Demonstration of a Humoral Inhibitor of the Na\textsuperscript{+}-K\textsuperscript{+} Pump in Some Models of Experimental Hypertension

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SUMMARY We have previously shown that ouabain-sensitive \textsuperscript{86}Rb uptake, a measure of Na\textsuperscript{+}-K\textsuperscript{+} pump activity, is decreased in the blood vessels of dogs with one-kidney, one wrapped hypertension and rats with one-kidney, DOCA, saline hypertension. We here extend the study to rats with one-kidney, one clip and reduced renal mass-saline hypertension. We also assayed supernates of boiled plasma from three of these models for ouabain-sensitive \textsuperscript{86}Rb uptake suppressing activity. Finally, we examined the influence of the anteroventral third ventricle (AV3V) lesion in the rat on vascular pump and plasma supernate activities. We found that ouabain-sensitive \textsuperscript{86}Rb uptake is suppressed in the tail arteries of rats with one-kidney, one clip and reduced renal mass hypertension and that plasma supernates from these rats and from dogs with one-kidney, one wrapped hypertension suppress \textsuperscript{86}Rb uptake when applied to tail arteries from normal rats. We also found that, in the volume-expanded state, rats with AV3V lesions had higher vascular ouabain-sensitive \textsuperscript{86}Rb uptake than rats with sham lesions and evidence for decreased inhibitory activity of the plasma. These findings suggest that reduced vascular Na\textsuperscript{+}-K\textsuperscript{+} pump activity is common to several models of experimental hypertension and that this defect results from a heat-stable ouabain-like agent in plasma that originates in or is influenced by the AV3V area of the brain. (Hypertension 3 (suppl II): II-96-II-101, 1981)

KEY WORDS • ouabain-like humoral factor • rubidium uptake • one-kidney, one clip hypertension • reduced renal mass hypertension • anteroventral third ventricle lesion • volume expansion

We have previously shown that ouabain-sensitive \textsuperscript{86}Rb uptake, a measure of Na\textsuperscript{+}-K\textsuperscript{+} pump activity, is decreased in the mesenteric arteries and veins of dogs with one-kidney, one wrapped hypertension\textsuperscript{1} and that a similar defect exists in the tail arteries of rats with one-kidney, DOCA, saline hypertension.\textsuperscript{2} In contrast to these models of hypertension, decreased activity of the vascular Na\textsuperscript{+}-K\textsuperscript{+} pump is not observed in the tail arteries of rats with glucocorticoid hypertension\textsuperscript{2} and two models of genetic hypertension.*\textsuperscript{1}\textsuperscript{2} We have furthermore shown that acute expansion of the extracellular fluid volume in dogs and rats also inhibits vascular Na\textsuperscript{+}-K\textsuperscript{+} pump activity and that supernates of boiled plasma from these animals inhibit Na\textsuperscript{+}-K\textsuperscript{+} pump activity when applied to tail arteries from normal rats.\textsuperscript{3} The plasma appeared to contain a heat-stable ouabain-like factor. We wondered whether suppressed vascular Na\textsuperscript{+}-K\textsuperscript{+} pump activity is also found in animals with other models of hypertension and whether the plasma of these animals contains an ouabain-like factor.

These questions are relevant because inhibition of the Na\textsuperscript{+}-K\textsuperscript{+} pump in the cardiovascular muscle of normal animals, with cardiac glycosides or low potassium, for example, causes increased cardiac contractility,\textsuperscript{4,5} increased vascular bed and total peripheral resistance,\textsuperscript{6,7} increased responsiveness of blood vessels to vasoactive agents,\textsuperscript{8-11} and increased arterial pressure\textsuperscript{12,13} particularly if diuresis cannot occur.\textsuperscript{14} These changes are like those seen in many forms of experimental hypertension.\textsuperscript{15,16} Thus, the hypertension may be in part related to the Na\textsuperscript{+}-K\textsuperscript{+} pump suppression.

This hypothesis, however, is difficult to reconcile with the findings of Brody et al.,\textsuperscript{20} which show that ablation of the anteroventral third ventricle (AV3V) in rats prevents and reverses the development of one-kidney renal and one-kidney, DOCA, saline hypertension, models in which we find decreased vascular Na\textsuperscript{+}-K\textsuperscript{+} pump activity.

The present study was therefore undertaken to determine whether: 1) decreased vascular Na\textsuperscript{+}-K\textsuperscript{+} pump activity also occurs in two other models of experimental hypertension; 2) decreased pump activity is

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accompanied by the appearance of the ouabain-like humoral factor; and 3) the AV3V lesion influences vascular Na⁺-K⁺ pump activity and the level of the ouabain-like factor in plasma.

