Nisoldipine Inhibition of Sodium Influx Into Aorta From Aldosterone-Salt-Hypertensive Rats

Jacquelyn M. Smith, Edward J. Cragoe, and Allan W. Jones

The purpose of this study was to determine whether increased sodium (Na) influx into the aorta was associated with aldosterone-salt hypertension in the rat and, if present, to determine what mechanisms contributed to the increase. Basal Na influx was elevated in aorta from the hypertensive rats (2.21±0.10 mmol/l cell H2O/min, n=25) compared with control-salt rats (1.75±0.04 mmol/l cell H2O/min, n=24). The calcium (Ca) antagonist nisoldipine inhibited the Na influx into aorta from hypertensive rats in a concentration-dependent manner. At 10 nM nisoldipine, the Na influx in hypertensive rats (1.52±0.14 mmol/l cell H2O/min, n=10) was similar to control rats (1.66±0.18 mmol/l cell H2O/min, n=7). The basal Na influx in aorta from hypertensive rats was not altered by dichlorobenzamil or ethylisopropylamiloride, selective inhibitors of Na-Ca and Na-H exchange, respectively. The Na influx was 2.21±0.10, 2.03±0.24, and 2.11±0.19 mmol/l cell H2O/min for basal (n=25), dichlorobenzamil (n=4), and ethylisopropylamiloride (n=11), respectively. Inhibition of Na influx in hypertensive rats by 0.1 μM nisoldipine (ΔNa influx=−0.72±0.18 mmol/l cell H2O/min, n=9) was not significantly altered when applied with dichlorobenzamil (−0.72±0.21 mmol/l cell H2O/min, n=4) or ethylisopropylamiloride (−0.55±0.15 mmol/l cell H2O/min, n=11). These agents did not alter Na influx in control aorta. Our results suggest that, in aorta from aldosterone-salt-hypertensive rats, an elevated Na influx exists that is dependent on Ca entry through potential-operated Ca channels. Na-Ca and Na-H exchange do not appear to contribute significantly to the elevated Na influx, which is suggested to result from the activity of a Ca-dependent cationic channel. (Hypertension 1989;13:676-680)

Vascular smooth muscle from the aldosterone-salt-hypertensive rat (AHR) exhibits an increased efflux of potassium (K) and chloride (Cl). This increase in KCl efflux was initially thought to result from a leaky membrane destabilized by reduction in membrane-bound calcium. However, the recent demonstration in this laboratory that the elevated K efflux can be reduced to control values by calcium (Ca) antagonists suggested that Ca-activated K channels (and possibly Cl channels) mediated the increase in K efflux in AHR.

Sodium transport was also altered in vascular smooth muscle from AHR. Although the measures of intracellular sodium (Na) concentration showed no changes, Na efflux was increased primarily by the active extrusion of Na via the Na-K pump. These findings would suggest that Na entry was also elevated in AHR. There have been, however, no direct measures of Na influx in this preparation. As in the case of K efflux, studies suggest that intracellular Ca may activate Na influx in some tissues. A Ca-regulated nonselective cation channel has been demonstrated in rat myocytes and mouse neuroblastoma cells. A Ca-dependent increase in Na influx has also been reported in arterial smooth muscle and skeletal muscle. Na influx may also be coupled to intracellular Ca via a Na-Ca exchange mechanism, which has been suggested to alter vascular function during hypertension. Recent evidence has also supported the concept that Na-H exchange was increased in response to agonist stimulation and acidification of vascular smooth muscle.

The focus of the present study was to determine whether Na influx was increased in the aorta from AHR and to evaluate contributions to increased Na influx by Ca entry and Na-Ca– and Na-H– exchanged mechanisms.

Materials and Methods

Animals

The AHR model has been described previously. The left kidney was removed from anesthetized
male Sprague-Dawley rats (150–170 g), an osmotic minipump (Alza Corp., Palo Alto, California) was implanted subcutaneously, and the rats were given 1% NaCl (supplemented with 0.3% KCl to maintain K balance) to drink. The d-aldosterone (Sigma Chemical Co., St. Louis, Missouri) was dissolved in polyethylene glycol and was infused at a rate of 0.25 μg/hour for a minimum of 2 weeks after which the pumps were replaced. This protocol produced a significant elevation in systolic blood pressure, which was determined by a tail-cuff method. Each AHR had a minimum systolic pressure of 180 mm Hg and was treated for 3–4 weeks. The control-salt (CS) rats were nephrectomized and given 1% NaCl to drink. Previous experiments indicated that infusion of the vehicle did not produce any detectable differences in the control group, so pump infusions of vehicle were omitted. The average systolic pressure of CS rats was 120 mm Hg.

