Plasma Kinin Concentration in Deoxycorticosterone-Salt Hypertension

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SUMMARY We investigated the status of circulating kinins in rats with severe hypertension caused by drinking 1% NaCl (saline) and treatment with deoxycorticosterone (DOC, 25 mg/kg/wk s.c.) for 5 weeks. Saline-drinking rats treated with DOC had a higher systolic blood pressure (210 ± 4 mm Hg) than did rats without DOC treatment drinking water (138 ± 3 mm Hg) or saline (141 ± 3 mm Hg). The concentration of kinins in the inferior vena cava plasma of DOC-salt hypertensive rats did not differ from the venous plasma kinin concentration in normotensive rats drinking water or saline. In contrast, the arterial plasma kinin concentration in DOC-salt hypertensive rats (7.0 ± 0.8 pg/ml) was lower (p< 0.002) than that in water-drinking controls (14.0 ± 2.2 pg/ml); it also was lower (p<0.005) in saline-drinking rats (8.1 ± 0.9 pg/ml) than in water-drinking controls. Infusion of bradykinin (20 μg/kg/min i.v.) increased arterial plasma kinins in all the groups. Nonetheless, the arterial plasma kinin concentration achieved during bradykinin infusion in DOC-salt hypertensive (1590 ± 130 pg/ml) and in saline-drinking rats (1540 ± 100 pg/ml) was lower than that in water-drinking rats (2140 ± 210 pg/ml). On the other hand, during infusion of the kininase II inhibitor captopril (80 μg/hr i.p.) for 3 days, neither DOC-salt hypertensive rats nor saline-drinking normotensive rats exhibited significant reduction of arterial plasma kinins relative to the level in water-drinking controls. These data indicate that high salt intake, irrespective of the level of blood pressure, causes the arterial plasma concentration of kinins to fall. The observed reduction of arterial plasma kinins in DOC-salt hypertensive rats and in saline-drinking normotensive rats may be the expression of increased kinin degradation. (Hypertension 11: 411-415, 1988)

KEY WORDS kinins hypertension dietary sodium kininases captopril

BRADYKININ and related kinins are vasodilator polypeptides released from plasma protein precursors, kallikreins, by plasma and glandular kallikreins.1 The kallikrein-kinin system may participate in the regulation of cardiovascular and renal functions. For example, endogenous kinins are thought to subserve blood pressure–lowering mechanisms, including the mediation of vasodilation, diuresis, and natriuresis.1 Recently, blood pressure was shown to increase in response to treatment with a kinin receptor antagonist,2,3 suggesting that kinin-mediated blood pressure–lowering mechanisms participate in setting the level of arterial blood pressure.

Abnormalities in urinary kallikrein excretion have been demonstrated in several forms of clinical and experimental hypertension,4 but the functional importance of altered urinary kallikrein in relation to the development of hypertension remains unknown. Kinins occur in circulating blood at concentrations that reflect the balance between kinin generation by kallikreins and kinin inactivation by kininases.3 The status and importance of blood kinins in relation to the development of hypertension are also unknown.6,7

According to a recent report,8 the arterial blood concentration of kinins in rats with severe one-kidney, one-clip hypertension is increased. Severe hypertension causes vascular injury and, consequently, may create a setting that favors activation of plasma prekallikrein and augmentation of blood kinin generation and levels.9 If so, severe hypertension, regardless of its cause, may be expected to increase circulating kinins. This report details the results of experiments aimed at contrasting the concentration of kinins in the plasma of normotensive rats and of rats with severe hypertension caused by treatment with deoxycorticosterone (DOC) and increased dietary sodium.

Materials and Methods

Experiments were performed on 91 male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) that had...
the left kidney removed through a left flank incision under ether anesthesia. One day after uninephrectomy, the animals were randomly divided into three treatment groups. Throughout the study, rats in Group 1 drank deionized water, rats in Group 2 drank 1% NaCl (wt/vol; saline), and rats in Group 3 drank 1% NaCl and received weekly subcutaneous injections of DOC (Percorten pivalate, CIBA; 25 mg/kg body weight). The animals were kept in a temperature-controlled (24°C) and humidity-controlled (50%) room that was illuminated between 0600 and 1800. They were fed ad libitum a standard chow (Purina 5001, Ralston Purina, St. Louis, MO, USA), and had the systolic blood pressure determined by tail sphygmography. The following three protocols were implemented 5 weeks after the onset of treatment.

