Renal arteriolar Na\(^+\)/Ca\(^{2+}\) exchange in salt-sensitive hypertension

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The Na\(^+\)/Ca\(^{2+}\) exchanger was first identified in heart and giant squid axon (17). Subsequent studies demonstrated its existence in a variety of other tissues including rod outer segment (9), vascular smooth muscle (1–3, 15, 29), and kidney (11, 12, 24). At the present time, two separate gene products have been identified (28). One is a Na\(^+\)/K\(^+\)/Ca\(^{2+}\) exchanger found in the outer rod segment of the eye (9, 25, 28), and the other gene product, called “cardiac Na\(^+\)/Ca\(^{2+}\) exchanger,” is expressed in a number of different tissues, including kidney (16, 17). In addition, different isoforms of this Na\(^+\)/Ca\(^{2+}\) exchanger have been identified, suggesting that the characteristics and regulation of exchanger activity may be tissue specific (16).

Recently, we have characterized Na\(^+\)/Ca\(^{2+}\) exchange activity in afferent and efferent arterioles isolated from kidneys of rabbits (11) using the Ca\(^{2+}\)-sensitive fluorescent probe fura 2. In these studies, extracellular Na (Na\(_e\)) was lowered, which resulted in Na\(^+\) exit and Ca\(^{2+}\) entry into the smooth muscle cells via the exchanger. This Na\(^+\)-induced increase in [Ca\(^{2+}\)]\(_i\) occurred through what is known as the “reverse-mode Na\(^+\)/Ca\(^{2+}\) exchange” and resulted in significant elevations in [Ca\(^{2+}\)]\(_i\) in both afferent and efferent arterioles. In response to a decrease in Na\(_a\), Na\(^+\)-induced increases in [Ca\(^{2+}\)]\(_i\) were dependent upon the presence of bath Ca\(^{2+}\) and were blocked by nickel, a nonspecific inhibitor of exchanger activity. The increase in [Ca\(^{2+}\)]\(_i\) with reduction in Na\(_a\) was augmented after arterioles were Na\(^+\) loaded with ouabain. In addition, changes in [Ca\(^{2+}\)]\(_i\) with reductions in Na\(_a\) were not blocked by voltage-dependent Ca\(^{2+}\) channel blockers, nor could they be attributed to changes in cell pH (24). These results are all consistent with the presence of the exchanger in renal arterioles. In other studies (11), we found that activation of protein kinase C (PKC) with phorbol 12-myristate 13-acetate (PMA) enhanced exchange activity, suggesting that PKC may be an important regulator of the exchanger. These results are consistent with previous work demonstrating enhanced Na\(^+\)/Ca\(^{2+}\) exchange activity in cultured aortic smooth muscle cells with PKC activation (34). Recent studies have also shown that PKC directly phosphorylates sites within the cytosolic loop of the Na\(^+\)/Ca\(^{2+}\) exchanger and that it has a modest stimulatory effect on NCX1 and NCX3 isoforms expressed in baby hamster kidney (BHK) cells (14, 19). Thus, previous results clearly support the presence of a PKC-regulated Na\(^+\)/Ca\(^{2+}\) exchanger in afferent arterioles, and it is likely that this exchanger plays an important role in controlling [Ca\(^{2+}\)]\(_i\) in these resistance elements.

Cytosolic calcium concentration ([Ca\(^{2+}\)]\(_i\)) is the principal determinant of contractility in vascular smooth muscle, and alterations in its regulation may play a role in the pathogenesis of hypertension. Several mechanisms controlling [Ca\(^{2+}\)]\(_i\) movement have been identified and include influx and efflux pathways located at the plasma membrane as well as intracellular storage sites. At the plasma membrane, Ca\(^{2+}\)-extrusion mechanisms include an ATP-dependent Ca\(^{2+}\) pump and a Na\(^+\)/Ca\(^{2+}\) exchanger. There has been considerable interest in the Na\(^+\)/Ca\(^{2+}\) exchanger in terms of the regulation of [Ca\(^{2+}\)]\(_i\) as well as its potential involvement in the pathogenesis of certain forms of hypertension (2, 3, 6, 27).

