, o-phenanthroline 1, and iodoacetamide 1. This suspension was mixed with an equal volume of gel sample buffer (125 mmol · L^{-1} Tris pH 6.8, 5% sodium dodecyl sulphate, 20% glycerol, 0.004% bromophenol blue solution) followed by boiling for 10 minutes. Aliquots (50 μg protein) were then resolved on 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. After electroblotting onto supported nitrocellulose, membranes were blocked with 10% low-fat milk powder in Tris-buffered saline containing 0.1% Tween 20. The membranes were then incubated with 1 μg/mL G116 antibody, followed by horseradish peroxidase-linked donkey antirabbit second antibody and detection with enhanced chemiluminescence reagents. The bands were visualized on preflashed x-ray film.

Statistics

Results are expressed as mean ± SEM, and comparisons were by ANOVA and Student's *t* test, performed using the software package Minitab (Minitab, Inc, State College, PA). Two-tailed *P* values less than .05 were considered significant and *P* values for multiple comparisons with the *t* test were subjected to the Bonferonni correction.

RESULTS

An example of VSMC cultures from WKY and SHR incubated for 48 hours in 2 or 8 mmol · L^{-1} [K^+] and then clamped to pH_i 6.0 is illustrated in Fig 1. Introduction of Na^+ containing HBSS at the arrow led to external Na^+-dependent H^+ efflux and thus intracellular alkalinization in all of the VSMCs. The rate of Na^+-dependent H^+ efflux in SHR or WKY cultures in 2 mmol · L^{-1} [K^+] was greater than that at 8 mmol · L^{-1} [K^+].

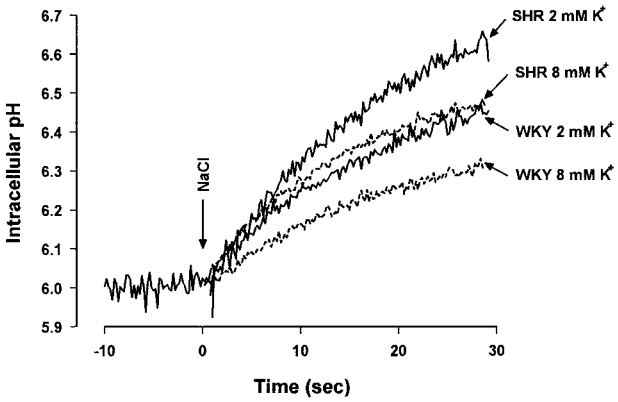

Fig 1. Intracellular pH measurements from SHR and WKY VSMCs cultured for 48 hours in 2 mmol · L^{-1} (continuous lines) or 8 mmol · L^{-1} [K^+] (dotted lines). The NaCl containing HBSS was introduced at the arrow onto VSMCs on cover slips following clamping of pH_i to 6.0.