Protocol 1
The purpose of the experiment was to contrast the arterial and venous plasma kinin concentrations in normotensive rats drinking water or 1% NaCl with the corresponding plasma kinin concentrations in rats made hypertensive by DOC treatment and saline drinking. Rats from Groups 1, 2, and 3 were anesthetized with ether; the abdomen was opened with a midline incision, and blood (4.0 ml) for assay of plasma kinins was rapidly drawn from the abdominal aorta, or from the inferior vena cava above the renal veins, into a chilled plastic syringe containing a mixture (0.4 ml) of kalliokrein and kininase inhibitors (aprotinin, 4,000 KIU; soybean trypsin inhibitor, 320 µg; 1,10-phenanthroline, 4 mg; disodium EDTA, 8 mg; and hexadimethrine, 1.6 mg; Sigma).10

Protocol 2
The purpose of the experiment was to contrast the arterial plasma kinin concentration in rats drinking water or saline and in rats with DOC-salt hypertension during the administration of exogenous bradykinin to minimize the contribution of endogenous kinins to circulating kinin levels. Rats from Groups 1, 2, and 3 were anesthetized with Inactin (100 mg/kg i.p.; BYK-Gulden Lomberg Chemische Fabrik) and placed on a heating pad to maintain body temperature at 37°C. Polyethylene cannulas were placed in the trachea (PE-260), in a femoral vein (PE-50) for drug infusion, and in the carotid artery (PE-50) for blood sampling and blood pressure measurement. After a 30- to 60-minute equilibration period, bradykinin triacetate (Sigma) was infused i.v. at 20 µg/kg/min, and blood for the determination of arterial plasma kinin concentration was sampled 2 minutes later as described for Protocol 1.

Protocol 3
The purpose of the experiment was to contrast, during blockade of kininase II by captopril, the arterial plasma kinin concentration in rats drinking water or 1% NaCl and in rats with DOC-salt hypertension. Captopril (Squibb) was infused at 80 µg/hr i.p. by an osmotic minipump (Model 2001, Alza, Palo Alto, CA, USA) implanted in the peritoneal cavity of rats from Groups 1, 2, and 3 under ether anesthesia. Three days later, blood for determination of arterial plasma kinins was drawn from the abdominal aorta of rats anesthetized with ether, as described for Protocol 1.

Assay of Kinins
The concentration of kinins in duplicate plasma samples was measured by radioimmunoassay as described by Shimamoto et al.,11 after sample purification by the method of Ando and Shimamoto.10 The bradykinin antibody was a gift from Dr. Kazuaki Shimamoto, Sapporo Medical College, Sapporo, Japan, and [125I]TyTyr3-bradykinin was obtained from New England Nuclear, Boston, MA, USA. The recovery of unlabeled bradykinin added to blood at the time of sampling was 82.9 ± 5.0%. The recovery of [125I]TyTyr3-bradykinin added to each plasma sample before purification was 66.0 ± 1.4%; the individual recovery values served to correct the estimates of the plasma kinin concentration for losses incurred during purification. The concentration of kinins in plasma is expressed as picograms per milliliter. The difference in kinin concentration of duplicate plasma samples averaged 12.0 ± 1.4% (n = 60).

Statistical Analysis
Results are expressed as means ± SEM. Comparisons among groups were made by analysis of variance using least-square procedures. The null hypothesis was rejected when the p value was less than 0.05.

