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COMMENTARY

NATRIURETIC HORMONE—THE MISSING LINK IN LOW RENIN HYPERTENSION?

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The mechanism of low renin hypertension is particularly obscure. The elevated pressure does not respond appreciably to angiotensin antagonists or converting enzyme inhibitors. While it does respond to slow volume depletion (low salt intake, diuretics), a sudden increase in volume in a normal subject does not immediately increase pressure. It does increase with time, however, suggesting some indirect mechanism.

Evidence for a ouabain-like humoral agent

In 1976, we reported that sodium–potassium pump activity, as estimated by ouabain-sensitive ^{86}Rb uptake, is suppressed in the arteries and veins of dogs with one model of low renin hypertension (one-kidney, one wrapped) [1]. We also observed that Na^+,K^+-ATPase activity is suppressed in cardiac microsomes (mostly sarcolemma) obtained from rats with another model of low renin hypertension (one-kidney, one clip) [2]. We had shown previously that suppression of sodium–potassium pump activity, by reducing the serum potassium level, increases the contractile activity of arterial smooth [3] and cardiac [4] muscle *in situ* and that ouabain produces a large increase in arterial pressure in the anesthetized dog prepared so that diuresis cannot occur [5]. An exhaustive search of the literature [6] revealed evidence dating back to 1940 for an unknown slowly acting pressor and sensitizing agent in the plasma of animals with low renin hypertension. It also revealed evidence suggesting that natriuretic hormone is a Na^+,K^+-ATPase inhibitor. We therefore hypothesized that the indirect mechanism is some humoral agent, perhaps natriuretic hormone, operating through the sodium–potassium pump on vascular and cardiac muscle [6]. Shortly thereafter, Blaustein [7] presented essentially the same hypothesis.

Subsequent studies in our laboratories tended to support the hypothesis. We observed the same changes in ouabain-sensitive ^{86}Rb uptake and Na^+,K^+-ATPase activity in other models of low renin hypertension (one-kidney, one clip; one-kidney, DOCA, saline; reduced renal mass-saline) [8–11] but not in two genetic models of hypertension. Furthermore, we found that the change in ouabain-sensitive ^{86}Rb uptake in blood vessels could be reproduced in normal animals with rapid volume expansion and that this defect could be transferred to the arteries of another animal via the plasma [12]. These data plus an even more exhaustive literature search led us to reaffirm our belief in the hypothesis and to extend it to include the adrenergic nerve terminals [13, 14], i.e. we suggested that the humoral agent also suppresses norepinephrine uptake. The data also encouraged us to examine the plasma in three models of low renin hypertension (one-kidney, one wrapped in the dog and one-kidney, one clip and reduced renal mass-saline in the rat) for sodium–potassium pump inhibiting activity. In each of the three models, ouabain-like activity was found in the plasma [9, 12].

As the potential significance of the hypothesis disseminated, data supporting the suggested link between natriuretic hormone and salt-dependent hypertension began to emerge from other laboratories, particularly those long engaged in natriuretic hormone research. Gruber *et al.* [15] reported that the same plasma extracts from salt-loaded dogs that contain natriuretic hormone also contain a factor that cross-reacts with antidigoxin antibodies and inhibits Na^+,K^+-ATPase, suggesting that natriuretic hormone is in fact an endogenous digoxin-like substance and therefore a potential vasoconstrictor. The same laboratory also reported that plasma extracts taken from salt-loaded dogs increase reactivity of the microcirculation in the rat cremaster muscle to norepinephrine [16]. In addition, in another study the laboratory found elevated plasma levels of the factor in monkeys with spontaneous and two-kidney, one clip hypertension [17]. Songu-Mize *et al.* [18] reported that plasma from rats with one-kidney, DOCA, saline hypertension inhibits ouabain-sensitive ^{86}Rb uptake by a tail artery taken from another rat, just as we reported for three other models of low renin hypertension. Kojima *et al.* [19] found that intravenous injection of antidigoxin antibodies reduces the pressure in the rat with one-kidney, DOCA, saline hypertension.

Poston and associates [20] presented evidence implicating a circulating inhibitor of Na^+,K^+-ATPase in human essential hypertension. As reported earlier by Edmondson *et al.* [21], these investigators found that active sodium transport in leukocytes from patients with essential hypertension is impaired [20], more so in the low renin variety [22]. Moreover, incubating leukocytes from normotensive subjects in serum obtained from patients with essential hypertension caused an impairment in sodium transport similar to that found in the leukocytes of hypertensive patients [20]. Similar evidence has been presented by Morgan *et al.* [23]; male hypertensive subjects with a high sodium intake (spontaneous or induced) had reduced ^{22}Na efflux from their red cells when they were incubated in their plasma. Note-

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worthy is the observation that natriuretic hormone is elevated in the plasma of subjects with primary aldosteronism [24].

Source of the ouabain-like agent

Working with Dr. James Buggy, we showed that ouabain-sensitive ^{86}Rb uptake by the tail artery was higher in the volume expanded rat with an anteroventral third ventricle (AV3V) lesion than in the volume expanded rat with a sham AV3V lesion [9]. This is the same lesion that prevents or ameliorates certain forms of low renin hypertension in rats [25, 26] and eliminates the appearance of antinatriferic activity in plasma on volume expansion [27]. In very recent experiments [28], we showed that the AV3V lesion prevents (1) reduced renal mass, saline hypertension in the rat, (2) the appearance of the ouabain-like humoral agent in the plasma of these animals, and (3) the pump suppression in the tail artery of these rats. Thus, we think the AV3V area produces or influences the production of the ouabain-like humoral agent. Songu-Mize *et al.* [18] reached the same conclusion because the AV3V lesion prevented both the hypertension and the pump suppression in the one-kidney, DOCA, saline model.