Nelson, Lawrence D., M. Tino Unlap, James L. Lewis, and P. Darwin Bell. Renal arteriolar Na\(^+\)/Ca\(^{2+}\) exchange in salt-sensitive hypertension. Am. J. Physiol. 276 (Renal Physiol. 45): F567–F573, 1999.—The present studies were performed to assess Na\(^+\)/Ca\(^{2+}\) exchange activity in afferent and efferent arterioles from Dahl/Rapp salt-resistant (R) and salt-sensitive (S) rats. Renal arterioles were obtained by microdissection from S and R rats on either a low-salt (0.3% NaCl) or high-salt (8.0% NaCl) diet. On the high-salt diet, S rats become markedly hypertensive. Cytosolic calcium concentration ([Ca\(^{2+}\)]\(_i\)) was measured in fura 2-loaded arterioles bathed in a Ringer solution in which extracellular Na (Na\(_e\)) was varied from 150 to 2 mM (Na was replaced with N-methyl-D-glucamine). Baseline [Ca\(^{2+}\)]\(_i\) was similar in afferent arterioles of R and S rats fed low- and high-salt diets. The change in [Ca\(^{2+}\)]\(_i\) (Δ[Ca\(^{2+}\)]\(_i\)) during reduction in Na\(_a\) from 150 to 2 mM was 80 ± 10 and 61 ± 3 nM (not significant) in afferent arterioles from R rats fed the low- and high-salt diet, respectively. In afferent arterioles from S rats on a high-salt diet, Δ[Ca\(^{2+}\)]\(_i\) during reductions in Na\(_a\) from 150 to 2 mM was attenuated (39 ± 4 nM) relative to the Δ[Ca\(^{2+}\)]\(_i\) of 79 ± 13 nM (P < 0.05) obtained in afferent arterioles from S rats on a low-salt diet. In afferent arterioles, baseline [Ca\(^{2+}\)]\(_i\), which was similar in R and S rats fed low- and high-salt diets and Δ[Ca\(^{2+}\)]\(_i\) in response to reduction in Na\(_a\) was also not different in afferent arterioles from R and S rats fed low- or high-salt diets. Differences in regulation of the exchanger in afferent arterioles of S and R rats were assessed by determining the effects of protein kinase C (PKC) activation by phorbol 12-myristate 13-acetate (PMA, 100 nM) on Δ[Ca\(^{2+}\)]\(_i\) in response to reductions in Na\(_a\) from 150 to 2 mM. PMA increased Δ[Ca\(^{2+}\)]\(_i\) in afferent arterioles from R rats but not from S rats. These results suggest that Na\(^+\)/Ca\(^{2+}\) exchange activity is suppressed in afferent arterioles of S rats that are on a high-salt diet. In addition, there appears to be a defect in the PKC-Na\(^+\)/Ca\(^{2+}\) exchange pathway that might contribute to altered [Ca\(^{2+}\)]\(_i\) regulation in this important renal vascular segment in salt-sensitive hypertension.
Blaustein and Hamlyn (6) initially suggested that the Na\(^{+}/Ca^{2+}\) exchanger may be involved in altered regulation of vascular resistance in hypertension. However, assessment of vascular smooth muscle Na\(^{+}/Ca^{2+}\) exchange activity in several models of hypertension has produced mixed results (2, 3, 21). In isolated aortic rings, Na\(^{+}/Ca^{2+}\) exchange activity was assessed using the rate of contraction while varying the Na\(^{+}\) gradient across smooth muscle cells. Exchanger activity was lower in rings obtained from Dahl salt-sensitive (S) rats fed a high-salt diet (8% NaCl) for 4 wk compared with rings from Dahl salt-resistant (R) rats (2) on the same diet. In another model of hypertension, we have recently reported that Na\(^{+}\)-induced changes in [Ca\(^{2+}\)] in arterioles from Wistar-Kyoto (WKY) rats or prehypertensive SHR (3). These results contradict data obtained from aortic rings of SHR and WKY rats where contraction was significantly higher (2-fold) in hypertensive SHR (24). These results were obtained from arterioles from Dahl/Rapp S and R rats maintained on a high-salt (8% NaCl) diet in S rats, blood pressure averaged 142 ± 6 mmHg (n = 24). In addition, as reported by Chen et al. (10), when 5- to 6-wk-old S rats were placed on a high-salt diet for 1 wk, they became hypertensive but did not, as yet, develop renal or glomerular pathology. Less than 10% of the rats became hypertensive but did not, as yet, develop renal or glomerular pathology. Less than 10% of the rats became hypertensive but did not, as yet, develop renal or glomerular pathology. Less than 10% of the rats became hypertensive but did not, as yet, develop renal or glomerular pathology. Less than 10% of the rats became hypertensive but did not, as yet, develop renal or glomerular pathology.