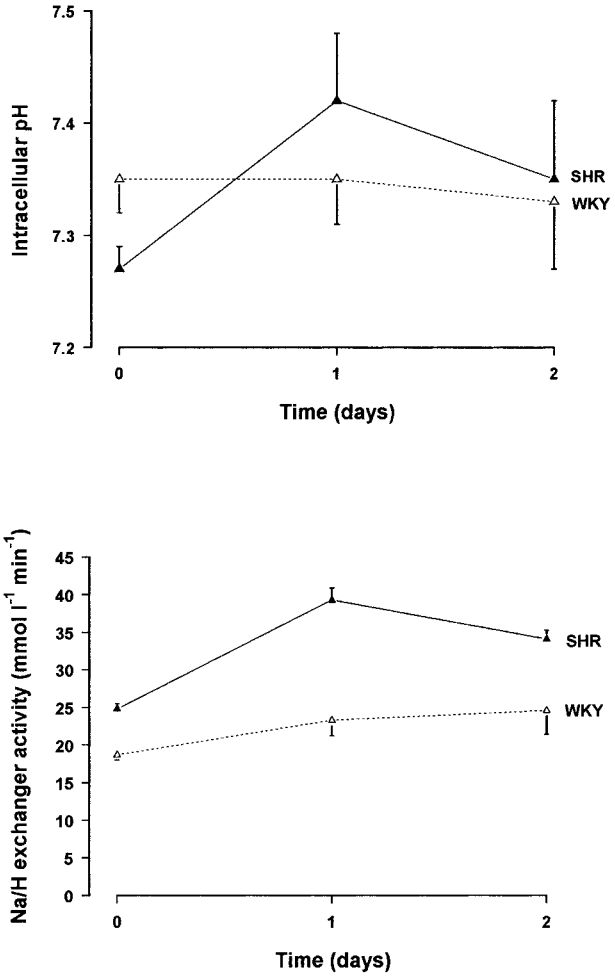

Fig 2. Effect of incubation of SHR and WKY VSMCs in growth media containing [K^+] of 2 mmol · L^{-1} for periods up to 48 hours on the measurements of pH_i and Na^+/H^+ exchanger activity. There were no significant changes in pH_i in WKY cultures, but SHR VSMCs exhibited a time-dependent change in pH_i and Na^+/H^+ exchanger activity (ANOVA, $P < .001$ for both, n = 12).

Culture of VSMC from SHR and WKY in 2 and 8 mmol · L^{-1} [K^+] were studied following 24 and 48 hours of incubation in the growth media (Fig 2). WKY VSMCs demonstrated no significant change in pH_i, and there was also no change in the Na^+/H^+ exchanger activity (P = not significant [NS] by ANOVA for both). In contrast, culture of the VSMC from SHR in 2 mmol · L^{-1} media led to a time-dependent change in pH_i (ANOVA $P < .02$). There was a transient increase in the pH_i within 24 hours ($P < .05$, n = 12), which was not sustained at 48 hours. This was associated with a marked activation of Na^+/H^+ exchanger activity (Fig 2) at both 24 and 48 hours ($P < .001$ by ANOVA, n = 12). NHE-1 activity in the SHR VSMCs cultured in 2 mmol · L^{-1} [K^+] was significantly different at 24 hours and 48 hours compared with the initial values (39.3 ± 1.6 and 34.1 ± 1.2 compared with 24.8 ± 0.7 mmol · L^{-1} min^{-1}, $P < .005$ for both). Measurements of Na^+/H^+ exchanger activity in SHR VSMCs in both 2 and 8 mmol · L^{-1} media at all time points remained significantly greater than those of WKY VSMCs ($P < .001$). This Na^+/H^+ exchanger activity was completely suppressed by 10 μmol · L^{-1} HOE-694, suggesting that it was mediated by NHE-1 (H^+ efflux rate in Na^+-free buffers, using N-methyl glucamine chloride to substitute for Na^+ being 12.1 ± 1.1 mmol · L^{-1} min^{-1} compared with H^+ efflux rate in HBSS containing 10 μmol · L^{-1} HOE-694, 14.2 ± 2.1 mmol · L^{-1} min^{-1} n =12, P, NS).

The VSMCs from WKY and SHR were cultured for 48 hours in media containing 2, 4, 6, and 8 mmol · L^{-1} [K^+] to investigate whether there was a concentration-related effect on NHE-1. In WKY, there was no significant trend in the pH_i or the Na^+/H^+ exchanger activity (Fig 3). SHR VSMCs, however, demonstrated a clear concentration-related effect of [K^+] with enhancement of Na^+/H^+ exchanger activity at lower [K^+]