For each experiment, the rats were decapitated, and the thoracic aorta was quickly removed and placed in dissection solution. Loose connective tissue and fat were dissected from the vessel, which was then slit lengthwise. The endothelial cells were removed by lightly stroking the intimal surface with moistened filter paper. The vessel was cut into two or four strips, and each strip was mounted on a stainless steel holder and placed in physiological salt solution (PSS) for 3 hours.

Sodium Influx

Experimental agents were added to the incubation solution during the last 30 minutes, followed by incubation for 60 seconds in an identical solution containing 24Na (20 μCi/ml, University of Missouri Research Reactor, Columbia, Missouri). Less than 20% of the cellular pool was labeled at 60 seconds, which made backflux corrections unnecessary. The influx was terminated by plunging the strip into tubes containing ice-cold (1° C) PSS for 10 minutes to clear the extracellular space of 24Na. An evaluation of this exchange was performed in aorta from control-salt rats (open bar, n=24) and aldosterone-salt-hypertensive rats (crosshatched bar, n=25). Mean+1 SEM (vertical line) is presented. *Values for aldosterone-salt–hypertensive rats were significantly greater than values for control-salt rats (p<0.001).

Solutions

Normal physiological solution had the following composition in mM: Na+ 146.2, K+ 5.0, Mg2+ 1.2, Ca2+ 2.5, Cl− 143.9, HCO3− 13.5, H2PO4− 1.2, and glucose 11.4. Solutions were gassed with a mixture of 97% O2–3% CO2 at 37° C to achieve a pH of 7.4. In some solutions 10 mM N-(2-hydroxyethyl)piperazine-N′-2-ethanesulfonic acid (HEPES) was used as a buffer in place of HCO3−-CO2.

Nisoldipine (NIS) (gift from Miles Pharmaceuticals, West Haven, Connecticut) was dissolved in 100% ethanol. The maximum concentration of ethanol in PSS was 0.1%. Dichlorobenzamil (DCB) and ethylisopropylamiloride (EIP-A) (gifts from Merck, Sharp and Dohme Research Laboratories) were dissolved in dimethylsulfoxide. Control experiments indicated that the solvent did not alter aortic Na influx.

Statistics

Student’s t test was used to evaluate the difference between two groups. The paired t test was used to evaluate the effect of an experimental agent on 24Na influx. Since only one observation was made per rat, n equals the number of rats throughout. Values of a p<0.05 were deemed to be significant.

Results

Calcium Antagonists and Sodium Influx

The basal Na influx (Figure 1) was 26% greater in aorta from AHR than CS rats (p<0.001). This elevated Na influx was decreased in a concentration-dependent manner by the Ca-antagonist nisoldipine (NIS) (Figure 2). At 10 nM NIS, the elevated basal Na influx in AHR was reduced to values (1.52±0.14 mmol/1 cell H2O/min, n=10) observed for the control aorta in the presence of NIS (1.66±0.18 mmol/l cell H2O/min, n=7, pv=NS). NIS did not alter the Na influx in aorta from CS rats except at the highest concentration (100 nM). These results indicated that elevated Na influx in aorta from AHR may be Ca dependent. Experiments were conducted to determine whether Na-Ca or Na-H exchange mediated the elevated Na influx.

Sodium-Calcium Exchange

The effect of DCB, a selective inhibitor of Na-Ca exchange, was evaluated on Na influx in the...
presence and absence of NIS. A high concentration of DCB (30 μM), which completely inhibited acetylcholine-induced relaxation of rat aorta, 17 did not alter the basal Na influx in AHR or CS rats (Table 1). DCB also did not modify the inhibition of Na influx in AHR by NIS (Table 1).