**METHODS**

**Animals.** The majority of male and female Dahl/Rapp salt-sensitive (S) and salt-resistant (R) rats were acquired from an internal colony of rats maintained at University of Alabama at Birmingham (UAB), by Dr. Jim Lewis. The genotypes and blood pressures of rats from this colony were monitored carefully to ensure genetic and phenotypic purity. The parental stock for this colony was obtained from Dr. John Rapp. R and S rats were placed on a high-salt (8% NaCl) diet for 5–8 days, and mean arterial blood pressures of awake unrestrained animals were measured through a catheter implanted into the aorta. In R rats on a high-salt diet, mean blood pressure averaged 108 ± 4.0 mmHg (n = 22), whereas in S rats, blood pressure averaged 142 ± 2.7 mmHg (n = 24). In addition, as reported by Chen et al. (10), when 5- to 6-wk-old S rats were placed on a high-salt diet for 1 wk, they became hypertensive but did not, as yet, develop renal vascular or glomerular pathology. Least than 10% of the rats used in this study were obtained from Harlan Sprague Dawley (Indianapolis, IN) after this colony was rederived following its contamination (18). S and R rats weighing ~50 g were placed on diets containing 0.3% or 8.0% NaCl (Dyets, Bethlehem, PA) and were used for experiments between days 5 and 8. Water was given ad libitum.

Isolation of afferent and efferent arterioles. For the study of isolated renal arterioles, rats were killed by decapitation, kidneys were removed, and thin (1 mm) coronal sections were obtained for microdissection. Kidney slices were placed in cold Ringer solution containing (in mM) 150 NaCl, 5 KCl, 1 MgSO\(_4\), 1.6 NaH\(_2\)PO\(_4\), 0.4 NaH\(_2\)PO\(_4\), 5.0 dextrose, and 1.5 CaCl\(_2\), as well as 7.0 \(\mu\)M of the acetoxymethyl ester of fura 2 (fura-2 AM). Glomeruli with intact segments of afferent and efferent arterioles and associated thick ascending limb were isolated by frehand dissection using a Wild M5A stereomicroscope. Criteria for identification of afferent and efferent arterioles was similar to that previously described (7, 24). Afferent and efferent arterioles were distinguished based on size and relative position to the thin ascending limb (7, 33). Afferent arterioles were also identified by the presence of concentric rings of smooth muscle cells.

