Humoral Sodium Transport Inhibitor in Acute Volume Expansion and Low Renin Hypertension

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SUMMARY This review summarizes our bioassay methods for determining the level of humoral sodium pump inhibiting factor after acute volume expansion in experimental animals and humans, and in low renin experimental and human essential hypertension. In brief, ouabain-sensitive 86Rb uptake and membrane potential in blood vessels from normal animals are measured after incubation in plasma supernate from experimental subjects and animals and their respective controls. The data show that humoral sodium pump inhibitor is elevated after acute volume expansion in normal animals (dogs and rats) and in normal humans. The level of inhibitor is also elevated in patients with low renin essential hypertension and in experimental animals with low renin, volume-dependent types of hypertension, namely, one-kidney, one wrapped hypertension in dogs, and one-kidney, one clip and reduced renal mass-saline hypertension in rats. Humoral sodium pump inhibiting factor inhibits the Na⁺-K⁺ pump in the cardiovascular system. Such inhibition by other means (hypokalemia, cardiac glycosides) activates the system. Therefore, we also discuss the possible role of humoral sodium pump inhibitor in low renin volume-dependent hypertension. (Hypertension 10 [Suppl I]: I-78–I-83, 1987)

KEY WORDS • hypertension • Na⁺-K⁺ pump • volume expansion • humoral sodium pump inhibitor

THE mechanisms of volume-dependent and salt-dependent hypertension are not clear. Increased salt intake or decreased salt excretion leads to elevated blood pressure. The pressure rises slowly, suggesting that the increase cannot be explained by volume expansion per se. In these types of hypertension, plasma renin activity is decreased, and converting enzyme inhibitors and angiotensin antagonists have minimal effects on blood pressure. Catecholamine blood levels are decreased after increased salt ingestion or decreased salt excretion and therefore cannot explain the elevated blood pressure.

Recent studies in our laboratory suggest that Na⁺,K⁺-adenosine triphosphatase (ATPase) and Na⁺-K⁺ pump activities are reduced in cardiovascular muscle cells of animals with experimental low renin hypertension1–6 and in animals after acute volume expansion.7 These findings are of interest because induced suppression of Na⁺,K⁺-ATPase and Na⁺-K⁺ pump activities in cardiovascular muscle of normal animals with cardiac glycosides produces some of the changes seen in experimental low renin hypertension. These changes include vasoconstriction,8–10 increased vascular sensitivity to vasoactive agents,11,12 increased cardiac contractility,13 and raised blood pressure.8–10,14 Using bioassay techniques, we have also generated evidence for an inhibitor of the vascular Na⁺-K⁺ pump in the plasma of these animals. Evidence has been reported from our laboratory for a similar inhibitor in the plasma of black patients with low renin essential hypertension and in the plasma of normotensive subjects after acute volume expansion.

In this review, we summarize the methodology used to elicit the evidence that suggests the presence of elevated levels of humoral sodium pump inhibiting factor (HPIIF) after acute volume expansion, and in low renin experimental and human essential hypertension.
We also summarize the findings in these conditions and the possible mechanism of action of HPIF in the pathophysiology of hypertension.

Methods

Acute Volume Expansion

Normotensive mongrel dogs and Wistar rats (anesthetized with pentobarbital) and normotensive male black and white humans (unanesthetized) underwent acute extracellular fluid volume expansion. In the dogs and rats, this was achieved by an initial rapid intravenous infusion of normal saline at the rates of 45 and 1.26 ml/min, respectively, for 20 to 30 minutes to expand fluid volume by 30%. The rate of infusion was then slowed to 3.2 and 0.09 ml/min in the dogs and rats, respectively, to maintain the volume expansion for an additional 2 hours. In the humans, acute volume expansion was achieved by intravenous infusion of normal saline, 33 ml/min for 30 minutes and then 10 ml/min for the next 150 minutes. The subjects also drank 1.5 L of tap water over the 3-hour period. Paired control animals or humans (age- and race-matched) were sham-infused. Blood was then collected from the expanded and sham-expanded dogs, rats, and humans, 160, 6, and 30 ml, respectively, for assay of endogenous HPIF.

Acute Volume Expansion of AV3V Lesioned and Sham-Lesioned Rats

In some rats, the anterior wall of the third ventricle (AV3V) was lesioned under pentobarbital anesthesia as previously described. Control rats were sham-lesioned. After hydration recovery was complete, typically within 4 to 8 weeks, a lesioned and a paired sham-lesioned animal were acutely volume-expanded and then their blood was examined for HPIF activity.

