Response of the Kallikrein-Kinin and Renin-Angiotensin Systems to Saline Infusion and Upright Posture

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ABSTRACT The possibility that bradykinin, a potent vasodilator, might be a physiological antagonist of the renin-angiotensin system was investigated. 11 normal subjects, ranging in age from 21 to 33 yr were studied. Seven of the subjects were given a 10 meq sodium, 100 meq potassium, 2,500 ml isocaloric diet. After metabolic balance was achieved, they were infused with either 1 liter of 5% glucose over 2 h or 2 liters of 0.9% saline over 4 h. During the infusions, plasma renin activity (PRA), angiotensin II (A II), prekallikrein, bradykinin, and aldosterone levels were frequently determined. Plasma prekallikrein and kallikrein inhibitor did not change during the infusion of either glucose or saline. In subjects receiving saline, plasma bradykinin fell from 3.9±1.5 (SEM) ng/ml at 0 min to 0.93±0.2 at 30 min and 0.95±0.3 at 120 min. These changes paralleled the decrease in PRA over the same period (7.9±1.3 ng/ml/h to 5.6±0.8 at 30 min and 3.5±0.7 at 120 min). Similarly, A II fell from 113±12 pg/ml to 62±10 and 48±5, respectively, at 30 and 120 min. In contrast, the control group infused with glucose showed no change in bradykinin, A II, or PRA.

Another four subjects were given a constant 200 meq sodium/100 meq potassium isocaloric diet. After metabolic balance was achieved, they were kept supine and fasting overnight. At 9 a.m. they assumed an upright position and began walking a fixed distance (200 ft) at a normal rate (3–4 ft/s). Plasma prekallikrein and kallikrein inhibitor did not change during the posture study. The plasma bradykinin rose from a base line of 0.54±0.01 (SEM) ng/ml to 0.96±0.13 at 20 min, 0.77±0.18 at 60 min, and 0.96±0.07 at 120 min. These changes parallel the increase in PRA over the same period (1.65±0.33 ng/ml/h to 3.6±0.85 at 20 min, 5.3±0.9 at 60 min, and 5.35±0.55 at 120 min). Likewise, the A II rose from 32.5±1.82 pg/ml to 50.8±3.6 at 20 min, 54.3±3.2 at 60 min, and 61.3±5.9 at 120 min.

Thus, in sodium-depleted individuals, saline infusion produces a rapid fall of plasma bradykinin at a rate similar to that observed for A II and PRA. Conversely, in sodium-loaded individuals, assumption of upright posture leads to a parallel rise in A II, PRA, and bradykinin. These studies indicate that there is a close correlation of bradykinin levels with renin activity and angiotensin II, in both acute sodium loading and assumption of upright posture, suggesting that these two systems may be physiologically interrelated.

INTRODUCTION

Angiotensin is the most potent endogenous vasoconstrictor agent identified (1). Its role in the maintenance of normal blood pressure and in the pathogenesis of hypertension, however, is disputed (2–4). For example, in normal individuals sodium depletion, while associated with high levels of circulating angiotensin

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II (A II),[1] is not correlated with consistent increases in blood pressure (2). Moreover, in several hypertentive disorders, plasma renin and/or angiotensin concentrations have been normal (5, 6). Finally, the administration of an antibody to A II to hypertensive animals does not necessarily correct the hypertension (7–9).

Conversely, there is also evidence suggesting that angiotensin in the sodium-depleted state does contribute to the maintenance of a normal or elevated blood pressure. Several studies support this concept. When a competitive inhibitor of A II was given to the sodium-depleted dog, a decrease in blood pressure occurred, while no such effect was produced in the sodium-loaded state (10). Similarly, in the one-kidney Goldblatt-hypertensive rat, a competitive antagonist of A II produced a fall in blood pressure only when the animals were sodium-restricted (11). One possible explanation for the differing results in these studies is that additional vasoactive substance(s) may be important in regulatory blood pressure. Both in vitro and in vivo studies suggest that bradykinin is the most potent mammalian vasodilator known (12).

