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Vascular Na^+-K^+ Pump Activity in Dahl S and R Rats[1] (41001)

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Abstract. Ouabain-sensitive ^{86}rubidium uptake was used to estimate sodium–potassium pump activity in the tail arteries of Dahl salt-sensitive and Dahl salt-resistant rats on normal and high oral intakes of salt. Uptake was increased in the salt-sensitive strain relative to the resistant strain at a given salt intake. It was also increased in a given strain when the salt intake was increased. In each case the increased ouabain-sensitive uptake was associated with increased ouabain-insensitive uptake which in part reflects the permeability of the cell membrane to rubidium. The results suggest that the increased pump activity is a secondary compensatory response to increased passive penetration of sodium. In this respect, the Dahl salt-sensitive rat is similar to SHR, another genetic model, but different from the other low-renin, presumably, volume-expanded models of hypertension we have studied.

We have previously measured ouabain-sensitive and ouabain-insensitive uptake of radioactive rubidium by blood vessels in several forms of experimental hypertension. Ouabain-sensitive uptake reflects Na^+-K^+ pump activity (1) and ouabain-insensitive uptake reflects distribution in extracellular space and passive penetration into cells (determined by surface area and permeability). We found that ouabain-sensitive ^{86}Rb uptake is reduced in the tail arteries of rats with one-kidney, one clip (2), one-kidney, DOCA, salt (3), and reduced renal mass (4) hypertension. We have also shown that Na^+-K^+ pump activity is reduced in the mesenteric arteries and veins of dogs with one-kidney, one wrap hypertension (5). All of these models of experimental hypertension are considered to be low-renin (6) and presumably volume-expanded types of hypertension. In contrast ouabain-sensitive ^{86}Rb uptake in tail arteries of spontaneously hypertensive rats (SHR) was found to be increased relative to Wistar Kyoto rats (WKY) but unchanged relative to normotensive Wistar rats (7). The finding of decreased pump activity is of interest because induced pump suppression, with the cardiac glycosides, for example, is known to produce vasoconstriction, increased blood vessel sensitivity to vasoactive agents, and increased cardiac contractility, changes not unlike those seen in experimental low renin hypertension (6, 8). Decreased pump activity could produce vasoconstriction via electrogenic depolarization (9) or the Na–Ca exchange mechanism (10).

The purpose of the present study was to determine whether or not a similar defect in pump activity occurs in salt-induced hypertension in Dahl salt-sensitive (S) rats.

Methods. Male Dahl salt-sensitive (S) and salt-resistant (R) rats from Brookhaven National Laboratory, Upton, New York, were used in this study. All rats consumed standard rat chow containing 0.4% NaCl from weaning until about 8–10 weeks of age. Both the S and R rats were then fed either a "normal" (0.4%) or a high (8%) salt (sodium chloride) diet (Agway Inc.) for 4 weeks. All animals drank tap water *ad libitum*. Systolic blood pressure (tail plethysmographic method) and body weight were recorded weekly beginning at age 8 weeks. At the end of the dietary period, the S rats on normal salt (S_{NS}) and high salt (S_{HS}) and the R rats on normal salt (R_{NS}) and high salt (R_{HS}) were anesthetized with sodium pentobarbital (75 mg/Kg body wt) intraperitoneally. Tail arteries were excised simultaneously from S_{NS} and S_{HS} and their

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respective controls R_{NS} and R_{HS} rats and immediately placed in Krebs–Henseleit solution at room temperature and aerated with O_2+CO_2 (95:5).

The uptake of ^{86}Rb was measured as in our previous studies (2–5, 7). The tissue was first incubated at 0° in K^+-free Krebs–Henseleit solution to depress the pump and load the cells with Na^+. Next, to stimulate the pump, the artery was incubated in 37° K^+ free Krebs–Henseleit solution containing 2 m*M* RbCl. Each tail artery was divided in half. One-half was incubated in medium without ouabain and the other half in medium containing 0.8 m*M* ouabain. ^{86}Rb (New England Nuclear) was added to each medium to a standard concentration of 0.01 m*M* and the incubation continued for 18 min. The media were oxygenated with O_2+CO_2 (95:5). At the end of the incubation period, tissues were rapidly washed in the K^+-free Krebs–Henseleit solution containing 2 m*M* RbCl, blotted to remove surface fluid, weighed, and placed in a scintillation counter (1185 Searle) to determine ^{86}Rb uptake (pmole/mg of tissue). The tissues were then placed in an oven at 100° for 24 hr and reweighed to determine dry weight. ^{86}Rb uptake was then expressed as picomoles per milligram of tissue dry weight.