Methods

Preparation of Hypertensive Dogs

One-kidney, one wrapped hypertension was produced in dogs according to methods previously described. After at least 4 weeks of sustained hypertension and after a similar time interval in the paired sham-wrapped control dog, mesenteric arteries and aortic blood were obtained from the pair under pentobarbital anesthesia. Ouabain-sensitive and insensitive ⁸⁶Rb uptakes were immediately measured in the arteries utilizing our standard technique.¹ The blood was subsequently assayed for ouabain-like activity (see below). Hematocrit and plasma electrolyte and creatinine concentrations were also measured.

Preparation of One-Kidney, One Clip Hypertension in Rats

Normotensive male Wistar rats weighing 150–200 g were randomly divided into experimental and control groups. Under light ether anesthesia, a constricting silver clamp (0.2 mm I.D.) was placed on the left renal artery of the experimental animals through a flank incision. A nonconstricting clamp (0.42 mm I.D.) was placed on the left artery of the paired control rats. One week later, both groups of animals underwent right nephrectomy through a flank incision, again utilizing light ether anesthesia. After 4 weeks of sustained hypertension (systolic pressure > 140 mm Hg), tail plethysmographic method) in an experimental animal and after a similar time interval in a paired control animal, blood and/or tail arteries were removed under pentobarbital anesthesia and examined for ouabain-like activity and ⁸⁶Rb uptake respectively. In some cases, plasma electrolyte concentrations were also measured.

Preparation of Rats with Reduced Renal Mass Hypertension

Normotensive male Wistar rats weighing 180–200 g were randomly divided into experimental and control groups. Under light ether anesthesia, subtotal nephrectomy was performed on both the experimental and control animals by removing the right kidney and 50% of the left kidney. The two poles of the left kidney were excised by incircling them with loops of 4-0 silk suture and then tightening the loops. This both cut the tissue and tied off the main arterial and venous channels with minimal blood loss. Following surgery, all animals consumed a sodium deficient diet (0.02% sodium, Bioservices, Inc.) but the experimental animals drank an iso-osmotic solution of sodium chloride at a rate of 1.26 ml/min for 20 to 30 minutes until extracellular fluid volume increased by 30%. The infusion was then maintained at a rate of 0.09 ml/min for an additional 2 hours. At the end of the infusion period, tail arteries were excised and blood collected for ⁸⁶Rb uptake.

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Assay for Ouabain-like Activity in Plasma

Aortic blood from an experimental animal and a paired control animal (dogs 160 ml, rats 6 ml) was collected through prechilled femoral cannulas into heparinized tubes in an ice bath. The blood was then centrifuged at 4°C in a Sorval refrigerated centrifuge at 1100 × g for 10 minutes. The plasma was separated and allowed to sit at room temperature for 30 minutes to enhance any ouabain-like activity.² The plasma was then boiled for 5 minutes at 100°C to precipitate serum proteins. The boiled plasma was then centrifuged at 3600 × g for 90 minutes. The supernate was harvested and immediately frozen for future use in the assay for ouabain-like humoral factor.

In this assay, tail arteries were excised from pentobarbital-anesthetized untouched normotensive rats. The arteries were quickly cleaned of adventitia and placed in Krebs-Hanseleit solution. Each artery was divided in half. One half was preincubated at room temperature in the supernate prepared from the boiled plasma of the hypertensive animal and the other half in the supernate prepared from the paired normotensive control animal. After 2 hours of preincubation, ouabain-sensitive and insensitive ⁸⁶Rb uptakes were measured as described previously.¹ In the case of rat supernates, only total ⁸⁶Rb uptake was measured for lack of sufficient supernate volume. The supernates from the dogs and from the rats with reduced renal mass were also examined chemically (see Results).

Vascular Na⁺-K⁺ Pump and Ouabain-like Humoral Factor Activities in AV3V Lesioned Rats

Lesions of the anterior wall of the third cerebral ventricle (AV3V) were produced in male normotensive Sprague-Dawley rats under anesthesia by Dr. James Buggy at the University of South Carolina, Columbia, as previously described.³ Sham-lesioned animals were also prepared. The rats were studied when hydration recovery was complete, typically 4-8 weeks after creating the AV3V lesion. At this time and after the same time interval in the sham-lesioned animal, tail arteries were excised under pentobarbital anesthesia for measurement of ⁸⁶Rb uptake.

Studies were also conducted in the volume-expanded state. Under pentobarbital anesthesia, the right external jugular veins of a lesioned animal and a paired sham-lesioned animal were cannulated. The animals were then intravenously infused with an iso-osmotic solution of sodium chloride at a rate of 1.26 ml/min for 20 to 30 minutes until extracellular fluid volume increased by 30%. The infusion was then maintained at a rate of 0.09 ml/min for an additional 2 hours. At the end of the infusion period, tail arteries were excised and blood collected for ⁸⁶Rb uptake.