A serendipitous observation of a normotensive patient with renal artery stenosis and elevated bradykinin and A II levels (unpublished observation) prompted us to investigate the physiological interrelationships between the renin-angiotensin and kallikrein-kinin systems. Since is has been previously shown that the renin-angiotensin system is rapidly suppressed by saline infusion (13) and activated by upright posture, the present study examines whether the kallikrein-kinin system is equally responsive.

METHODS

Selection of subjects. 11 normal subjects (four men and seven women), ranging in age from 21 to 33 yr, were studied in the clinical research centers of either the Peter Bent Brigham Hospital or the Thorndike Memorial Laboratory, Boston City Hospital, Boston, Mass. All had normal physical examinations and routine laboratory tests. No medication including sedatives, diuretics, or any antihypertensive agents had been taken by the patients. Informed written consent was obtained in each case.

All subjects were maintained on a constant activity pattern simulating their daily activity outside the hospital until the day of study. The diet for seven of the subjects consisted of a constant 10 meq sodium/100 meq potassium isocaloric diet (13–16). Another four subjects were given a constant 200 meq sodium/100 meq potassium isocaloric diet. Studies were performed after the subjects achieved sodium balance, usually on the 5th or 6th day of dietary sodium restriction. 24-h urines were collected daily and analyzed for sodium, potassium, and creatinine. All studies were begun at 8 a.m. after an overnight fast, and the subjects were supine for at least 12 h.

Intravenous saline infusion. Four sodium-restricted subjects (two men and two women) were given normal saline intravenously. 2 liters of normal saline (0.9%) were infused at a constant rate of 500 ml/h for 4 h. Blood samples were obtained for renin activity (PRA), A II, aldosterone, prekallikrein, kallikrein inhibitor, and bradykinin. After two base-line samples, blood was drawn at 10, 20, and 30 min, and at 1, 2, and 4 h after the start of the infusion.

Intravenous glucose infusion. Three sodium-restricted subjects (two women and one man) received intravenous glucose as a sham or control procedure. 1 liter of 5% dextrose in water was infused at a constant rate of 500 ml/h for 2 h as in the intravenous saline study. After two base-line plasma determinations were obtained, all of the above parameters (except aldosterone) were measured at 30 min and at 2 h.

Upright posture study. Four subjects in balance on a 200 meq sodium/100 meq potassium diet were kept supine and fasting overnight. At 9 a.m. they assumed an upright position and began a constant activity program, walking a fixed distance (200 ft) at a normal rate (3–4 ft/s). Blood samples were taken before 20, 60, and 120 min after assumption of the upright position.

Laboratory procedures

Plasma kallikrein and kinin measurements. Serial blood samples were collected and processed separately for prekallikrein and bradykinin measurement. Determinations of plasma prekallikrein were made by arginine esterase assay (17), and bradykinin was measured by radioimmunoassay, as previously described (18). Excellent correlation between the esterase assay of kallikrein and assay by bradykinin has been demonstrated (19). Kallikrein inhibitors were measured by two different methods. The first utilizes the TAMe (tosyl arginine methyl ester) esterase method as described (17). In the second method, the kallikrein inhibitors were assayed by incubating the subjects' plasma with purified kallikrein by a modification of a procedure previously reported (20). Plasma (75 μl) was incubated at 25°C for 5 min with 25 μl of purified human kallikrein at an initial concentration sufficient to hydrolyze 80 μmol of TAMe/h. Under these conditions, the percent inhibition = $[E - (E1-C)]/E \times 100\%$, where E = initial enzyme activity (purified kallikrein activity), $E1$ = residual enzyme activity after incubation with subjects' plasma, and C = control subject plasma alone. The enzyme incubated with buffer for 5 min showed no decrease of activity and no correction was necessary. The normal for plasma prekallikrein (mean±SEM) is 97±4 μmol/ml/h, normal plasma kallikrein inhibitor activity by the TAMe esterase method was 0.99±0.03 U (measured on 36 patients), and by the enzyme incubation method was 55±4.2% (8 patients). The normal plasma bradykinin level with ad libitum sodium intake was 1.38 ±0.45 ng/ml (14 patients). Plasma kininase activity was measured by quantitating the destruction of [^{3}H, ^{5}phe]-bradykinin as previously reported (21).