**Measurement of arteriolar calcium concentrations.** Isolated glomeruli with arterioles were incubated for more than 1 h in 7.0 \(\mu\)M fura 2-AM (100 \(\mu\)M MgSO\(_4\), 1.6 NaH\(_2\)PO\(_4\), 5.0 dextrose, and 1.5 CaCl\(_2\)) regularly applied in close proximity via a 1-ml syringe and 26-gauge needle. After 1 h of incubation in fura 2-AM, single glomeruli with arterioles were transferred to a chamber mounted on a Leitz inverted microscope. Glass pipettes attached to micromanipulators were used to gently hold the glomeruli and vascular structures at the floor of the bathing chamber. Temperature of the chamber was maintained at 37°C by continuous superfusion (bath exchange) with prewarmed Ringer solution at a rate of 1.5 ml/min. For precise temperature control, hot water was continuously circulated through tubing located around the circumference of the chamber. The composition of the bathing solution was identical to the dissection solution (denoted as 150 Na\(^{-}\)-Ringer solution), whereas the 2 mM Na\(^{-}\)-Ringer solution was achieved by substituting N-methyl-D-glucamine for Na\(^{+}\). Bath solutions were bubbled with O\(_2\), pH was 7.4, and temperature was maintained at 37°C. Osmolality of solutions was frequently measured and found to be 297 ± 2 mosmol/kgH\(_2\)O. An adjustable optical window was positioned over the arteriole within which photon emissions were collected and measured using a Leitz compact photometer modified to perform photon counting. Wavelengths for excitation of fura 2 were 340 and 380 nm (xenon 75-W light source), while emitted fluorescence was passed through a 510 ± 10 nm band-pass filter. Background fluorescence was determined prior to the experimental protocol and rarely exceeded 5% of the total fluorescence. In addition, there was no evidence for dye leakage across the surface of the experiments. Hardware, including dual monochrometers and chopper, and software were obtained from Photon Technology International (PTI Deltascan System, Princeton, N J). Data were collected at 20 points/s.

Baseline fura 2 ratios were measured in afferent or efferent arterioles that were bathed in 150 Na\(^{+}\)-Ringer solution for at least 100 s, and experiments were performed only if the ratio remained stable. To evaluate exchanger activity, Na\(_{e}\) was reduced to 2 mM. This resulted in a rapid increase in [Ca\(^{2+}\)], followed by a return of the ratio toward baseline values. Na\(_{e}\) was then increased to 150 mM to obtain recovery measurements only after a stable ratio had been achieved at low Na\(_{e}\). Experiments were also performed to test the effects of 100 nM PMA (Sigma Chemical, St. Louis, MO) on Na\(^{+}/Ca^{2+}\) exchanger activity. Measurement of exchanger activity, i.e., reductions in Na\(_{e}\) were initiated after the arterioles were exposed to PMA for 15 min. A control series was also performed to ensure that the effects seen with PMA were not due to the DMSO used as the solvent for PMA.

[Ca\(^{2+}\)]\(_i\) was determined using the ratio of fluorescence obtained at 340- and 380-nm excitation wavelengths and calculated using the equation described by Grynkiewicz et al. (13)

\[
[Ca^{2+}]_i = K_d \times [(R - V \times R_{min})/(V \times R_{max} - R)](S_2/S_0)
\]

where \(K_d\) is the effective dissociation constant of fura 2, \(V\) is the viscosity correction factor (0.85) described by Poenie (26), \(R_{min}\) and \(R_{max}\) are the minimum and maximum ratios at 340 and 380 nm in the presence and absence of Ca\(^{2+}\), respectively, and \(S_0\) and \(S_2\) are the emissions at 380 nm in the presence...
and absence of saturating Ca\textsuperscript{2+}. The value of 224 nM was utilized as the $K_d$ for fura 2 (13). Calibration was conducted in vitro. Composition of the calibration solution was chosen to approximate the intracellular milieu and consisted of (in mM) 115 KCl, 20 NaCl, 10 MOPS, 1.1 MgCl\textsubscript{2}, 1 fura 2 pentapotassium salt (Molecular Probes, Eugene, OR), and either 3 CaCl\textsubscript{2} or no Ca\textsuperscript{2+} and 3 EGTA. $R_{\min}$ and $R_{\max}$ values averaged 0.57 ± 0.01 (n = 21) and 3.36 ± 0.23 (n = 21), respectively, whereas $S_0/S_{120}$ averaged 3.45 ± 0.22. We and others have established the validity of using in vitro calibration parameters for studies in renal arterioles (7, 11, 24) because of the difficulties in obtaining accurate in situ calibrations.

Data are presented as the mean ± SE. Student's paired and unpaired t-tests were used, and statistical significance for all comparisons was set at a P value of 0.05.

