Vascular sodium–potassium pump activity in various models of experimental hypertension

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Summary
1. Ouabain-sensitive 86Rb uptake was used to assess sodium–potassium pump activity in vascular smooth muscle of animals with various types of experimental hypertension.
2. The findings suggest that pump activity is suppressed in the non-genetic low renin, presumably volume-expanded forms of hypertension.
3. By contrast, pump suppression does not appear to be involved in spontaneously hypertensive rats or in salt-induced hypertension in Dahl's salt-sensitive rats. In these genetic models the primary defect may be increased cell membrane permeability.

Key words: Dahl's salt-resistant rats, Dahl's salt-sensitive rats, low renin hypertension, spontaneous hypertension, sodium–potassium pump.

Introduction
It is now widely believed that membrane-bound Na⁺,K⁺-dependent ATPase is the enzymatic machinery for active transport of sodium and potassium across cell membranes (Wallick, Lane & Schwartz, 1979). The recent demonstration that ouabain-inhibitable, ATP-dependent Na⁺–K⁺ transport can be measured in vesicles reconstituted from purified Na⁺,K⁺-ATPase and phospholipids provides direct evidence that Na⁺,K⁺-ATPase is in fact the Na⁺–K⁺ pump (Wallick et al., 1979).

It has been further demonstrated that suppression of Na⁺–K⁺ pump activity in normal cardiovascular muscle increases the contractility of heart (Brace, Anderson, Chen, Scott & Haddy, 1974), constricts blood vessels (Anderson, 1976) and increases the responsiveness of blood vessels to vasoactive agents (Brender, Vanhoutte & Shepherd, 1969). These changes are not unlike those seen in certain forms of experimental hypertension.

The purpose of these studies was therefore to determine whether the Na⁺–K⁺ pump activity of blood vessels is altered in various forms of experimental hypertension.

Methods
Ouabain-sensitive radioactive rubidium (86Rb) uptake was used to measure Na⁺–K⁺ pump activity in blood vessels of hypertensive and paired control animals. Rubidium is known to substitute for potassium in the operation of the pump. The ouabain-sensitive 86Rb uptake is that related to active transport and is therefore a measure of Na⁺–K⁺ pump activity. The ouabain-insensitive 86Rb uptake reflects distribution in extracellular space and passive penetration into cells.

The methods used to measure 86Rb uptake by vascular tissues were adapted from techniques employed for studying myocardium (Ku, Akera, Pew & Brody, 1974), erythrocytes and brain tissue (Bernstein & Israel, 1970). In brief, after at least 4 weeks of sustained significant hypertension in experimental animals, and at a similar time interval in the paired control animals, vascular tissue was obtained simultaneously from the paired control and experimental animals for the measurement of 86Rb uptake. The tissue was first incubated at 0°C in K⁺-free Krebs–Henseleit solution to depress the pump and load the cells with sodium. Next, to stimulate the pump, the artery was incubated in 37°C K⁺-free Krebs–Henseleit solution containing 2 mmol of rubidium chloride/l. Each vessel was divided in half: one half was incubated in a medium without ouabain and the other half in a medium containing 0.8 mmol of ouabain/l. 86Rb (New
England Nuclear) was added to each medium to a standard concentration of 0.01 mmol/l and the incubation continued for 18 min. The media were oxygenated with O₂ + CO₂ (95:5). At the end of incubation, tissues were rapidly washed with K⁺-free Krebs–Henseleit solution containing 2 mmol of rubidium chloride/l, blotted to remove surface fluid, weighed and placed in a scintillation counter (1185 Searle) to determine ⁸⁶Rb uptake (pmol/mg of tissue). The tissues were then placed in an oven at 100°C for 25 h and reweighed to determine dry weight.

Ouabain-sensitive ⁸⁶Rb uptake was calculated as the difference between ⁸⁶Rb uptake without and with ouabain. The paired t-test (Sokal & Rohlf, 1973) was used to compare group means of uptakes. P values ≤0.05 were considered significant.

Results

Table 1 shows ouabain-sensitive and ouabain-insensitive ⁸⁶Rb uptakes by blood vessels from animals with four types of low renin (presumably volume-expanded) hypertension. Ouabain-sensitive ⁸⁶Rb uptake was significantly decreased in the blood vessels of all four types of hypertension, namely in the mesenteric arteries and veins of one-kidney, one wrapped hypertensive dogs and in the tail arteries of rats with one-kidney, one-clip, one-kidney, DOCA–salt and reduced renal mass hypertension. The ouabain-insensitive uptake in these vessels, however, was not significantly different compared with uptakes in respective control vessels, except in tail arteries from one-kidney, DOCA–salt hypertensive rats, where it was significantly increased.

In contrast (Table 1) ouabain-sensitive ⁸⁶Rb uptake was significantly increased in tail arteries from spontaneously hypertensive (SH) rats relative to normotensive Wistar–Kyoto (WK) rats. It was also increased in tail arteries from hypertensive Dahl’s salt-sensitive rats on a high (8%) dietary NaCl intake relative to normotensive salt-sensitive rats on a normal (0-4%) dietary NaCl intake and normotensive Dahl’s salt-resistant rats on a high (8%) dietary NaCl intake. In each case the ouabain-insensitive uptake was also increased.