Renin, angiotensin, and aldosterone measurements. The serial blood samples were processed for PRA, A II, and aldosterone. PRA and A II were measured by a double-antibody radioimmunoassay (22), plasma aldosterone was measured by a displacement analysis technique as previously described (23). In salt-depleted man, the normal fasting supine value for PRA was 5.28±1 (SEM) ng/100 ml/3 h, for angiotensin II, 57±7 ng/ml, and aldosterone, 33±3 ng/

[1] *Abbreviations used in this paper:* A II, angiotensin II; PRA, plasma renin activity; TAMe, tosyl arginine methyl ester.

100 ml. In sodium-loaded man, the normal fasting supine value for PRA was 1.5±0.5 ng/ml/h, and for angiotensin II was 15±2 pg/ml.

Statistical method. The statistical analyses were performed as described by Snedecor and Cochran (24) with a Mathatron 4280 computer (Mathatronics Div., Barry Wright Corp., Waltham, Mass.). The zero time values for all hormone determinations were arbitrarily set at 100%. This value was then calculated. The means of the percentage change at the different time intervals were compared to the base line (100%) by Student's t test with Bessel's correction for small sample size. The null hypothesis that the curves describing the changes in hormonal levels after saline infusion were similar was tested by the paired Student's t test.

For this study, the level of significance is taken as $P < 0.05$ and value reported as mean±SEM unless otherwise stated.

RESULTS

Sodium restriction values. The mean, fasting, supine, 8 a.m. levels of A II, renin activity, and aldosterone were not significantly different from those of the group of normal sodium-depleted subjects previously reported from our laboratory (13–16). The mean ±standard error of the hormones was as follows: PRA = 5.61±0.82 ng/ml/h; A II = 64±12 pg/ml; aldosterone (four subjects only) = 42±5 ng/100 ml. The mean prekallikrein = 99±4.5 μmol/ml/h, and percent kallikrein inhibition = 56±4.2 are not significantly different from normal, nonsalt-depleted subjects. The bradykinin level of 3.56±1.0 ng/ml is higher than that of 1.38±0.45 ng/ml in normal, nonsodium-depleted subjects.

Sodium loading values. The mean, fasting, supine 8 a.m. levels of A II and renin activity were not significantly different from those of the group of normal sodium-loaded subjects previously reported from our laboratory (15). The mean±standard error of the hormones was as follows: PRA = 1.7±0.33 ng/ml/h; A II = 32.5±1.8 pg/ml. The mean prekallikrein = 86.4±6.3 μmol/ml/h was not significantly different from that of normal, nonsalt-depleted subjects. The bradykinin level of 0.54±0.01 ng/ml was significantly lower than 3.56 ±1.0 ng/ml in the normal, sodium-depleted subjects.

Intravenous saline infusion. The results of the saline infusion study are summarized in Table I and Fig. 1. The base-line levels of prekallikrein in these four subjects were normal. Serial blood samples showed no significant change in prekallikrein or kallikrein inhibitor values in any of these subjects. On the other hand, there was a rapid decline of bradykinin levels during the first 30 min of the saline infusion. Bradykinin levels fell significantly by 10 min after the start of saline infusion and reached a stable plateau value of about 40% of the base-line concentration ($p < 0.001$). The fall in bradykinin paralleled the fall in PRA, A II, and aldosterone (Fig. 1 and Table I). The rate of change in the latter three parameters is similar to those previously described (16). Furthermore, the rate of change of the four parameters is significantly correlated ($p < 0.01$). The kininase values were determined in three of the four subjects studied. There was no detectable change during the study.

Intravenous glucose infusion. The base-line prekallikrein levels of subjects in this group were normal. After glucose infusion, there was no significant change of prekallikrein or kallikrein inhibitor. Furthermore, there was no alteration in bradykinin, PRA, or A II levels (Fig. 2). This was in distinct contrast to the group of subjects with saline infusion who exhibited a rapid decline in bradykinin, PRA, and A II.