RESULTS

Baseline [Ca\textsuperscript{2+}] was measured in arterioles bathed in the 150 Na\textsuperscript{+}-Ringer solution, and data are shown in Table 1. There were no differences in baseline [Ca\textsuperscript{2+}] in afferent and efferent arterioles obtained from R rats on either the low-salt (0.3% NaCl) or high-salt (8% NaCl) diet. In afferent arterioles obtained from S rats, [Ca\textsuperscript{2+}] was significantly lower (132 ± 10 nM) in afferent arterioles from S rats on high-salt diet compared with that of rats on low-salt diet (192 ± 27 nM). This finding was unexpected and may have been due to the rather large variability in the results obtained in afferent arterioles from S rats on low-salt diet. No differences in [Ca\textsuperscript{2+}] were observed in efferent arterioles of S rats on low-salt diet. The regulation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange system in renal afferent and efferent arterioles was examined to determine whether the differences in [Ca\textsuperscript{2+}] were observed in efferent arterioles of S rats fed low- or high-salt diet.

Initial characterization of renal arteriolar Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange was conducted in afferent and efferent arterioles from R and S rats on low-salt diet. This was accomplished by reducing Na\textsubscript{e} and measuring the increase in [Ca\textsuperscript{2+}], i.e., Δ[Ca\textsuperscript{2+}], which was the difference between [Ca\textsuperscript{2+}] at 150 mM and the maximum increase in [Ca\textsuperscript{2+}] obtained at 2 mM of Na\textsubscript{e}. As shown in previous work (11, 23), reductions in Na\textsubscript{e} result in a monophasic increase in [Ca\textsuperscript{2+}]. All conclusions reached in this study are exactly the same if, instead of Δ[Ca\textsuperscript{2+}], the data were expressed as the initial rate of change in [Ca\textsuperscript{2+}].

The results in Fig. 1 indicate that baseline Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity was higher in afferent than efferent arterioles. The effects of high-salt diet (8% NaCl) on exchange activity were examined in afferent and efferent arterioles of R and S rats after 5–8 days on a high-salt diet. Reducing Na\textsubscript{e} from 150 to 2 mM resulted in similar Δ[Ca\textsuperscript{2+}] of 80 ± 10 (n = 22) and 61 ± 3 nM (n = 15) in afferent arterioles of R rats on low- and high-salt diet, respectively (Fig. 2). In afferent arterioles of S rats on a high-salt diet, Δ[Ca\textsuperscript{2+}] was significantly lower (39 ± 4 nM, n = 22; P < 0.001) compared with afferent arterioles (79 ± 13 nM, n = 15) of S rats on a low-salt diet (Fig. 2). There were no significant differences in the Δ[Ca\textsuperscript{2+}] values in response to reduction in Na\textsubscript{e} in efferent arterioles from R and S rats on either high- or low-salt diets (Fig. 3). Therefore, the results indicate that exchanger activity was only attenuated in afferent arterioles of S rats on high-salt diet.

The regulation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger in afferent arterioles was examined to determine whether the varied response to high-salt diet might be due, in part, to differential regulation of the exchanger in afferent arterioles of R and S rats (Fig. 4). Afferent arterioles

Table 1. Baseline [Ca\textsuperscript{2+}] in afferent and efferent arterioles

<table>
<thead>
<tr>
<th>[Ca\textsuperscript{2+}] nM</th>
<th>Afferent Arterioles</th>
<th>Efferent Arterioles</th>
</tr>
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<tbody>
<tr>
<td>Salt-resistant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Na</td>
<td>147 ± 12 (22)</td>
<td>133 ± 15 (11)</td>
</tr>
<tr>
<td>High Na</td>
<td>139 ± 11 (15)</td>
<td>134 ± 15 (18)</td>
</tr>
<tr>
<td>Salt-sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Na</td>
<td>192 ± 27 (15)</td>
<td>137 ± 11 (13)</td>
</tr>
<tr>
<td>High Na</td>
<td>132 ± 10 (22)</td>
<td>120 ± 8 (13)</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 experiments. Numbers in parentheses refer to number of arterioles in each group. Afferent and efferent arteriolar cytosolic calcium concentration ([Ca\textsuperscript{2+}]) under basal conditions in Dahl salt-resistant and salt-sensitive rats fed low (0.3%) and high (8.0%) dietary sodium were measured as described in METHODS. *P < 0.05.
from R and S rats on a low-salt diet were treated with PMA (100 nM) for 15 min followed by activation of the exchanger by reducing Na\textsubscript{e} from 150 to 2 mM. Δ[Ca\textsuperscript{2+}] in afferent arterioles from R rats increased significantly from 58 ± 16 nM before to 130 ± 31 nM after PMA treatment (n = 8; P < 0.05). In contrast, PMA treatment did not significantly alter Δ[Ca\textsuperscript{2+}] in afferent arterioles of S rats in response to Na\textsubscript{e} reduction (98 ± 14 before and 68 ± 20 nM after PMA treatment; n = 6), indicating that there was indeed differential regulation of exchanger activity in afferent arterioles of R and S rats.