Mechanism of the vascular action of the ouabain-like humoral agent

Working with David Harder, we showed that the smooth muscle cell in tail arteries taken from rats with one-kidney, one clip hypertension is depolarized relative to the cell in tail arteries taken from control animals [29]. The measurements were made some 90 min after placing the tail arteries in a modified Krebs solution, and the findings were not different whether or not the solution contained phentolamine and 6-hydroxydopamine. Furthermore, supernate of boiled plasma from the one-kidney, one clip hypertensive animal quickly depolarized the smooth muscle cell in the tail artery taken from a normal rat [29]. The degree of depolarization was the same as produced by ouabain. These findings are compatible with the hypothesis that the humoral agent binds quickly but tightly to smooth muscle cells producing electrogenic pump suppression and hence depolarization. Since Ca^{2+} permeability is sensitive to the membrane potential, this should result in calcium influx and vasoconstriction.

Nature of the ouabain-like humoral agent

Other studies tend to strengthen the possibility that the ouabain-like humoral agent is natriuretic hormone. Both the humoral suppressor of the vascular sodium–potassium pump observed in our studies and natriuretic factor are heat stable. The appearance of natriuretic hormone in the plasma of rats during acute volume expansion is blocked by an AV3V lesion [27] and, as pointed out above, ouabain-sensitive ^{86}Rb uptake by the tail artery is higher and plasma ouabain-like activity, assayed on the tail artery, is lower than in sham lesioned animals [9]. Natriuretic factor reduces short circuit current in the toad bladder; so does plasma supernate from dogs with one-kidney, one wrapped hypertension (Chen *et al.*, unpublished observation). The humoral pump suppressor observed in our studies does not appear to be vasopressin or the natriuretic factor recently extracted from rat atria [30] because they fail to reproduce the effect of supernates of boiled plasma from hypertensive or volume expanded animals when added to our assay system (tail artery from a normal rat) [31].

Natriuretic hormone is a small molecule (< 500 mol. wt) which is resistant to heat. Its chemical structure, however, is still unknown. de Wardener, who provided the first convincing bioassay evidence for the presence of a circulating natriuretic factor in animals, has more recently concentrated the efforts of his laboratory [32, 33] on purifying and identifying a natriuretic factor found in human urine where it increases with increased sodium intake. On intravenous injection in the rat, the purified preparation has a rapid onset and short duration of action. Its characteristics include a molecular weight of less than 500, resistance to hydrolysis and proteolytic enzymes, and an absence of amino acids. It is a polar substance and appears to be a sugar attached to a ring structure. It binds to digoxin antibodies ten times better than digoxin and inhibits Na^+,K^+-ATPase many times more than ouabain. It also stimulates glucose-6-phosphate dehydrogenase activity in kidney slices, which they believe also signifies a decrease in Na^+,K^+-ATPase activity.

An agent with a similar activity and characteristics has been extracted from bovine hypothalamus by Haupert and Sancho [34]. This agent inhibits active sodium transport across anuran membranes, ouabain binding to frog urinary bladder and Na^+,K^+-ATPase from kidney. It appears to be a low molecular weight, basic, nonpeptide. Fishman [35] prepared a fraction of guinea pig brain containing a substance which blocks ouabain binding to Na^+,K^+-ATPase and inhibits uptake of ^{86}Rb into human erythrocytes. It has a low molecular weight and withstands acid hydrolysis. Lichtstein and Samuelov [36] extracted a ouabain-like compound from rat brain. It inhibits ouabain binding and Na^+,K^+-ATPase activity in rat brain synaptosomes. It has a low molecular weight and its activity is not influenced by treatment with protease, trypsin and other proteolytic enzymes.

On the other hand, Gruber and Buckalew [37] have purified an agent with natriuretic and antinatriferic activity from the plasma of salt-loaded dogs which appears to be a heat stable, low molecular weight, acidic peptide derived from a larger precursor molecule (see above for other studies from this laboratory).

Future directions

There is a need to determine whether the ouabain-like humoral factor has, in addition to a direct effect on the muscle cells, an indirect effect on the muscle cells via actions on the sympathetic nerve endings. Norepinephrine uptake into adrenergic nerve terminals is sodium and potassium dependent and inhibited by ouabain [14]. Thus, consideration should be given to the possibility that the agent suppresses the uptake of norepinephrine by the nerve terminals. This, particularly if accompanied by increased norepinephrine release, should initially raise the concentration of norepinephrine in the cleft and eventually lead to depletion

of norepinephrine in the nerve terminal. The endogenous norepinephrine contents of arteries and heart are, in fact, decreased in animals with experimental low renin hypertension and, while the blood vessels respond more vigorously to norepinephrine application, they respond less well to nerve stimulation [14]. Application of plasma from the normal dog to the isolated saphenous vein reduces its ability to take up norepinephrine [38].

Attempts to purify and identify the biochemical structure of natriuretic hormone, which are already in progress in several laboratories in the United States and Europe, should perhaps be expanded and pressed more vigorously. Success would permit the study of its physiological actions on the cardiovascular, autonomic nervous and renal systems and allow the measurement of its levels in various types of hypertension.

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