Upright posture study. After assumption of the upright posture in the subjects on a 200 meq sodium/100 meq potassium diet, plasma prekallikrein and kallikrein inhibitor did not change (Table II). In contrast, plasma bradykinin rose significantly from 0.54±0.01 (SEM) ng/ml to 0.96±0.13 at 20 min and remained in this range to 120 min. The PRA rose from 1.65±0.33 ng/ml/h to 3.6±0.85 at 20 min, and 5.3±0.9 at 60 min, and remained in this range to 120 min. Similarly, A II rose from 32.5±1.82 pg/ml to 50.8±3.6 at 20 min, and gradually rose to 61.3±5.9 at 120 min.

DISCUSSION

This study demonstrates that in sodium-depleted individuals, saline infusion produces a rapid fall of plasma bradykinin at a rate similar to that observed for A II and PRA. In the control subjects infused with glucose and water, no significant changes occur. Conversely, after dietary sodium loading, upright posture leads to a parallel rise in PRA, A II, and bradykinin. These studies thus indicate a close correlation of bradykinin levels with renin activity and A II, in studies which acutely suppress or activate the renin-angiotensin system. The results of the present study are in agreement with those of Streeten, Keer, Keer, Prior, and Dalakos (25) where plasma bradykinin concentration was found to be significantly higher after 2 h of upright posture when compared to the corresponding supine fasting value. Presumably, plasma renin activity and A II levels of these patients were also higher in the upright posture.

The mechanism responsible for the changes in bradykinin levels in these studies is not known. To explain this phenomenon, it is first necessary to examine whether the changes in bradykinin levels are due to altered production, a different rate of destruction, or both. As the formation of angiotensin is accompanied by a rise in the releasing enzyme, renin, one might expect that a rise in bradykinin levels would be accom-

TABLE I

Response of the Kallikrein-Bradykinin and Renin-Angiotensin Systems to Saline Infusion in Normal Subjects on a 10 meq/100 meq K Diet; Normal Saline Infused at 500 ml/h for 4 h

	0 Time	10 min	20 min	30 min	60 min	120 min	240 min
pre KKn, *μmol/ml/h*							
G. M.	117	104	98	98	92	78	117
R. E.	110	96	99	108	97	114	102
M. E.	89	79	95	87	93	96	73
P. E.	108	105	111	91	113	86	87
Mean	106	96	101	96	99	94	95
SE	5	5	3	4	4	7	8
KKn Inhibit, %							
G. M.	73	74	64	61	63	71	64
R. E.	52	51	54	45	34	41	50
M. E.	53	48	49	51	57	44	60
P. E.	60	62	63	61	52	62	57
Mean	59	59	57	54	52	55	58
SE	4	5	3	3	5	6	3
BK, *ng/ml*							
G. M.	7.80	4.12	2.14	1.31	1.12	0.94	1.45
R. E.	5.56	3.12	1.62	1.28	2.02	1.78	0.94
M. E.	1.28	0.60	0.50	0.50	0.40	0.40	0.50
P. E.	0.91	0.91	0.50	0.62	0.40	0.69	0.75
Mean	3.89	2.19	1.19	0.93	0.98	0.95	0.91
SE	1.46	0.74	0.36	0.19	0.34	0.26	0.18
KA							
G. M.	27.7			30.6		26.1	
R. E.	32.8			30.4		31.8	
M. E.	33.5			25.6		28.9	
Mean	31.3			29		29	
SE	1.5			1.3		1.1	
PRA, *ng/ml/h*							
G. M.	4.6	3.4	2.4	3.3	2.5	2.2	2.8
R. E.	7.2	4.3	3.8	4.9	4.0	2.7	2.6
M. E.	7.7	7.0	—	6.0	3.1	3.0	2.2
P. E.	12.0	—	9.5	8.0	7.5	6.0	4.0
Mean	7.9	4.9	5.2	5.6	4.3	3.5	2.9
SE	1.3	0.9	1.8	0.8	1.0	0.7	0.3
A II, *pg/ml*							
G. M.	112	58	54	47	45	38	25
R. E.	88	78	65	60	45	49	40
M. E.	100	62	57	45	44	42	34
P. E.	154	149	104	94	75	65	69
Mean	113	87	70	62	52	48	42
SE	12	18	10	10	7	5	8
Aldo, *ng/100 ml*							
G. M.	43	46	36	34	19	17	19
R. E.	28	24	24	23	14	15	15
M. E.	37	37	31	23	23	22	11
P. E.	58	51	64	51	34	52	23
Mean	42	40	39	33	22	26	17
SE	5	5	8	6	4	7	2