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measurement and ouabain-like factor assay, respectively. At the conclusion of most experiments, accurate placement of the lesion was verified histologically.

Statistics

The paired Student t test was used for ouabain-sensitive and insensitive uptakes. The unpaired test was used in all other cases.

Results

Hypertensive Dogs and Rats

All experimental and control animals used in the analysis appeared healthy during the study period. The experimental groups became hypertensive relative to their own control groups. The blood pressures in mm Hg ± SEM were 167 ± 8 mean (control 117 ± 5) for the one-kidney, one wrapped dogs, 186 ± 4 systolic (control 123 ± 2) for the one-kidney, one clip rats, and 167 ± 1 systolic (control 116 ± 3) for the reduced renal mass rats. Serum sodium, potassium, calcium, magnesium, and creatinine concentrations did not differ. Body weight was lower only in the reduced renal mass hypertensive rats (279 ± 9 vs 305 ± 9 g), as was hematocrit (29 ± 2% vs 41 ± 1%).

Ouabain-sensitive 42Rb uptake by mesenteric arteries from dogs with one-kidney, one wrapped hypertension was significantly decreased whereas ouabain-insensitive uptake was not significantly different relative to the sham-operated control group, thus confirming our earlier study.1 Figures 1 and 2 show that the same was the case for the rats with one-kidney, one clip and reduced renal mass hypertension.

Figure 3 shows that incubation of tail arteries from normal rats in plasma supernates from dogs with one-kidney, one wrapped hypertension caused a decrease in ouabain-sensitive 42Rb uptake but was without effect on ouabain-insensitive 42Rb uptake, just as was the case in the arteries of the hypertensive donor animal. Similarly, incubation of tail arteries from normal rats in plasma supernates from rats with one-kidney, one clip and reduced renal mass hypertension reduced total 42Rb uptake (fig. 4). The osmolality and the concentration of Na+, K+, Ca++, Mg++, Cl-, creatinine, nonprotein nitrogen, and protein in the super-

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**Figure 1. Ouabain-sensitive and insensitive 42Rb uptakes by tail arteries from one-kidney, sham-clipped normotensive (1-K, NT) and one-kidney, one clipped hypertensive (1-K, 1-C HT) rats.**

**Figure 2. Ouabain-sensitive and insensitive 42Rb uptakes by tail arteries from reduced renal mass normotensive (RRM NT) and reduced renal mass hypertensive (RRM HT) rats. The former drank distilled water while the latter drank a 1% solution of sodium chloride.**

**Figure 3. Ouabain-sensitive and insensitive 42Rb uptakes by tail arteries from normal rats incubated in plasma supernates from one-kidney, sham wrapped normotensive and one-kidney, one wrapped hypertensive dogs.**
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Figure 4. Total $^{86}$Rb uptake by tail arteries from normal rats incubated in plasma supernates from one-kidney, sham clipped normotensive (1-K NT) and one-kidney, one clipped hypertensive (1-K, 1-C HT) rats (left) and from reduced renal mass normotensive (RRM NT) and reduced renal mass hypertensive (RRM HT) rats (right)

Figure 5. Ouabain-sensitive and insensitive $^{86}$Rb uptakes by tail arteries from volume-expanded sham-lesioned (SL) and AV3V-lesioned (L) rats (left) and total $^{86}$Rb uptake by tail arteries from normal rats incubated in plasma supernates from these volume-expanded sham-lesioned and AV3V-lesioned rats (right)

In the absence of volume expansion, $^{86}$Rb uptake by the tail artery was not significantly different in the sham-lesioned and AV3V-lesioned rats. Values for ouabain-sensitive $^{86}$Rb uptake were 4281 and 5019 pmoles/mg tissue dry weight ($D \pm \Delta D = 738 \pm 538$, $p > 0.2$, $n = 8$) respectively and for ouabain-insensitive uptake were 970 and 967 pmoles/mg tissue dry weight ($D \pm \Delta D = 3 \pm 100$, $p > 0.9$, $n = 8$) respectively. However, in the presence of acute volume expansion, ouabain-sensitive $^{86}$Rb uptake was higher in the arteries of the lesioned rats than in the arteries of the sham-lesioned rats (fig. 5). Furthermore, plasma supernates from these lesioned animals raised total $^{86}$Rb uptake relative to supernates from the sham-lesioned animals when applied to arteries taken from normal untouched rats (fig. 5).