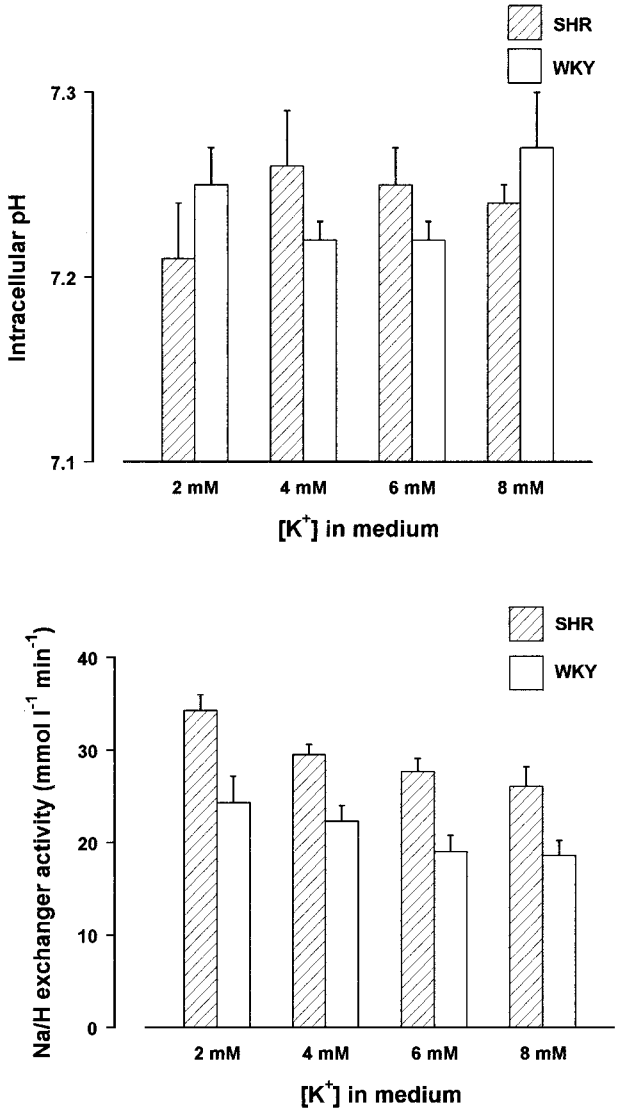

Fig 3. The effect of incubation of SHR and WKY VSMCs in growth media containing 2, 4, 6, or 8 mmol · L^{-1} [K^+] for 48 hours on the pH_i and Na^+/H^+ exchanger activity. The changes in Na^+/H^+ exchanger activity in the SHR with different [K^+] was significant (ANOVA, $P < .001$, n = 12).

(Fig 2, $P < .006$ by ANOVA). Na^+/H^+ exchanger activity at $[K^+]$ of 2 mmol · L^{-1} was significantly higher than that at $[K^+]$ of 6 or 8 mmol · L^{-1} (Fig 3, $P < .02$).

We examined whether this enhanced NHE-1 activity in the SHR VSMC cultures was associated with an increased NHE-1 protein expression. Identical cultures in varying $[K^+]$ from 2 to 8 mmol · L^{-1} were extracted, resolved on SDS-PAGE gels, and blotted onto supported nitrocellulose for immunodetection using specific NHE-1 antibodies (G116). A typical Western blot is shown in Fig 4, in which it is clear that there is no significant $[K^+]$-dependent increase in NHE-1 protein expression in either the SHR or the WKY VSMC cultures in the different $[K^+]$ media. Similar results were obtained on 3 other occasions, and the mean results on 4 lines from each of the WKY and SHR are presented in Table 1. Densitometry results of the NHE-1 protein were normalized to the WKY value at 4 mmol · L^{-1} $[K^+]$. There were no significant differences in NHE-1 abundance between cultures in the different $[K^+]$ media in either rat strain.

Table 1. Abundance of NHE-1 Protein in VSMC Extracts From SHR and WKY Cells

Potassium mmol · L^{-1} for 48 hr	WKY NHE-1 Abundance ± SEM	SHR NHE-1 Abundance ± SEM
2	1.02 ± 0.04	1.18 ± 0.11
4	1.00 ± 0.00	1.16 ± 0.25
6	1.04 ± 0.05	1.23 ± 0.18
8	0.97 ± 0.04	0.87 ± 0.07

NOTE. Results have been normalized to the WKY densitometric result at 4 mmol · L^{-1} $[K^+]$, which was assigned a value of 1. There were no significant differences between the different $[K^+]$ between the 2 rat strains (n = 4).

DISCUSSION

We and others have previously demonstrated that activity of the Na^+/H^+ exchanger[14-19,22] was increased in different tissues derived from the SHR and also from hypertensive humans.[23] The etiology of this intermediate phenotype was not dependent on increased NHE-1 protein expression and may be due to increased turnover of NHE-1.[19,22,23] Although some in vitro effects of K^+ depletion have been demonstrated, such as increased proliferation of kidney epithelial cells[6] and vascular myocytes,[7] it had been suggested that a transient increase in intracellular Na^+ due to reduced Na^+ efflux via the Na^+,K^+-ATPase was necessary. However, the increase in intracellular Na^+ ($[Na^+]_i$) could also be derived from an increased Na^+ influx.[6] Because NHE-1 is a major contributor to Na^+ influx in a variety of cells, we examined its response to K^+ depletion in VSMCs from both normotensive and hypertensive rats.