Preparation of Experimental Hypertension

One-kidney, one wrapped (1K1W) hypertensive and paired one-kidney, sham wrapped (1KSW) normotensive control dogs; one-kidney, one clip (1K1C) hypertensive and one-kidney, sham clip (1KSC) normotensive control rats; and reduced renal mass–saline drinking (RRM-S) hypertensive and reduced renal mass–distilled water drinking (RRM-DW) normotensive control rats were produced as previously described. After 4 weeks of sustained significant hypertension (systolic pressure > 140 mm Hg) in an experimental hypertensive animal and a similar time interval in a paired control normotensive animal, blood was removed under pentobarbital anesthesia for assay of endogenous HPIF level.

Human Essential Hypertension

Black male patients with mild to moderate uncomplicated essential hypertension (age 35–55 years, taking no medication for 4–6 weeks prior to study) were paired with respect to age, sex, and race with normotensive control subjects. After an overnight fast, 30 ml of blood was obtained from a hypertensive and a control subject and assayed for HPIF level.

Preparation of Boiled Plasma Supernates for Assay of HPIF

Aortic blood from an experimental animal and a paired control animal (dogs, 160 ml; rats, 6 ml), and venous blood (30 ml) from human experimental and control subjects was collected into prechilled heparinized tubes in an ice bath. The blood was then centrifuged at 4°C in a Sorvall refrigerated centrifuge (Du- pont, Newton, CT, USA) at 1100 g for 10 minutes. The plasma was separated and allowed to sit at room temperature for 30 minutes to enhance HPIF activity, if any. The plasma was then boiled for 5 minutes at 100°C to precipitate serum proteins and to inactivate known vasoactive hormones. The boiled plasma was then centrifuged at 36,000 g for 90 minutes. The supernate was harvested and used immediately or frozen at −70°C for future use in the assay for HPIF.

Assays for HPIF Activity in Plasma

Two different bioassays were used to estimate the levels of HPIF in experimental animals relative to control animals. The bioassays used were 1) the effect of boiled plasma supernates obtained from experimental and control animals on ouabain-sensitive ⁸⁶Rb uptake by tail arteries obtained from normal untouc hed rats, and 2) the effect of these plasma supernates on the transmembrane potentials of vascular smooth muscle cells in tail arteries from experimental, control, and normal untouc hed rats.

⁸⁶Rb Uptake Assay

A tail artery was excised from an untouched normal rat and divided in half. One half was preincubated at room temperature in the boiled plasma supernate obtained from the experimental animal and the other half in the boiled plasma supernate obtained from the paired control animal. After 2 hours of preincubation, the test tubes were transferred to a shaking water bath at 37°C. After 2 minutes, ⁸⁶Rb was added to each tube to a standard concentration of 0.01 mM in the presence and absence of 1 mM ouabain, and the incubation continued for 18 minutes. At the end of this incubation period, tissues were rapidly washed with Krebs-Henseleit solution containing 2 mM cold RbCl, blotted to remove surface fluid, and placed in a gamma counter, and ⁸⁶Rb uptake was determined on the basis of dry weight (pmol/mg tissue dry weight). In the case of rat supernates, only total ⁸⁶Rb or ouabain-insensitive ⁸⁶Rb uptake was measured in any given assay for lack of sufficient supernate volume.

Transmembrane Potential Assay

This bioassay has thus far been used only in two models of experimental hypertension, namely, 1K1C and RRM-S hypertensive rats.

Transmembrane potentials of vascular smooth muscle cells in tail arteries from 1K1C and RRM-S hypertensive and appropriate control rats were measured with glass microelectrodes filled with KCl solution (3 M) with tip resistance of 30 to 80 MΩ and tip potential of less than 5 mV. Microelectrode impalements were
made from the adventitial side of the unopened tail arteries in vitro, which were constantly suffused with physiological salt solution (in mM: NaCl, 118.3; KCl 4.7; CaCl$_2$, 2.5; KH$_2$PO$_4$, 1.2; NaHCO$_3$, 1.2; glucose, 11) aerated with 95% O$_2$, 5% CO$_2$. After recording transmembrane potentials in hypertensive and normotensive arteries, boiled plasma supernate from the hypertensive or normotensive rat was added to the bathing solution and its effect on resting transmembrane potentials was recorded.

Results

$^{86}$Rb Uptake Assay

In all studies, ouabain-insensitive uptakes were the same when the tail artery halves were incubated in the plasma supernates obtained from experimental and control animals. However, uptake of ouabain-sensitive $^{86}$Rb by the tail artery half incubated in the plasma supernate from the volume-expanded dog was significantly less than that by the other tail artery half incubated in the supernate from the sham volume-expanded dog (Figure 1). The same was the case when the tail artery halves were incubated in the plasma supernates from the volume-expanded rats and humans.