**Sodium-Hydrogen Exchange**

Na-H exchange had been previously identified in rat aorta exposed to a HEPES-buffered solution. 18 Therefore, it was important to determine whether Na-H exchange could be observed in a HCO3-CO2-buffered PSS as used in our protocols. The initial studies compared Na-H exchange in aorta from CS rats buffered with HCO3-CO2 and with HEPES. Basal Na influxes in HCO3-CO2 (1.75±0.04 mmol/l cell H2O/min, n=24) (Figure 1) were similar to those in HEPES (1.92±0.14 mmol/l cell H2O/min, n=6, p=NS). Na-H exchange was stimulated by intracellular acidification using a standard protocol in which tissues were incubated in NH4Cl (15 mM) followed by a wash in NH4-free solution. 19 This procedure elevated Na influx during the second minute of wash in HCO3-CO2 (2.91±0.11 mmol/l cell H2O/min, n=5, p<0.001) as well as in HEPES (2.94±0.23 mmol/l cell H2O/min, n=5, p<0.001). Repetition of the NH4Cl protocol in the presence of the selective Na-H inhibitor EIP-A (30 μM) 18 prevented the stimulation of Na influx in both HCO3-CO2 and HEPES (1.35±0.12 and 1.17±0.06 mmol/l cell H2O/min, n=5 and n=6, respectively; p<0.001). These initial studies demonstrated the presence of an EIP-A-sensitive Na-H exchange in acidified aorta from CS rats. When evaluated on hypertensive aorta, however, EIP-A (30 μM) did not alter the basal Na influx in AHR or CS rats when normalized in terms of either cell H2O or dry weight (Table 1). In addition, EIP-A did not modify the NIS inhibition of Na influx, which was similar in the absence or presence of the Na-H-exchange inhibitor (Table 1).

**Discussion**

These results show that Na influx is elevated in aorta from AHR under basal conditions (Figure 1) and that the increase is consistent with previous reports of increased Na efflux during aldosterone or deoxycorticosterone-induced hypertension. 17,20 The elevation in Na influx was equivalent to the increase in Na efflux via the Na-K pump. 7 The elevation in Na influx appeared to be a Ca-dependent process since it was inhibited by the Ca channel antagonist NIS (Figure 2). The effective NIS concentrations (1–10 nM), which either reduced or eliminated the elevation in Na influx, were in the range of the NIS (3 nM) required for 50% inhibition of Ca entry through potential-operated (slow) Ca channels. 21 Additional effects of Ca antagonists on vascular smooth muscle function required much higher concentrations. 22 Comigration of Na and Ca

**TABLE 1. Effects of 30 μM Dichlorobenzamil, 30 μM Ethylisopropylamiloride, and 0.1 μM Nisoldipine on Sodium Influx in Aorta From Control-Salt Rats and Aldosterone-Salt-Hypertensive Rats**

<table>
<thead>
<tr>
<th>Condition</th>
<th>No.</th>
<th>Total 24Na influx (mmol/l cell H2O/min)</th>
<th>DCB (mmol/l cell H2O/min)</th>
<th>DCB + NIS (mmol/l cell H2O/min)</th>
<th>NIS† (mmol/kg dry wt/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>3</td>
<td>1.76±0.10</td>
<td>-0.01±0.24</td>
<td>-0.26±0.06*</td>
<td></td>
</tr>
<tr>
<td>AHR</td>
<td>4</td>
<td>2.14±0.23</td>
<td>-0.01±0.24</td>
<td>-0.26±0.06*</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>7</td>
<td>1.80±0.06</td>
<td>-0.03±0.11</td>
<td>-0.26±0.09*</td>
<td>-0.26±0.06*</td>
</tr>
<tr>
<td>AHR</td>
<td>11</td>
<td>2.19±0.16</td>
<td>-0.09±0.18</td>
<td>-0.55±0.15*</td>
<td>-0.72±0.18*</td>
</tr>
<tr>
<td>CS</td>
<td>7</td>
<td>1.42±0.04</td>
<td>-0.03±0.09</td>
<td>-0.20±0.04*</td>
<td>-0.21±0.04*</td>
</tr>
<tr>
<td>AHR</td>
<td>11</td>
<td>2.32±0.14</td>
<td>-0.09±0.18</td>
<td>-0.58±0.15*</td>
<td>-0.76±0.18*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. DCB, dichlorobenzamil; NIS, nisoldipine; CS, control-salt rats; AHR, aldosterone-salt-hypertensive rats; EIP-A, ethylisopropylamiloride.