Ouabain-sensitive ^{86}Rb uptake was calculated as the difference between the ^{86}Rb uptake without and with ouabain. The paried "*t*" test was used to compare group mean uptakes: $P \leq 0.05$ were considered significant.

Results. Table I presents means ± SEM of arterial blood pressures and body weights in the four groups of animals at the time of sacrifice following 4 weeks of dietary treatment. S rats on the high salt (8% NaCl) diet showed a progressive elevation in mean systolic pressure relative to the S rats on the normal salt (0.4% NaCl) diet and by the fourth week of dietary treatment the blood pressures of S rats on high salt (S_{HS}) and S rats on normal salt (S_{NS}) were 179 ± 5.2 and 135 ± 2.9 (mm Hg), respectively ($P < 0.001$). By contrast, R rats on high salt (R_{HS}) did not show elevation in blood pressure when compared to R rats on normal salt diet (R_{NS}). There was, however, a small but significant difference in the mean systolic blood pressures of S and R rats maintained on normal salt diet, the pressure being about 16 mm Hg higher in the S rats. Thus our results regarding blood pressure in relation to salt intake in these two strains of animals are in agreement with results from several other laboratories (11, 12). The mean body weights of these groups of animals were not significantly different.

Compared with the arteries from normotensive R_{NS} rats, both ouabain-sensitive and ouabain-insensitive ^{86}Rb uptakes by arteries from S_{NS} rats were increased (Fig. 1). Similarly, both ouabain-sensitive and ouabain-insensitive uptakes were significantly higher in tail arteries of hypertensive S_{HS} compared to the uptakes in normotensive R_{HS} rats (Fig. 2). Furthermore, when we compared the ^{86}Rb uptakes in arteries of S rats on normal salt and S rats on high salt, again both the ouabain-sensitive and ouabain-insensitive ^{86}Rb uptakes were significantly increased in arteries of the S rats on the high salt diet (Fig. 3). Similarly, ouabain-sensitive and ouabain-insensitive uptakes were higher in arteries of R rats on high salt compared to arteries of R rats on normal salt diet (Fig. 4).

TABLE I

	Normal salt diet (0.40%)		High salt diet (8%)	
	R rats	S rats	R rats	S rats
Mean systolic blood pressure (mm Hg)	119 ± 2.8	135 ± 2.9[a]	120 ± 1.4	179 ± 5.2[b]
Mean body weights (g)	430 ± 15.7	417 ± 16.2	435 ± 10.6	406 ± 15.6

[a] Significantly different from R rats on normal salt ($P < 0.01$).
[b] Significantly different from S rats on normal salt ($P < 0.001$).

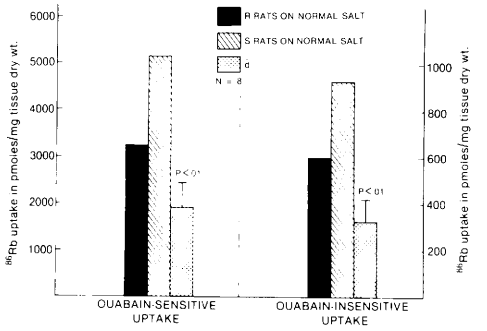

FIG. 1. ^{86}Rb uptake by tail arteries of Dahl S and R rats on normal salt diet. The cross-hatched bars represent mean of the differences ($\bar{d}$), and vertical lines indicate SEM of the differences.

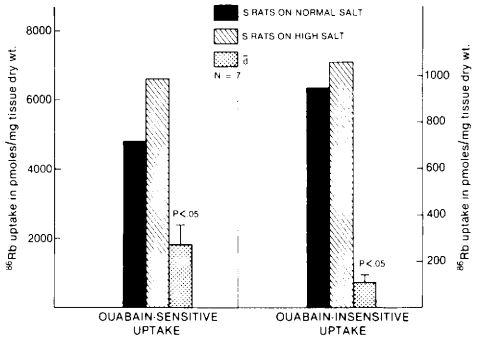

FIG. 3. ^{86}Rb uptake by tail arteries of S rats on normal and high salt diets. As in Fig. 1.