pre KKn, prekallikrein; KKn Inhibitor, kallikrein inhibitor activity by enzyme incubation expressed in percent inhibition; BK, bradykinin; Aldo, plasma aldosterone in ng/100 ml; KA, kininase activity expressed as percentage destroyed.

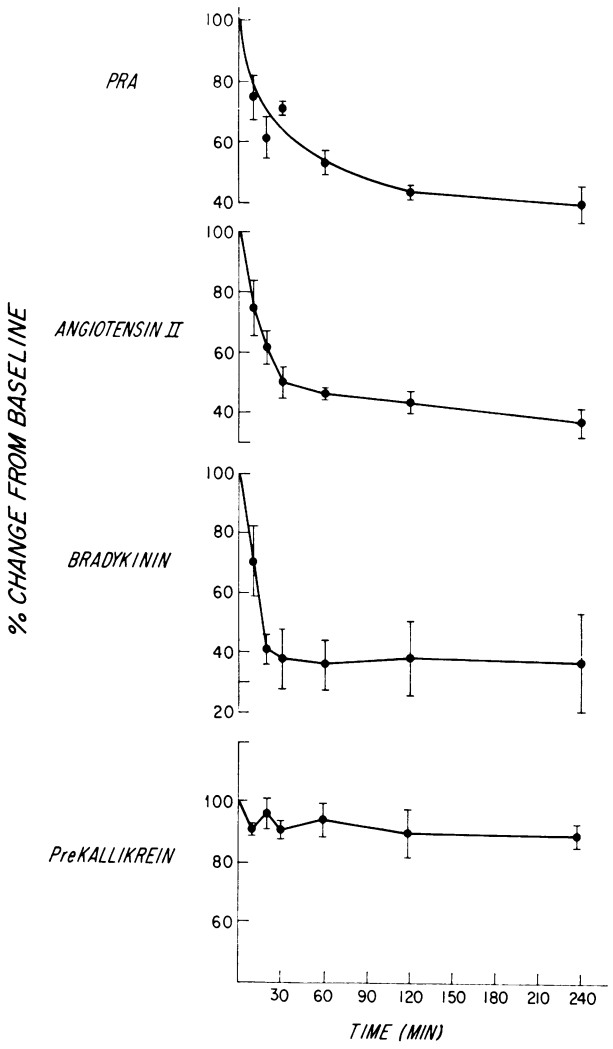


FIGURE 1 Response of the kallikrein-bradykinin and renin-angiotensin systems to saline infusion, expressed as percent of base-line value in normal subjects on a 10 meq Na/100 meq K diet; normal saline infused at 500 ml/h for 4 h.

panied by an increase in one of the major enzymes responsible for its production, plasma kallikrein. Kallikrein is a proteolytic enzyme that releases bradykinin from a plasma α_2 globulin, termed kininogen. Free kallikrein (determined by spontaneous esterase activity) was not decreased in the plasma with saline infusion or increased with upright posture. Since in the plasma, kallikrein arises from the action of activated Hageman factor or its fragments on the precursor prekallikrein, one might expect to find a decrease in prekallikrein if the enzyme were converted to the active form, i.e. with sodium restriction or upright posture. Although there are no published reports of plasma prekallikrein in normal sodium-depleted subjects, the mean plasma prekallikrein of 98±11.6 SE μmol/ml/h is essentially the same as that of our published normal value of 97±4 SE μmol/ml/h in normal subjects with normal salt intakes (17). This would suggest that plasma prekallikrein levels do not change with salt intake or changes in posture. This is confirmed both by the base-line sodium-restricted and sodium-loaded levels and by the constant prekallikrein levels during saline infusion while bradykinin was falling, and during upright posture when bradykinin was rising. Once activated, kallikrein combines stoichiometrically with C1 esterase inactivator, resulting in depletion of both enzyme and inhibitor. However, the mean kallikrein inhibitor during the saline and glucose infusion and upright posture is not significantly different from that of the mean base-line value, suggesting there has been no change in activation of prekallikrein during these acute studies. Thus, no apparent change in plasma kallikrein accounts for the change of bradykinin after sodium infusion and upright posture.