Potassium depletion led to only small statistically insignificant increases in Na^+/H^+ exchanger activity in WKY VSMCs. In SHR VSMCs, in which the Na^+/H^+ exchanger activity was already significantly elevated compared with WKY in physiologic media, K^+ depletion led to further marked increases in Na^+/H^+ exchanger activity, which were not associated with any transient intracellular acidosis. Indeed, there was a transient intracellular alkalinization in the SHR VSMCs, consistent with the activation of NHE-1. However, this was not sustained. This increased Na^+/H^+ exchanger activity was not due to an elevation in NHE-1 protein expression, although sensitivity to the highly specific NHE-1 inhibitor, HOE-694, confirmed that this isoform was primarily involved.[21] Thus, turnover of NHE-1 was increased in the SHR VSMCs cultured in low $[K^+]$ media.

A further mechanism that may be considered is the increase in intracellular calcium ($[Ca^{2+}]_i$) that could be expected due to the increase in $[Na^+]_I$ resulting from transient reduction in the Na^+ efflux via the Na^+,K^+-ATPase. It is also known that NHE-1 possesses a Ca^{2+}-calmodulin binding domain in its cytoplasmic C-terminal region,[24] which has an autoinhibitory function on the exchanger. However, in these VSMCs, we had previously demonstrated that an increase in $[Ca^{2+}]_I$ stimulated NHE-1 activity in WKY cells with no significant stimulatory effect on NHE-1 activity in SHR cells.[25] In addition, calmodulin beads bound more NHE-1 extracted from WKY than SHR VSMCs,[25] suggesting that the SHR NHE-1 may not be as sensitive to changes in $[Ca^{2+}]_i$. Thus, the upregulation of NHE-1 activity by K^+ depletion in SHR VSMCs is unlikely to have resulted from an elevation in Ca^{2+}-calmodulin interacting directly with NHE-1.

NHE-1 activity may also be dependent on other accessory proteins interacting with the C-terminal cytoplasmic region of the exchanger.[26] In particular, agonist-dependent enhancement of NHE-1 activity may be mediated via these proteins, and it remains a possibility that the increased NHE-1 activity of SHR VSMCs cultured in low K^+ media is mediated by altered interactions of these proteins with NHE-1. Before this hypothesis can be examined further, these proteins will have to be identified and characterized as necessary and sufficient for the activation of NHE-1.

In conclusion, we have demonstrated that the NHE-1 activity of VSMC derived from SHR exhibits an exaggerated enhanced response to culture in low K^+ media. Due to the important permissive role of NHE-1 in cell proliferation[11] and DNA synthesis,[10] these changes in NHE-1 activity in SHR vascular myocytes during K^+ depletion may stimulate or support VSMC proliferation and could contribute to the maintenance or further elevation of blood pressure due to vascular remodelling.

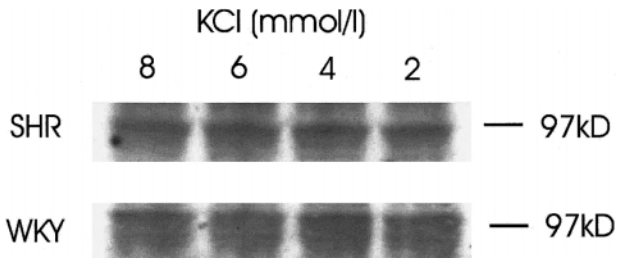

Fig 4. Western blot showing extracts (50 μg protein) of SHR and WKY VSMCs cultured in growth media containing 2, 4, 6, or 8 mmol · L^{-1} $[K^+]$ for 48 hours resolved on 7.5% SDS-PAGE gels, electroblotted, and detected with rat NHE-1–specific antibodies (G116). No significant differences in NHE-1 protein expression of SHR or WKY VSMCs were seen in the different $[K^+]$ growth media.