**DISCUSSION**

S rats presented with a diet rich in NaCl (8.0%) for 1 wk develop hypertension, whereas R rats remain normotensive (10). After 2 wk on 8.0% NaCl, glomerular filtration rate (GFR) begins to decline and mean arterial pressure remains elevated in S rats. Apparent by the 3rd wk of high-NaCl diet are renal vascular and glomerular abnormalities in S rats (10). Thus elevated arterial blood pressure in the S rat is associated with increased renal vascular resistance and decreased GFR. In the present studies, S rats were utilized within 5–8 days of being placed on a 8.0% NaCl diet; at this period of time, S rats are markedly hypertensive, but there is no evidence for renal vascular damage (10). Nearly all of the rats used in this work came from a colony of S and R rats maintained at UAB. Blood pressure in S rats on a high-NaCl diet for 1 wk from this colony averaged 142 mmHg. Blood pressure in R rats from this colony did not increase on a high-NaCl diet.

In previous work, it has been found that S rats challenged with high dietary NaCl exhibit renal vascular hyperactivity to, among other agonists, angiotensin II, norepinephrine, and vasopressin (27). These vascular alterations may result from multiple pathologies, including a defect in the intrinsic and/or neurohumoral regulation of [Ca\textsuperscript{2+}]; in vascular smooth muscle. It should be stated that there were no obvious or interpretable differences in baseline [Ca\textsuperscript{2+}]; between S and R arterioles on a low- or high-salt diet. Since these studies were conducted in arterioles that had been removed from the normal milieu, there is no reason to assume that these measured [Ca\textsuperscript{2+}] values reflect [Ca\textsuperscript{2+}] values in arterioles in vivo. In addition, ambient [Ca\textsuperscript{2+}] is a summation of the various Ca\textsuperscript{2+} regulatory processes that control influx, efflux, sequestering, and mobilization of this ion. The role of the exchanger in controlling resting [Ca\textsuperscript{2+}] is not known.

One of the mechanisms that participates in the control of [Ca\textsuperscript{2+}] in vascular smooth muscle cells, especially in terms of lowering agonist-induced increases in [Ca\textsuperscript{2+}], is the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. It has also been suggested that this exchanger may be important in the development of hypertension (5, 6). The present studies were designed to obtain evidence for exchanger activity and to study the effects of high-salt diet and PKC activation on Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity in arteriolar segments from R and S rats. Although previous studies in genetic models of hypertension have examined Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity in larger conductance vessels (1–3, 20, 22), it is likely that characteristics of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger may vary between tissues. These previous studies, therefore, may not necessarily be representative or predictive of exchange activity in renal resistance vessels.