In addition, $^{86}$Rb uptake by the tail artery half incubated in the plasma supernate from the expanded rat with an AV3V lesion was significantly greater than uptake by the half incubated in supernate from the volume-expanded rat with a sham AV3V lesion.

Ouabain-sensitive $^{86}$Rb uptake by the tail artery half incubated in the plasma supernate from the 1K1W hypertensive dog was significantly less than uptake by the other artery half incubated in the plasma supernate from the paired 1KSW normotensive control dog (Figure 2). Uptake of $^{86}$Rb by the tail artery was also inhibited by plasma supernates from both black male patients with low renin essential hypertension and 1K1C and RRM-S hypertensive rats relative to uptake by arteries incubated in supernates from appropriate control subjects and rats. In addition, a prior AV3V lesion or central sympathectomy by intraventricular injections of 6-hydroxydopamine in RRM-S hypertensive rats prevented development of hypertension and eliminated the evidence of elevated HPIF. Furthermore, after reversal of the hypertension in the 1K1C and RRM-S hypertensive rats, the inhibition of $^{86}$Rb uptake was no longer seen. Similarly, inhibition of $^{86}$Rb uptake did not occur if the arteries were incubated in plasma supernates from black male patients with essential hypertension unselected for their renin status.

Transmembrane Potential Assay

Table 1 shows data from the 1K1C hypertensive and 1KSC normotensive rats. The vascular smooth muscle cells in the hypertensive artery were depolarized by about 7 mV relative to those in the control artery. The supernate from control normotensive rats did not significantly affect the resting membrane potentials of the vascular smooth muscle cells in these arteries. Plasma supernate from hypertensive animals depolarized these cells in the control artery, however, but was without effect on the already depolarized cells in the hypertensive artery. In other experiments, the depolarization of vascular smooth muscle cells in the tail arteries from the hypertensive rats relative to cells in the arteries from the control rats was no longer present after unclipping of the sole remaining renal artery in 1K1C hypertensive rats. In contrast to 1K1C hypertensive rats, vascular smooth muscle cells in tail arteries from RRM-S hypertensive rats had the same membrane potential as those in arteries from normotensive RRM-DW control rats. In addition, plasma supernates from
these hypertensive and control normotensive rats produced similar changes in the membrane potentials of vascular smooth muscle cells in the arteries from these hypertensive and normotensive rats. When supernate from RRM-S hypertensive rats was applied to a tail artery half from a normal animal, however, the cells quickly depolarized (from $-50.0 \pm 1.3$ to $-44.3 \pm 1.2; n = 8; p < 0.05$). The cells in the other tail artery half did not depolarize (from $-50.0 \pm 1.3$ to $49.6 \pm 1.4; n = 8, p > 0.5$) when exposed to supernate from RRM-DW normotensive control rats.

**Discussion**

Decreased ouabain-sensitive $^{86}$Rb uptake by tail artery halves incubated in plasma supernates from acutely volume-expanded dogs, rats, and humans, and its absence when tail artery halves are incubated in the plasma supernates from acutely volume-expanded rats with AV3V lesions suggest that acute volume expansion in some way causes release of HPIF and that the AV3V area of the brain is involved in the synthesis or release of HPIF. Decreased ouabain-sensitive $^{86}$Rb uptake by arteries incubated in plasma supernates from animals and humans with low renin hypertension but not by arteries incubated in supernates obtained from respective controls and from patients with essential hypertension unselected with respect to renin status suggests that elevated level of HPIF is a characteristic of low renin hypertension and that a link may exist between volume and hypertension. Induction of depolarization of vascular smooth muscle cells in normotensive tail arteries by plasma supernates from 1K1C and RRM-S hypertensive rats also suggests elevated HPIF in these low renin types of experimental hypertension.

Unclipping of the sole remaining renal artery in 1K1C hypertensive rats and substituting distilled water for saline as the drinking fluid in the RRM-S hypertensive rats reversed both the elevated HPIF and the elevated blood pressure, suggesting a possible role of HPIF in the pathophysiology of the hypertension.