Discussion. These studies show that ouabain-sensitive ^{86}Rb uptake by the tail artery is greater in Dahl S than R rats on a given dietary sodium intake ($S_{NS} > R_{NS}$, $S_{HS} > R_{HS}$) and greater with a higher than normal sodium intake in a given strain ($R_{HS} > R_{NS}$, $S_{HS} > S_{NS}$). In each case, the increased ouabain-sensitive ^{86}Rb uptake was accompanied by increased ouabain-insensitive ^{86}Rb uptake. These findings are similar to those previously reported for SHR relative to WKY (7) but differ from those previously reported for four models of experimental low renin, presumably volume-expanded hypertension (2–5). In the latter models, ouabain-sensitive ^{86}Rb uptake is decreased without a change, in three of four instances, in the ouabain-insensitive ^{86}Rb uptake.

The possibility that the increased ^{86}Rb uptake results from structural changes should be considered, particularly since there is indirect evidence that the relative content of cells in the arterial wall increases with time in another model (one-kidney, DOCA, salt) of rat hypertension (14). This, however, is not a satisfactory explanation since increased ^{86}Rb uptake, both ouabain-sensitive and insensitive, was observed in the absence of hypertension (R_{HS} vs R_{NS}) or when hypertension had barely developed (S_{NS} vs R_{NS}). Furthermore, in the four low-renin varieties of hypertension studied previously, structural changes were almost surely also present (hypertension had been present for at least 4 to 5 weeks), yet ouabain-sensitive ^{86}Rb uptake was decreased and ouabain-insensitive ^{86}Rb uptake was mostly unchanged rather than being increased.

It seems more likely that the increased

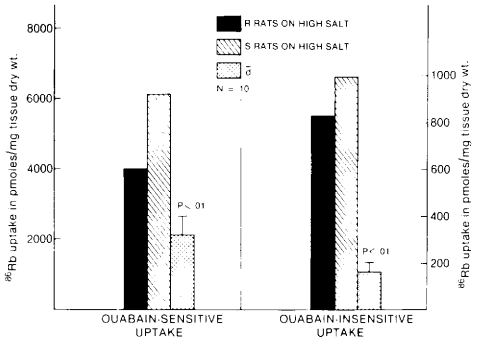

FIG. 2. ^{86}Rb uptake by tail arteries of S and R rats on high salt diet. As in Fig. 1.

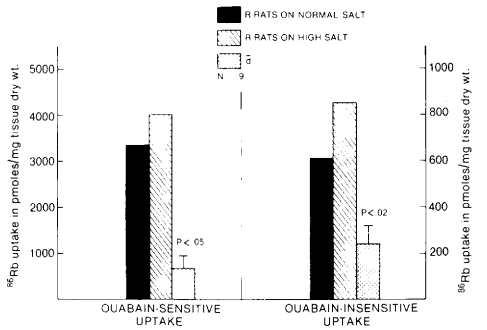

FIG. 4. ^{86}Rb uptake by tail arteries of R rats on normal and high salt diets. As in Fig. 1.

ouabain-sensitive ^{86}Rb uptake reflects increased activity of the sodium–potassium pump. Rubidium is an ion which can substitute for potassium in active transport by the pump across cell membranes. It is used in the study of pump activity because its radioactive form has a longer half-life and a lower-energy emission than the radioactive form of potassium. The ouabain-sensitive ^{86}Rb uptake is that uptake related to active transport because ouabain inhibits the sodium–potassium pump which is responsible for active transport. The ouabain-sensitive ^{86}Rb uptake would therefore be greater if the sodium–potassium pump were more active. Thus it appears that the pump is more active in Dahl S relative to Dahl R regardless of the salt intake and that a high salt intake increases pump activity in both Dahl S and Dahl R.

The increased pump activity could be due to an increase in the concentration of intracellular sodium or to an increase in the number of pumps/unit area of membrane or both. Pump activity is directly related to intracellular sodium concentration. The preincubation conditions were chosen to raise intracellular sodium concentration. If the level at the end of the preincubation period was higher in Dahl S relative to Dahl R on either normal or high dietary sodium or higher in a given strain on the high salt diet, pump activity and hence ouabain-sensitive rubidium uptake would also be higher. On the other hand, pump activity at a given intracellular sodium concentration would also be greater if the number of pump sites/unit area were greater.