In the present studies, exchanger activity in afferent and efferent arterioles was examined by reducing Na\textsubscript{e} from 150 to 2 mM and measuring changes in [Ca\textsuperscript{2+}]. This so-called reverse-mode Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange resulted in a prompt and linear increase in Δ[Ca\textsuperscript{2+}], in both afferent and efferent arterioles. These results are consistent with our recent findings in rabbit and rat (WKY and SHR) arterioles (11, 24) and further support the notion that changes in [Ca\textsuperscript{2+}], with manipulation of the transmembrane Na\textsuperscript{+} gradient is through the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. Although there is evidence from human fibroblasts that removal of Na\textsubscript{e} can directly mobilize [Ca\textsuperscript{2+}] (31), similar observations have not been reported in vascular smooth muscle. As shown in
previous studies by us (11, 24) and others (4), the responses of [Ca\textsuperscript{2+}]\textsubscript{i}, to changes in the Na\textsuperscript{+} transmembrane gradient are consistent with the operation of a Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger. Also in our studies in afferent arterioles (11), increases in [Ca\textsuperscript{2+}]\textsubscript{i}, with elimination of bath Na\textsuperscript{+} occurred strictly through Ca\textsuperscript{2+} entry and not Ca\textsuperscript{2+} mobilization. In other studies, elimination of Nae only increased [Ca\textsuperscript{2+}]\textsubscript{i} by prior Na\textsuperscript{+} loading (32). In contrast, we found that reducing Nae from 150 to 2 mM elicited increases in [Ca\textsuperscript{2+}]\textsubscript{i} without prior Na\textsuperscript{+} loading. This finding may indicate that, in renal microvessels, [Na\textsuperscript{+}]\textsubscript{i}, was sufficiently high to provide a driving force for Na\textsuperscript{+} to exit through the exchanger in the presence of 2 mM Na\textsubscript{e}. Presumably, in other studies, basal [Na\textsuperscript{+}]\textsubscript{i}, was lower, and therefore it was necessary to first raise [Na\textsuperscript{+}]\textsubscript{i}, to observe exchanger activity during reductions in bath Na\textsuperscript{+}\textsubscript{e}. It should also be noted that we did not measure [Na\textsuperscript{+}]\textsubscript{i} in this study due to the poor quantum yield of sodium-binding benzofuran isophthalate (SBFI) in renal arterioles. Therefore, we cannot entirely eliminate the possibility that all or part of the differences in the measured increases in [Ca\textsuperscript{2+}]\textsubscript{i} found in present studies with elimination of bath Na\textsubscript{e} are due to differences in [Na\textsuperscript{+}]\textsubscript{i}. Finally, in the continued presence of 2 mM Na\textsubscript{e} [Ca\textsuperscript{2+}]\textsubscript{i}, returned toward baseline (data not shown) but remained significantly greater than control values, indicating that other Ca\textsuperscript{2+}-regulatory mechanisms are not capable of fully restoring [Ca\textsuperscript{2+}]\textsubscript{i} to control levels and supporting the notion that the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger plays an important role in regulating [Ca\textsuperscript{2+}]\textsubscript{i} in these vascular segments. Taken together, these results support the existence of a Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger in renal arterioles. Although other Ca\textsuperscript{2+}-regulatory mechanisms are clearly present in these cells, the majority of the initial linear increase in [Ca\textsuperscript{2+}]\textsubscript{i}, that occurs as a function of reducing Nae involves transport of Ca\textsuperscript{2+} into these cells via the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger.

The changes in [Ca\textsuperscript{2+}]\textsubscript{i}, with reductions in bath Na\textsuperscript{+}\textsubscript{e} were greater in afferent vs. efferent arterioles. Similar findings were found in studies (11, 24) of rabbit and rat (WKY and SHR). The reason for this difference is unknown but could involve a higher level of exchanger activity in afferent compared with efferent arterioles or differences in [Na\textsuperscript{+}]\textsubscript{i}. The effect of a high-salt diet on exchanger activity was examined in afferent and efferent arterioles of R and S rats that were on a high-salt diet for 5–8 days and compared with arterioles from rats on a low-salt diet for the same period of time. In R rats, dietary NaCl did not influence exchanger activity in afferent or efferent arterioles. However, in S rats on a high-salt diet, there was an attenuation of exchanger activity in afferent arterioles. Interestingly, exchanger activity was not different in efferent arterioles of S rats between a low- or high-NaCl diet. The reason for the difference in results between afferent and efferent arterioles in S rats is unknown. It could be due to differences in the regulation of the exchanger between these two segments. Alternatively, it could be due to the lower Ca\textsuperscript{2+} responsiveness of the efferent arteriole to reduced Nae, which might mask or tend to minimize differences in \(\Delta[Ca^{2+}]\) with reductions in Nae in efferent arterioles from S rats. These results are similar to our recent studies in SHR-WKY rats, where the age-related increase in blood pressure in the SHR did not affect \(\Delta[Ca^{2+}]\) in response to reduced Nae in efferent arterioles (24).