Discussion

This study shows that ouabain-sensitive ⁸⁶Rb uptake and therefore Na⁺–K⁺ pump activity in arteries are reduced only in non-genetic, low renin, presumably volume-expanded forms of hypertension. These include one-kidney, one wrapped hypertension in the dog and one-kidney, one-clip, one-kidney, DOCA–salt and reduced renal mass hypertension in the rat. Since ouabain-sensitive ⁸⁶Rb uptake was also reduced in the mesenteric veins of the dogs, these findings do not appear to be related to elevated pressure but rather to some underlying basic defect in these forms of hypertension.

In separate studies we have shown that myocardial microsomal Na⁺,K⁺-ATPase activity (for technical reasons, it has not been possible to measure Na⁺,K⁺-ATPase activity in microsomes prepared from vascular smooth muscle cells) is also significantly reduced in the left and right ventricles of one-kidney, one-clip hypertensive rats (Clough, Pammrni & Haddy, 1977) and in the left ventricles of one-kidney, DOCA–salt (Clough, Pammrni & Haddy, 1978) and

<table>
<thead>
<tr>
<th>Type of hypertension</th>
<th>Species</th>
<th>Vessel</th>
<th>Ouabain-sensitive ⁸⁶Rb uptake</th>
<th>Ouabain-insensitive ⁸⁶Rb uptake</th>
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<tbody>
<tr>
<td>Low renin, induced</td>
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<tr>
<td>One kidney, one wrapped</td>
<td>Dog</td>
<td>Mesenteric arteries</td>
<td>†(380 ± 138, *P &lt; 0.02)</td>
<td>—(36 ± 26, *P &gt; 0.1)</td>
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<tr>
<td>One kidney, one wrapped</td>
<td>Dog</td>
<td>Mesenteric veins</td>
<td>†(981 ± 336, *P &lt; 0.02)</td>
<td>—(123 ± 60, *P &gt; 0.1)</td>
</tr>
<tr>
<td>One kidney, one clip</td>
<td>Rat</td>
<td>Tail artery</td>
<td>†(1122 ± 355, *P &lt; 0.01)</td>
<td>—(42 ± 45, *P &gt; 0.05)</td>
</tr>
<tr>
<td>One kidney, DOCA–salt</td>
<td>Rat</td>
<td>Tail artery</td>
<td>†(1704 ± 606, *P &lt; 0.02)</td>
<td>—(358 ± 130, *P &lt; 0.02)</td>
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<tr>
<td>Reduced renal mass</td>
<td>Rat</td>
<td>Tail artery</td>
<td>†(3403 ± 418, *P &lt; 0.02)</td>
<td>—(10 ± 68, *P &gt; 0.2)</td>
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<tr>
<td>Genetic</td>
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<td>Spontaneous hypertension</td>
<td>Rat</td>
<td>Tail artery</td>
<td>†(5033 ± 595, *P &lt; 0.01)</td>
<td>—(53 ± 122, *P &gt; 0.01)</td>
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<td>(relative to WK rats)</td>
<td>(relative to WK rats)</td>
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<tr>
<td>Hypertensive Dahl’s salt-sensitive rat on high salt</td>
<td>Rat</td>
<td>Tail artery</td>
<td>†(1777 ± 596, *P &lt; 0.05)</td>
<td>—(100 ± 36, *P &gt; 0.05)</td>
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<td>(relative to normotensive salt-sensitive rat)</td>
<td>(relative to normotensive salt-sensitive rat)</td>
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<tr>
<td>Hypertensive Dahl’s salt-sensitive rat on high salt</td>
<td>Rat</td>
<td>Tail artery</td>
<td>†(2109 ± 589, *P &lt; 0.01)</td>
<td>—(162 ± 46, *P &gt; 0.01)</td>
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<td>(relative to salt-resistant rat on high salt)</td>
<td>(relative to salt-resistant rat on high salt)</td>
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</table>

* Means of differences in uptake ± SEM for the differences.
reduced renal mass hypertensive rats (unpublished data). These data therefore further support our hypothesis that the activity of the Na⁺–K⁺ pump in cardiovascular muscle is suppressed in the low renin presumably volume-expanded forms of hypertension.

This, however, does not seem to be the case for SH rats and hypertensive Dahl's salt-sensitive rats. In both of these models of hypertension, ouabain-sensitive ⁸⁶Rb uptake was significantly increased. In each case, this was accompanied by an increase in ouabain-insensitive uptake, suggesting increased permeability or leakiness of the cell membrane to rubidium. If the permeability to Na⁺ is also increased, the observed increase in Na⁺–K⁺ pump activity could be a compensatory response (Thomas, 1972) subsequent to a rise in intracellular sodium concentration (the possibility that the increase in Na⁺–K⁺ pump activity also results from increased pump sites or increased turnover per pump site cannot be ruled out). Increased intracellular [Na⁺] due to increased permeability to Na⁺ would lead to vasoconstriction, either through a voltage-dependent increase in Ca²⁺ influx or through decreased Ca²⁺ efflux via the Na–Ca exchange mechanism (Blaustein, 1977; Sweander & Goldin, 1980). The end result of increased permeability to sodium would therefore be the same as decreased Na⁺–K⁺ pump activity, i.e. vasoconstriction and hypertension.

Increased permeability to sodium may be a common genetically linked abnormality which could explain the genetic predisposition of these two strains to hypertension.

It is apparent, however, that pump suppression, which appears to be a common feature in the non-genetic, low renin models of hypertension, does not play a role in SH rats or in salt-induced hypertension in Dahl's salt-sensitive rats.

References