Discussion

These findings suggest that reduced vascular Na+-K+ pump activity is common to several varieties of experimental hypertension and that the defect results from a heat-stable ouabain-like agent in the plasma which originates in or is influenced by the AV3V area of the brain.

Rubidium is an ion that substitutes 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 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 Na+-K+ pump which is responsible for active transport. The ouabain-insensitive uptake, i.e., the $^{86}$Rb uptake in the presence of ouabain, reflects distribution in extracellular space and passive penetration into cells (determined by cell wall permeability and surface area).

The control values for ouabain-sensitive $^{86}$Rb uptake differ somewhat from experiment to experiment. This is in part expected because in one case the test object differed (mesenteric arteries of the dog rather than tail artery of the rat) and in several cases the test conditions differed (Krebs-Henseleit solution vs plasma supernate). We also know that the state of hydration of the animal donating the arteries or the plasma supernate also influences uptake. This might
explain, for example, the difference in the control values in figure 4. The control supernate illustrated in the right panel came from animals with a lesser renal mass than the supernate illustrated in the left panel. In any event, the control and experimental animals were treated identically except for the experimental variable, and then studied simultaneously.

Decreased vascular ouabain-sensitive \( ^{86}\text{Rb} \) uptake seems to be peculiar to the varieties of experimental hypertension thought to have low plasma renin activity; decreased uptake has been observed in all such models thus far tested \(^1,4\), while increased uptake has been found in all other hypertensive models tested (SHR relative to WKY, Dahl S relative to Dahl R, one-kidney, dexamethasone relative to one-kidney rats). \(^1,4\)

The decreased pump activity seems to be related to a heat-stable ouabain-like humoral factor because supernates of boiled plasma from dogs with one-kidney, one wrapped hypertension reduced ouabain-sensitive \( ^{86}\text{Rb} \) uptake without affecting ouabain-insensitive \( ^{86}\text{Rb} \) uptake when applied to tail arteries from normal rats, thus mimicking the findings in the dogs' own arteries. Furthermore, supernates from rats with one-kidney, one clip and reduced renal mass hypertension reduced total \( ^{86}\text{Rb} \) uptake in the same assay system. Compatible with these findings is our earlier observation that ouabain-sensitive \( ^{86}\text{Rb} \) uptake is also reduced in the veins of dogs with one-kidney, one wrapped hypertension \(^1\) and the observation of Simon \(^2\) that serum from dogs with this model of hypertension increases the sodium content of rabbit aortic media explants.

The heat-stable ouabain-like factor may originate in or be influenced by the AV3V area of the brain. Brody et al. \(^20\) and Buggy et al. \(^22\) found that ablation of the AV3V area in rats prevents and ameliorates one-kidney, renal and one-kidney, DOCA, saline hypertension, models in which we find decreased vascular ouabain-sensitive \( ^{86}\text{Rb} \) uptake. We then reported that acute volume expansion of rats with an iso-osmotic solution of sodium chloride or mannitol reduces tail artery ouabain-sensitive \( ^{86}\text{Rb} \) uptake without affecting ouabain-insensitive uptake and that supernates of boiled plasma from these volume expanded rats or from volume expanded dogs reduces total \( ^{86}\text{Rb} \) uptake when applied to tail arteries from normal rats. \(^4\) Bealer et al. \(^4\) then showed impaired plasma antinatriferic activity on volume expansion of rats with AV3V lesions. We here show that this is associated with impaired suppression of ouabain-sensitive \( ^{86}\text{Rb} \) uptake by the tail artery and impaired ability of plasma supernates to suppress total \( ^{86}\text{Rb} \) uptake when applied to tail arteries from another rat. Finally, supernates of boiled plasma from acutely volume-expanded dogs and dogs with one-kidney, one wrapped hypertension exhibit increased antinatriferic activity (Chen et al., unpublished observation).

We have not characterized the ouabain-like humoral agent observed in these studies. We know only that it is heat stable. The humoral antinatriferic and natriuretic factor observed by Gruber and Buckalew \(^21\) and presumably by Bealer et al. \(^4\) appears to be a heat-stable, low molecular weight, acidic peptide formed from a precursor molecule. It apparently sensitizes the arteriole to the vasoconstrictor action of norepinephrine. \(^28\) On the other hand, Haupert and Sancho \(^26\) have extracted a heat-stable, low molecular weight, basic nonpeptide with antinatriferic activity from hypothalamus. We have previously reviewed \(^22\) the findings in the old and recent literature which indicate that the plasma of animals with low renin hypertension contains a slowly acting, heat-stable, low molecular weight, pressor and sensitizing agent. The relation between this agent, natriuretic factor, and the ouabain-like factor reported here deserves additional study.

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