These findings do not rule out the release of a tissue kallikrein. Nearly 40 yr ago (26) it was first reported that urinary kallikrein was reduced in patients with hypertension. These early findings were recently confirmed by Margolius, Geller, Pisano, and Sjoerdsma (27), who also reported that patients with essential hypertension have decreased urinary kallikrein excretion.

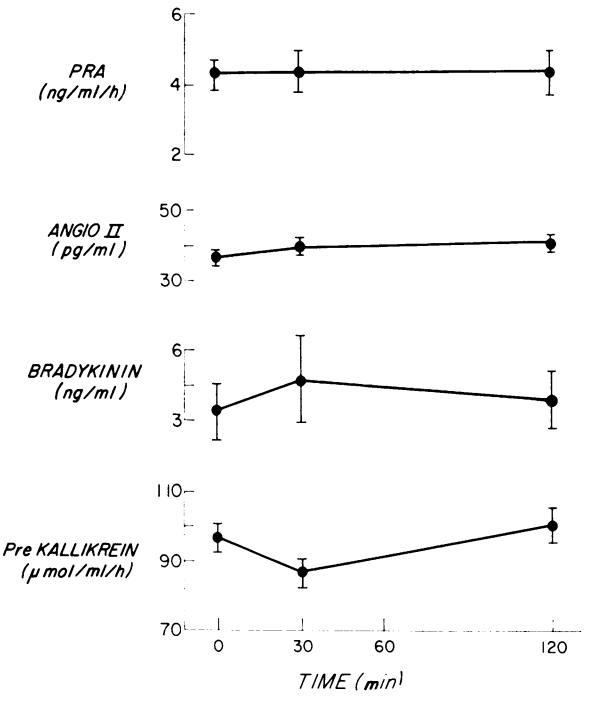


FIGURE 2 Response of the kallikrein-bradykinin and renin-angiotensin systems to 5% glucose infusion, expressed as percent of base-line value in normal subjects on a 10 meq Na/100 meq K diet; 5% glucose solution infused at 500 ml/h for 2 h.

TABLE II

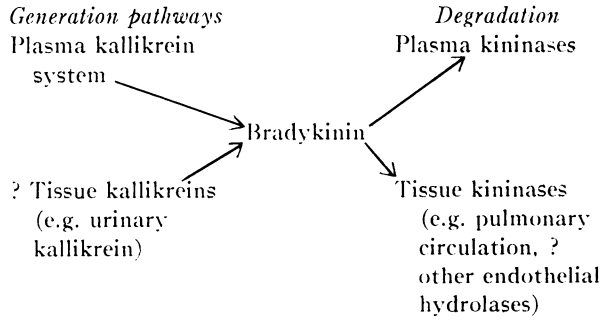
Response of Kallikrein-Bradykinin and Renin-Angiotensin Systems to Upright Posture in Normal Subjects on a 200 meq Na/100 meq K Diet

	0 Time	20 min	60 min	120 min
Prekallikrein, *μmol/ml/h*				
A. B.	99	87.3	104	92.3
Y. L.	84.4	87.0	88.8	108.1
K. H.	67	75.1	61.2	64.5
K. S.	95.5	79.4	108.4	87.0
Mean	86.4	82.2	90.6	87.98
SE	6.3	2.5	9.25	7.8
Bradykinin, *ng/ml*				
A. B.	0.54	1.0	0.91	1.0
Y. L.	0.58	0.62	0.49	1.0
K. H.	0.51	0.88	1.28	0.72
K. S.	0.51	1.34	0.39	1.12
Mean	0.54	0.96	0.77	0.96
SE	0.01	0.13	0.18	0.07
PRA, *ng/ml/h*				
A. B.	2.4	2.5	3.4	4.0
Y. L.	1.6	4.5	6.9	6.0
K. H.	0.6	1.4	3.6	4.6
K. S.	2.0	5.8	7.2	6.8
Mean	1.65	3.6	5.3	5.35
SE	0.33	0.85	0.9	0.55
A II, *pg/ml*				
A. B.	38	48	50	50
Y. L.	31	48	60	81
K. H.	28	44	46	56
K. S.	33	63	61	58
Mean	32.5	50.8	54	61.3
SE	1.82	3.6	3.2	5.9