Several other laboratories have also reported elevated HPIF in the plasma of animals with low renin hypertension. Using essentially the same $^{86}$Rb uptake bioassay system as ours, Songu-Mize et al. reported increased levels of HPIF in rats with one-kidney deoxycorticosterone acetate (DOCA)-salt hypertension of 5 to 6 weeks' duration. One-kidney DOCA-salt hypertension is also a low renin model of hypertension. Furthermore, as in our

**TABLE 1. Membrane Potentials Recorded in Arteries from 1K1C Hypertensive and 1KSC Normotensive Rats and the Effect of Plasma Supernates on These Potentials**

<table>
<thead>
<tr>
<th>Source of tail artery</th>
<th>1KSC normotensive rat supernate</th>
<th>1K1C hypertensive rat supernate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1KSC normotensive rat</td>
<td>$-52.0 \pm 1.4$</td>
<td>$-48.0 \pm 2.4$</td>
</tr>
<tr>
<td>1K1C hypertensive rat</td>
<td>$-45.0 \pm 1.4^\dagger$</td>
<td>$-47.0 \pm 1.3$</td>
</tr>
</tbody>
</table>

Membrane potential (Em) values (in mV) are means ± SE. SC = sham clip. $^\dagger p < 0.05$, control 1KSC normotensive Em vs Em after effect of 1K1C hypertensive rat supernate.
RRM-S hypertensive rats, prior AV3V lesions in one-kidney DOCA-salt hypertensive rats prevented the development of the hypertension and the increase in HPIF. More recently, Magargal and Overbeck, using our ⁸⁶Rb uptake bioassay on cultured vascular smooth muscle cells (instead of a tail artery), also reported increased levels of HPIF in 1K1C hypertensive rats.

We boiled the plasma and assayed HPIF activity in the plasma supernates in our studies of control and experimental animals or human subjects. This is a standard procedure in our and other laboratories. Boiling the plasma eliminates proteolytic enzymes and known vasoactive agents (e.g., norepinephrine) that may directly or indirectly affect ouabain-sensitive ⁸⁶Rb uptake, our measure of Na⁺-K⁺ pump activity. Valdes, using radioimmunoassay to quantitate digoxinlike immunoactivity, showed that digoxinlike immunoactivity in plasma is greater in hypertensive pregnant women (third trimester) than in normotensive pregnant women (third trimester), and that boiling the plasma enhances its immunoactivity in both control and hypertensive subjects. The difference in the immunoactivity between hypertensive and normotensive plasma remains, however. Thus the HPIF activity measured by us in boiled plasma may not represent quantitatively the HPIF activity in situ, but the differences in HPIF activity between volume-expanded and sham volume-expanded plasma and hypertensive and normotensive plasma observed by us are not affected by boiling the plasma.

The assay systems used by other investigators vary widely and include effects on 1) short-circuit current in the toad bladder, 2) ouabain-sensitive ³²Na efflux from white and red blood cells, and 3) glucose-6-phosphate dehydrogenase (G6PD) activity in kidney slices (which correlates inversely with Na⁺-K⁺-ATPase activity). Using the G6PD assay, MacGregor et al. reported greater activity of the enzyme when the slices were exposed to plasma from patients with essential hypertension, particularly of the low renin variety. Also of interest is the observation that antibodies to digoxin lower blood pressure in rats with one-kidney, DOCA-salt, and chronic aortic coarctation hypertension.

Our findings of elevated levels of HPIF in plasma supernates of dogs with 1K1W hypertension and in rats with 1K1C and RRM-S hypertension may help explain our earlier observation that the Na⁺-K⁺ pump activity in the cardiovascular muscle cells in these models of experimental hypertension is decreased. This could lead to increased contractile activity of these cells, thereby contributing to the hypertension by both direct and indirect mechanisms. The possible direct mechanisms are 1) depolarization of muscle cells leading to increased calcium influx through voltage-sensitive calcium channels and 2) altered sodium-calcium exchange leading to decreased calcium efflux. An indirect mechanism may be decreased reuptake of norepinephrine by sympathetic nerve terminals, leaving more norepinephrine in the neuromuscular cleft. Data from our experiments indicate that HPIF probably acts through depolarization of vascular smooth muscle cells. However, this does not rule out the possibility that by inhibiting vascular Na⁺-K⁺ pump activity, HPIF also alters sodium-calcium exchange leading to decreased calcium efflux. This mode of action may be more important in cardiac muscle cells where resting membrane potential is higher and sodium-calcium exchange is better established.

Membrane potential in the RRM model deserves further comment and study. Depolarization of vascular smooth muscle cells in tail arteries from untreated normotensive rats, but not of cells in tail arteries from RRM-DW normotensive control rats, by plasma supernates with elevated level of HPIF from RRM-S hypertensive rats suggests that HPIF binds less well to these cells in RRM-DW rats. Unlike the 1K1C hypertensive rat and its control 1K1C normotensive rat, both hypertensive and normotensive rats with reduced renal mass are mildly uremic. Perhaps uremia influences binding of HPIF to vascular smooth muscle cells. This could explain why, unlike these cells in in vitro tail arteries from 1K1C hypertensive rats, cells in in vitro tail arteries from RRM-S hypertensive rats have normal membrane potentials.

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