Results
DOC-treated rats (25 mg/kg/wk s.c.), drinking 1% NaCl for 5 weeks, had a higher systolic blood pressure (210 ± 4 mm Hg; p < 0.01) than did rats drinking either water (138 ± 3 mm Hg) or 1% NaCl (141 ± 3 mm Hg), without steroid treatment. The concentration of kinins in inferior vena cava plasma of rats with DOC-salt hypertension (21.0 ± 2.1 pg/ml) did not differ from the kinin concentration in the venous plasma of normotensive rats drinking water (18.6 ± 2.2 pg/ml) or 1% NaCl (23.3 ± 3.0 pg/ml). In contrast, as shown in Figure 1, the concentration of kinins in the arterial plasma of rats with DOC-salt hypertension (7.0 ± 1.3 pg/ml) was 50% lower (p < 0.002) than the arterial plasma kinin concentration in water-drinking controls (14.0 ± 1.4 pg/ml). Rats drinking 1% NaCl without DOC treatment also had a lower (p < 0.005) arterial plasma kinin concentration (8.1 ± 1.3 pg/ml) than did normotensive control rats drinking water (see Figure 1). But the arterial plasma kinin concentration of rats with DOC-salt hypertension and of normotensive rats drinking 1% NaCl did not differ significantly (see Figure 1).

Mean arterial blood pressure before and 2 minutes after the onset of bradykinin infusion (20 µg/kg/min i.v.) was, respectively, 120 ± 5 and 108 ± 5 mm Hg (p < 0.01) in water-drinking rats, 123 ± 4 and 125 ± 7 mm Hg in saline-drinking rats, and 147 ± 11 and 132 ± 12 mm Hg (p < 0.01) in DOC-salt hypertensive rats. Figure 2 contrasts, during infusion of bradykinin,
the arterial plasma kinin concentration of DOC-salt hypertensive rats and of rats without steroid treatment drinking water or 1% NaCl. In all three groups of animals the infusion of bradykinin established arterial plasma kinin levels that were more than 100-fold higher than the levels measured in rats without infusion of exogenous bradykinin. Nonetheless, the concentration of kinins in the arterial blood of rats with DOC-salt hypertension (1590 ± 150 pg/ml) was about 25% lower (p<0.02) than the arterial plasma kinin concentration in water-drinking rats (2140 ± 150 pg/ml). Rats drinking 1% NaCl without DOC treatment also had a lower (p<0.02) arterial plasma kinin concentration (1540 ± 130 pg/ml) than did control rats drinking water. The arterial plasma kinin concentration in rats with DOC-salt hypertension and in rats drinking 1% NaCl without DOC treatment did not differ.

During infusion of the kininase II inhibitor captopril (80 μg/hr i.p.), the arterial plasma kinin concentration in saline-drinking rats with (13.9 ± 1.3 pg/ml) or without (13.1 ± 1.5 pg/ml) DOC treatment did not differ significantly from the concentration of kinins in the arterial plasma of water-drinking controls (16.0 ± 1.5 pg/ml; Figure 3). Comparison of arterial plasma kinin levels in rats with (see Figure 3) and without (see Figure 1) captopril pretreatment suggests that the kininase II inhibitor caused elevation of arterial plasma kinins in DOC-salt hypertensive rats and in rats drinking 1% NaCl, but not in water-drinking controls. However, the administration of captopril had no effect on blood pressure; that is, the systolic blood pressure measured before and on the third day of captopril infusion was, respectively, 140 ± 5 and 135 ± 4 mm Hg in water-drinking rats, 146 ± 4 and 148 ± 4 mm Hg in rats drinking 1% NaCl, and 222 ± 6 and 228 ± 7 mm Hg in DOC-salt hypertensive rats.

**Discussion**

Reports that the plasma concentration of high molecular weight kininogen is depressed in patients with malignant hypertension12 and that the concentration of arterial blood kinins is increased in renal hypertensive rats8 raise the possibility that the vascular injury accompanying severe hypertension creates conditions that are conducive to augmentation of blood kinin generation and levels. Our present study does not support such a view, as the arterial plasma concentration of kinins in rats with severe DOC-salt hypertension of 5 weeks' duration was reduced rather than increased. Since the concentration of arterial plasma kinins was also decreased in normotensive rats drinking 1% NaCl, the lowering of arterial plasma kinin levels in DOC-salt hypertensive rats appears related to the increase in dietary sodium rather than to an excess of mineralocorticoids or hypertension. The concentration of kinins measured in peripheral venous blood was also reported to fall in response to saline infusion6,13 or increased dietary sodium14 in humans.