Our results in afferent arterioles are also consistent with studies in aortic rings removed from S and R rats fed a high-salt (8% NaCl) diet for 4 wk. In this study, development of tension was utilized as an indirect assay of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity. Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity, assessed by measurement of the rate of contraction elicited in a 1.2 mM Na\textsuperscript{+} solution, was lower in aortic rings from hypertensive S rats compared with R rats (2). In the SHR, we recently reported that Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity was suppressed in afferent arterioles from the SHR at 9 wk (hypertensive) of age compared with arterioles obtained at 3 wk of age. In this study, \(\Delta[Ca^{2+}]\) from WKY afferent arterioles were not different at 3 and 9 wk of age. Thus the results obtained in both the S rats and SHR indicate a suppression of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity in the afferent arteriole with the development of hypertension. In contrast to the results found in aortic rings from S rats (2) and in afferent arterioles from SHR, Ashida et al. (3) observed a twofold greater rate of tension development in rings from SHR exposed to 1.2 mM bath Na\textsuperscript{+}. These differences in experimental findings may reflect intrinsic differences in the regulation of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger activity expressed in different blood vessels and different models of hypertension and underscore the importance of examining blood vessels that specifically determine vascular resistance.

The current studies do not provide information concerning the mechanism responsible for the reduced exchanger activity in the afferent arterioles of S rats, nor do they provide evidence for a role of the exchanger in the development and/or maintenance of hypertension. However, a reduced Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity as found in afferent arterioles of S rats on a high-NaCl diet may lead to dysfunctional regulation of [Ca\textsuperscript{2+}]\textsubscript{i}, in this important resistance element. It is generally thought (35) that the exchanger functions to lower [Ca\textsuperscript{2+}]\textsubscript{i} in response to agonist-induced elevations in [Ca\textsuperscript{2+}]\textsubscript{i}. Thus a reduced ability to extrude Ca\textsuperscript{2+} from the cell via the exchanger may result in increased vascular resistance, diminished GFR, and, over time, an increase in blood pressure.

PKC, which is activated by acute PMA and related phorbol esters (8), has been shown to modulate Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity in aortic smooth muscle (34), in afferent arterioles from rabbit kidney (11) and in BHK cells transfected with several isoforms of the exchanger (19). In addition, angiotensin II and norepinephrine have been postulated to facilitate Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange in aortic myocytes by a mechanism involving PKC activation (23, 35). PKC activation of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange occurs through phosphorylation of sites on the cytoplasmic loop of the exchanger (14). To examine the possibility that attenuation of exchanger activity in
afferent arterioles of S but not R rats was due to differential regulation of the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange in these vessels, the effect of acute PMA treatment on afferent arterioles of R and S rats was examined. These studies were performed only in arterioles obtained in R and S rats on a low-NaCl diet, since we were looking for intrinsic differences in exchange regulation. These studies demonstrate that acute PMA treatment induced exchange activity in afferent arterioles of R but not S rats. These results are consistent with studies in cultured mesangial cells (see companion study, Ref. 19a) where PMA increased Δ[Ca\textsuperscript{2+}] in response to reductions in Na\textsuperscript{+} in R but not S mesangial cells. The fact that similar findings were found in cultured mesangial cells and freshly isolated afferent arterioles strengthens the notion that this is an intrinsic difference in exchange regulation between S and R renal contractile cells.

Thus, in both afferent arterioles and mesangial cells, there may be a defect in the PKC-Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange pathway. As stated, this defect could lead to a reduced ability of renal contractile cells from the S rat to lower [Ca\textsuperscript{2+}]\textsubscript{i} in response to agonist-induced elevations in [Ca\textsuperscript{2+}]\textsubscript{i}. Whether this defect is responsible for the reduction in Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange activity in afferent arterioles in S rats on a high-salt diet remains to be determined.

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