These same authors have recently reported that sodium-restricted normal subjects excrete more urinary kallikrein than sodium-loaded patients (28). These studies are in contrast to those of Adetuyibi and Mills (29) and Marin-Grez, Cottone, and Carretero (30), who had previously shown that urinary kallikrein excretion is positively correlated with sodium excretion, i.e., the greater the sodium excretion, the higher the kallikrein level. While the relationship of urinary kallikrein to plasma bradykinin is unclear, these reports suggest a possible role for renal kallikrein and kinins in blood pressure regulation and sodium excretion.

If changes in production of kinin are not the major cause of the decrease of kinin with saline infusion or its increase with upright posture, it is necessary to consider the pathways of bradykinin inactivation. There is a striking parallel between bradykinin removal and the conversion of A I to A II in the circulating blood, as well as in the pulmonary capillary bed.

Recently, the plasma angiotensin-converting enzyme and kininase II (a dipeptidyl-peptidase) have been shown to be identical (31). This enzyme converts A I to A II by removing the COOH-terminal His-Leu and destroys bradykinin by removing its COOH-terminal Phe-Arg. Along the pulmonary capillary endothelial surface there seems to be a membrane-bound enzyme similarly capable of these two actions (32). In addition, bradykinin is destroyed at this site by at least four other peptide hydrolases (33, 34). Thus, a change in kininase activity might account for the acute changes in bradykinin. The absence of any change in kininase activity during the saline infusion would make this an unlikely possibility. However, one could postulate that changes in renin and the formation of A II are primary. In this case an increasing level of renin, and thus of A I in salt depletion or upright posture might lead to competitive inhibition of kininase II (converting enzyme), i.e. a change in its function without a change in activity. Thus, if the affinity of A I for this enzyme is high, and the levels of the peptide are elevated, inhibition of kinin destruction might occur. However, this is unlikely to provide a complete explanation, since under normal circumstances kininase II accounts for only about 10% of the total circulating kininase activity; the rest is due to kininase I.[2] In the pulmonary vascular bed other potent kinin-destroying enzymes are also readily available (33). Thus, whether the changes in bradykinin in these studies are secondary to the release of a tissue kallikrein (as discussed) or to a change in the function of the kininases without a change in the measured activity remains to be clarified (Scheme).



The significant parallel changes in A II and bradykinin levels in response to acute manipulation would suggest that these hormones are physiologically interrelated. These substances have opposite effects on the vascular smooth muscle. Infusion of A II at doses in

[2] R. C. Talamo. Unpublished observation.

the range of 5 ng/kg/min in normal subjects usually produces vasoconstriction (35). On the other hand, infusion of bradykinin at levels of 500–1,500 ng/kg/min produces vasodilation (36, 37). This gives an effective dose ratio of angiotensin to bradykinin of appoximately 1 : 100–1 : 500, levels that are close to the ratios of these two hormones in the plasma of our normal subjects. The physiological significance of this relationship is unclear. However, it is known that in sodium-depleted man the high plasma levels of A II are not associated with a rise in blood pressure and furthermore, with sodium restriction, the vascular response to A II is blunted both in man and in the experimental animal (35–38). The mechanisms previously proposed for this phenomenon include a change in total blood volume or a change in the affinity of angiotensin for its vascular receptor (2). An alternative proposal on the basis of the present study would be a decreased vascular sensitivity due to an increase in bradykinin levels.

Since in this study, the changes in bradykinin correlated not only with changes in A II, but also with plasma aldosterone, it is possible that alterations in bradykinin levels are related to changes in concentration of aldosterone, as suggested by Margolius et al. (28), rather than of A II.

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