REFERENCES

1. Khaw KT, Barrett-Connor E: Dietary potassium and blood pressure in a population. Am J Clin Nutr 39:963-968, 1984
2. Krishna G, Miller E, Kapoor S: Increased blood pressure during potassium depletion in normotensive men. N Engl J Med 320:1177-1182, 1989
3. Siani A, Strazzullo P, Russo L, et al: Controlled trial of long-term oral potassium supplements in patients with mild hypertension. BMJ 294:1453-1456, 1987
4. Khaw KT, Barrett-Connor E: Dietary potassium and stroke-associated mortality: A 12-year prospective population study. N Engl J Med 316:235-240, 1987
5. Tobian L, Lange J, Ulm K, et al: Potassium reduces cerebral haemorrhage and death rate in hypertensive rats, even when blood pressure is not lowered. Hypertension 7:I110-I114, 1985 (suppl 1)
6. Walsh-Reitz MM, Aithal HN, Toback FG: Na regulates growth of kidney epithelial cells induced by lowering extracellular K concentration. Am J Physiol 247:C321-326, 1984
7. McCabe RD, Yound DB: Potassium inhibits cultured vascular smooth muscle cell proliferation. Am J Hypertens 7:346-350, 1994
8. Lescale-Matys L, Putnam DS, McDonough AA: Surplus Na^+ pumps: How low K^+-incubated LLC-PK1 cells respond to K^+ restoration. Am J Physiol 265:C887-892, 1993
9. Mahnensmith RL, Aronson PS: The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. Circ Res 56:773-788, 1985
10. Vairo G, Cocks BG, Cragoe EJ, et al: Selective suppression of growth-factor induced cell cycle gene expression by Na^+/H^+ antiport inhibitors. J Biol Chem 267:19043-19046, 1992
11. Bobik A, Grooms A, Little PJ, et al: Ethylisopropylamiloride sensitive pH control mechanisms modulate vascular smooth muscle growth. Am J Physiol 260:C581-588, 1991
12. Sardet C, Franchi A, Pouyssegur J: Molecular cloning, primary structure, and expression of the human growth factor-activatable Na^+/H^+ antiporter. Cell 56:271-280, 1989
13. Orlowski J, Kandasamy RA, Shull GE: Molecular cloning of putative members of the Na/H exchanger gene family. J Biol Chem 267:9331-9339, 1992
14. Ng LL, Dudley C, Bomford J, et al: Leucocyte intracellular pH and Na^+-H^+ antiport activity in human hypertension. J Hypertens 7:471-475, 1989
15. Canessa M, Morgan K, Goldszer R, et al: Kinetic abnormalities of the red blood cell sodium-proton exchange in hypertensive patients. Hypertension 17:340-348, 1991
16. Berk BC, Vallega G, Muslin AJ, et al: Spontaneously hypertensive rat vascular smooth muscle cells in culture exhibit increased growth and Na^+/H^+ exchange. J Clin Invest 83:822-829, 1989
17. Davies JE, Ng LL, Ameen M, et al: Evidence for altered Na^+/H^+ antiport activity in skeletal muscle cells and vascular smooth muscle cells of the spontaneously hypertensive rat. Clin Sci 80:509-516, 1991
18. Rosskopf D, Dusing R, Siffert W: Membrane sodium-proton exchange and primary hypertension. Hypertension 21:607-617, 1993
19. Siczkowski M, Davies JE, Ng LL: Na^+/H^+ antiporter protein in normal Wistar-Kyoto and spontaneously hypertensive rat. J Hypertens 12:775-781, 1994
20. Soleimani M, Bergman JA, Hosford MA, et al: Potassium depletion increases luminal Na^+/H^+ exchange and basolateral Na^+: $CO=_3$:$HCO-_3$ cotransport in rat renal cortex. J Clin Invest 86:1076-1083, 1990
21. Scholz W, Albus U, Lang HJ, et al: HOE 694, a new Na^+/H^+ exchange inhibitor and its direct effect in cardiac ischaemia. Br J Pharmacol 109:562-568, 1993
22. Kelly MP, Quinn PA, Davies JE, et al: Activity and expression of Na^+/H^+ exchanger isoforms 1 and 3 in kidney proximal tubules of hypertensive rats. Circ Res 80:853-860, 1997
23. Ng LL, Sweeney FP, Siczkowski M, et al: Na^+/H^+ antiporter phenotype, abundance and phosphorylation of immortalized lymphoblasts from humans with hypertension. Hypertension 25:971-977, 1995
24. Bertrand B, Wakabayashi S, Ikeda T, et al: The Na^+/H^+ exchanger isoform 1 (NHE1) is a novel member of the calmodulin-binding proteins. Identification and characterization of calmodulin-binding sites. J Biol Chem 269:13703-13709, 1994
25. Siczkowski M, Quinn PA, Ng LL: Calcium-induced activation of the rat vascular myocyte Na^+/H^+ exchanger isoform 1. Metabolism 46:250-256, 1997
26. Wakabayashi S, Bertrand B, Shigekawa M, et al: Growth factor activation and "H^+-sensing" of the Na^+/H^+ exchanger isoform 1 (NHE1). J Biol Chem 269:5583-5588, 1994