The concentration of kinins in circulating blood expresses the balance between kinin generation by kallikreins and inactivation by kininases.5 Therefore, the lowering of arterial plasma kinin levels we noted in
both the DOC-salt hypertensive rats and saline-drinking normotensive rats may be the expression of deficient kinin generation, excessive kinin degradation, or a combination of both. Our study argues against the possibility that a deficit in kinin generation is responsible for the reduction of arterial plasma kinins in saline-drinking rats with and without DOC treatment and hypertension. First, the concentration of kinins in inferior vena cava plasma was not diminished in DOC-salt hypertensive rats or in rats drinking 1% NaCl. Second, even during infusion of exogenous bradykinin, to greatly diminish the relative contribution to circulating kinin levels of endogenously produced kinins, DOC-salt hypertensive rats and saline-drinking normotensive rats continued exhibiting diminished arterial plasma kinin levels relative to the levels in the appropriate water-drinking controls. Third, in animals treated with captopril to inhibit kinin degradation by kininase II, the concentration of kinins in arterial plasma was not reduced in DOC-salt hypertensive rats or in rats drinking 1% NaCl relative to the levels in water-drinking rats. Indeed, these three observations are compatible with the notion that the reduction of arterial plasma kinins in DOC-salt hypertensive rats and in saline-drinking rats reflects augmentation of kinin degradation. If excessive kinin degradation is responsible for the diminished concentration of kinins in the arterial plasma of DOC-salt hypertensive rats and saline-drinking normotensive rats, our finding that the concentration of kinins in inferior vena cava plasma is not similarly diminished implies that the anatomical site of increased kinin degradation is in between the sites of venous and arterial blood sampling, presumably in the pulmonary circulation.

Bradykinin and related peptides are degraded on passage through the pulmonary circulation by a peptidylidipeptidase, kininase II or angiotensin I converting enzyme, occurring in the vascular endothelium. The lung, the activity of this enzyme measured in vitro is not affected by variations in dietary sodium or DOC treatment. However, the enzymatic activity of peptidylidipeptidase in vivo, examined by studying the ratio of angiotensin I to angiotensin II pressor responses, was reported to increase in DOC-treated dogs fed a high sodium diet. The blood pressure-lowering effect of bradykinin injected intravenously is increased in rats infused intravenously with angiotensin I, a finding that may be indicative of a competitive interaction between angiotensin I and bradykinin at the level of their metabolism by the peptidylidipeptidase. This interpretation is in agreement with reports that angiotensin I and bradykinin can inhibit each other’s hydrolysis by the peptidylidipeptidase. But a role for angiotensin I in inhibiting the degradation of bradykinin and related kinins by kininase II in a physiological setting is open to question because the preferred substrate for the enzyme is bradykinin rather than angiotensin I. This consideration notwithstanding, the diminution of plasma renin activity and angiotensin I levels accompanying increases in dietary sodium and treatment with mineralocorticoids may create a setting that favors the metabolism of plasma kinins by kininase II, thus reducing arterial plasma kinin levels.

Reports of the effect of captopril on blood pressure in DOC-salt hypertensive rats area conflicting. In the present study, captopril had no effect on blood pressure in DOC-salt hypertensive rats, even though the arterial plasma level of kinins was increased relative to the values in hypertensive rats without captopril treatment. Concerning this point, it has been reported that blood pressure does not fall in intact rats receiving bradykinin infusion until the arterial blood kinin concentration greatly exceeds the basal kinin level. Accordingly, in terms of blood pressure regulation, the functional importance of small changes in arterial plasma kinins is not apparent.

In conclusion, the results of this study indicate that high salt intake, irrespective of the level of blood pressure and mineralocorticoid activity, causes the arterial plasma concentration of kinins to decrease in rats. We suggest that the reduction of arterial plasma kinins in DOC-salt hypertensive and saline-drinking rats is the expression of increased kinin degradation.

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