*p<0.05 for change compared with basal values.

†NIS data are from Figure 2.
Throughout the same channel has been reported in some tissues. However, this mechanism is an unlikely explanation for these results. Recent voltage clamp studies in smooth muscle demonstrated that Ca channels conducted Na only under conditions of low extracellular Ca \((<1 \mu M)\). At physiological Ca concentrations, Ca channels were highly selective for Ca over Na. These results support the concept that a component of Na influx into vascular smooth muscle of AHR is indirectly linked to Ca entry through potential-operated (slow) Ca channels as shown in the schematic model (Figure 3).

Two exchange processes for Na, Na-Ca, and Na-H exchange were modulated by increased Ca. And, therefore, could contribute to the Ca-dependent Na entry in AHR. Also the cellular Ca concentration appeared to be elevated under basal conditions in AHR aorta as exemplified by increased spontaneous contraction and Ca-dependent efflux of \(42^K\) (Figure 3). Both of these responses were returned toward control levels by Ca antagonists. Several observations, however, make it unlikely that coupled Na-Ca exchange underlies the elevated basal Na influx in AHR (Figure 3). An inhibitor of Na-Ca exchange, DCCB, did not alter basal Na influx nor modify the inhibition of AHR by NIS (Table 1). In addition, the increased Na entry into the AHR aorta, 460 \(\mu mol/l\) cell \(H_2O/min\) (Figure 1), exceeded the increased Ca entry (20–50 \(\mu mol/l\) cell \(H_2O/min\), unpublished observations) by 10-fold or more. A similar ratio was reported for norepinephrine-stimulated Na influx and Ca efflux from rabbit aorta. In contrast, the proposed stoichiometry for the Na-Ca exchanger was 3 to 1.

Although agonist stimulation of vascular smooth muscle was reported to stimulate Na-H exchange, the activity of this exchanger under basal conditions has only been demonstrated in rat aorta that was incubated with ouabain in a HEPES-buffered solution. The HCO\(_3\)-Cl exchange mechanism would not be operative under such conditions, thereby reducing the capacity for intracellular buffering. Our experiments demonstrated that Na-H exchange could be stimulated in the presence of a physiological HCO\(_3\)-CO\(_2\) buffer system. However, we found no evidence that the Na-H exchanger transported significant amounts of Na under basal conditions in either AHR or CS rat aorta. The selective Na-H inhibitor EIPA, which effectively blocked the stimulation of Na-H exchange by intracellular acidification, did not alter basal Na influx or modify the inhibition by NIS (Table 1). Therefore, it is unlikely that the Na-H exchanger underlies the elevated basal Na influx observed in aorta from AHR (Figure 3). It is also unlikely that altered Na-Na exchange was responsible for increased Na influx in AHR. This exchange does not appear to be Ca-dependent, and the Na influx into K-free solution (active Na-K transport inhibited) was unchanged in AHR under conditions of both normal and high cell Na.

An alternative explanation for these results is that Na entry through the potential-operated (slow) calcium channels elevates intracellular Ca, which in turn activates Ca-dependent channels that conduct Na. Na conductance in smooth muscle differs from that in nerve as exemplified by low sensitivity to tetrodotoxin. There is also evidence that Ca modulates Na entry. Ca stimulation of Na efflux from Na-loaded arterial muscle has been demonstrated, along with activation of Na entry into cultured cardiac cells, barnacle muscle, and arterial smooth muscle. Ca has also been reported to regulate a nonselective cation channel in several tissues. These findings suggest that elevated Na entry in aorta from AHR results from a Ca-activated cation channel that conducts mainly Na under physiological conditions (Figure 3). Voltage clamp studies, however, are needed to verify this suggestion.

In summary this study demonstrated an elevated Na influx in the aorta from AHR, which could be reduced by the Ca-channel antagonist NIS. The Na-Ca and Na-H exchangers did not contribute significantly to the elevated basal Na influx. Evidence was presented that indicated a Ca-activated cation channel might be involved.

Acknowledgment

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Key Words: vascular smooth muscle, sodium-calcium exchange, sodium-hydrogen exchange, nisoldipine, sodium intake
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