The practical problems associated with the measurement of intracellular sodium concentration and the isolation of sarcolemma for the measurement of Na^+,K^+-ATPase activity in vascular smooth muscle are formidable. However, several indirect observations suggest that both mechanisms may be involved. First ouabain-insensitive ^{86}Rb uptake was also always higher in Dahl S relative to Dahl R, suggesting increased intracellular penetration of this ion. If this were also the case for the sodium ion, higher intracellular values for sodium concentration at the end of the preincubation period would be expected. The increased pump activity would therefore be secondary to increased sodium penetration, perhaps due to increased permeability of the membrane to sodium. Second, a high sodium diet is known to increase the sodium content of the arterial wall in the common laboratory rat (15) perhaps because of increased extracellular sodium concentration. Thus the intracellular sodium concentration in the animals on a high salt diet might have been higher at the time of excision of the arteries and this could have been carried on through the preincubation period. This could explain the increased pump activity in R_{HS} vs R_{NS} and S_{HS} vs S_{NS}. Here again the increased pump activity would be secondary to increased sodium penetration. Third, while it is not currently possible to measure Na^+,K^+-ATPase activity in sarcolemma of vascular smooth muscle from the rat tail artery (the tissue and thus the sarcolemmal yield are too small), this is not the case for rat heart. Measurements in myocardial microsomes have been made and show that Na^+,K^+-ATPase activity is greater in Dahl S than Dahl R, though this difference disappears when both strains are placed on a high salt diet (Clough, D.L., unpublished observation). Just the opposite is the case in the low-renin types of rat hypertension, i.e., myocardial Na^+,K^+-ATPase activity, like vascular ouabain-sensitive ^{86}Rb uptake, is uniformly low (6). If the findings in heart can be extrapolated to blood vessels, these observations suggest that the increased pump activity observed in this study may also be related to an increase in maximal pump activity (number of pump sites/unit area and turnover/site) at a concentration of sodium sufficient to saturate the pump sites. Additional studies are obviously necessary to settle these questions.

Regardless of the mechanism, the study clearly shows that oubain-sensitive ^{86}Rb uptake is increased in the Dahl S rat, just as it is in SHR, rather than decreased, as it is in the low renin models of hypertension. The hypertension in both the Dahl S rat and SHR is believed to be genetically mediated. Increased ouabain-insensitive ^{86}Rb uptake associated with increased ouabain-sensitive ^{86}Rb uptake (which may in part be compen-

satory in nature) in both the Dahl S rat and SHR suggest a common genetically linked abnormality which may help explain the predisposition of these strains to hypertension. This abnormality may be increased permeability of the smooth muscle cell membrane to sodium. On the other hand, the hypertension in the other models studied were investigator induced by interfering with the capacity of the kidney to excrete salt, either organic (one-kidney, one wrapped; one-kidney, one clip; reduction of renal mass) or both organic and functional (one-kidney, DOCA, salt), and here a common abnormality appears to be reduced sodium–potassium pump activity.

If the increased pump activity observed in the two genetic models fails to compensate for the increased influx of sodium, partial depolarization of vascular smooth muscle cells would result thereby leading to increased calcium influx via voltage-dependent calcium channels and vasoconstriction. Alternately, the increased intracellular sodium concentration could lead to increased intracellular calcium concentration and vasoconstriction via the Na–Ca exchange mechanism (the reduced transmembrane sodium gradient would provide less energy for calcium efflux). Thus the end result of increased penetration of sodium would be the same as decreased Na^+-K^+ pump activity, i.e., vasoconstriction and hypertension. Theoretically, increased intracellular sodium concentration could result from increased permeability at the same extracellular sodium concentration or from increased extracellular sodium at the same permeability. The former could explain the small but significant elevation in the arterial pressure in S rats relative to R rats even when both strains are fed normal salt. If the increased pump activity completely compensates for the increased sodium penetration, intracellular sodium concentration would not rise. This could account for the absence of a rise in blood pressure in R rats on the high salt diet.

We presently lack evidence to support the possibility that the increased pump activity is insufficient to compensate the increased penetration. We therefore cannot rule out the possibility that other factors play a dominant role in the genesis of the hypertension in S_{HS} rats. It is apparent, however, that pump suppression, which appears to be a common feature in the nongenetic, investigator induced, low-renin models of hypertension, does not play a role in the Dahl salt-sensitive genetic form of hypertension.

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