Plasma Angiotensin, Serum 'Angiotensinase Activity', and Blood Pressure Response during Angiotensin II Amide Infusions in Normal Volunteers[1]

During incremental infusion of synthetic angiotensin in normal volunteers, it would be expected that a) blood pressure response would correlate directly with the log dose of peptide[2]; b) pressor response would correlate directly with serum sodium levels or sodium balance[3]; c) plasma peptide levels would correlate directly with blood pressure change; and d) in line with current concepts of tissue inactivation of angiotensin, serum 'angiotensinase activity' would not relate to other factors[4]. The development of accurate radioimmunological means to measure plasma angiotensin II has permitted more precise analysis of the relationships among these various parameters in normal volunteers given angiotensin II amide infusions under controlled conditions. The results proved the validity of the assumptions a) and b), but showed that the assumptions c) and d) could not be so simply stated. Rather, the determinant of blood pressure response seemed to involve not only plasma peptide levels but also receptor site turnover and a possible role for serum peptidase activity at such sites.

Methods. 5 males and 2 females were studied while receiving ad libitum diets. 5 of these same subjects were studied again after stimulation of the renin-angiotensin system by mild salt restriction and administration of 50 mg of hydrochlorothiazide twice daily for 3 days.

Subjects were recumbent and an infusion of 5% dextrose in water was begun. Control specimens were taken after 30 min when blood pressure and pulse were stable. Angiotensin II amide was then given at constant rates of 0.5, 1.0, and 2.0 μg/min for periods of 20–30 min each. Total volume given during each experiment was less than 50 ml. Blood specimens for plasma angiotensin were taken at the end of each period, again at a time when blood pressure and pulse were stable. Plasma renin activity was determined at the beginning of each experiment, and serum 'angiotensinase activity', sodium and potassium determined at the beginning and end.

Plasma angiotensin was determined radioimmunologically by the method of BOYD and PEART[5], slightly modified. Plasma renin activity was estimated by use of SKINNER's method[6]. Serum 'angiotensinase activity' was determined by the in vitro degradation of angiotensin II amide[7], the same substrate used for infusions. This was expressed as angiotensin half-life, so that an increased 'angiotensinase activity' was reflected by a shortened half-life and vice versa.

Results and discussion. Blood pressure response was related to the log dose of angiotensin II amide, as shown in Figure 1 where the raw data are expressed by a curve drawn from the least squares equation. On cessation of infusion, blood pressure fell to control levels within five min in every instance.

Change in mean blood pressure during infusion correlated directly with serum sodium concentration, even in these normal subjects on an ad libitum diet, as shown in Figure 2. At the 2 higher infusion rates, this correlation was statistically significant ($r = 0.86,\ 0.71$; $p < 0.05$, < 0.1).

Plasma angiotensin levels rose during incremental infusion of angiotensin II amide, as shown in the Table. Mean blood pressures in the seven cases rose concomitantly, but further analysis of results at each infusion rate revealed that the correlation between plasma angiotensin II concentration and change in mean blood pressure was poor at lower and inverse ($r = -0.63$)[8] at the highest infusion rate. This last correlation approached significance ($p < 0.1$), and the unexpected relationship raised the possibility that another factor, perhaps receptor site uptake, influenced plasma peptide level and blood pressure response.

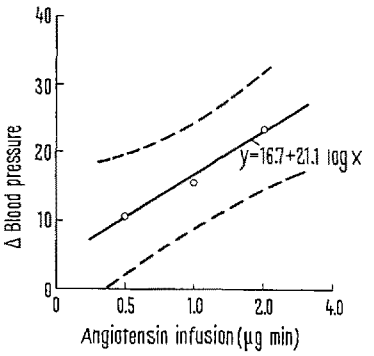

Fig. 1. Relationship of blood pressure response to the log dose of angiotensin II amide. Confidence limits (95%) are shown by the dotted lines.

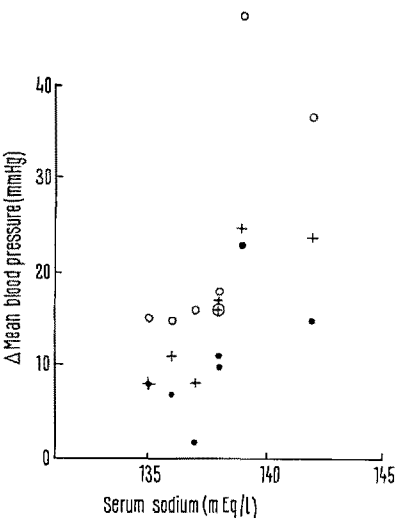

Fig. 2. Relationship of blood pressure response to serum sodium concentration during angiotensin II amide infusion. Infusion rates are shown by ●, 0.5 μg/min; +, 1.0 μg/min; ○, 2.0 μg/min.

[1] A preliminary report of this work was presented at the Eastern Section Meeting of the American Federation for Clinical Research, Washington, D.C., December 13, 1969.

[2] E. DE BONO, G. DE J. LEE, F. R. MOTTRAM, G. W. PICKERING, J. J. BROWN, H. KEEN, W. S. PEART and P. H. SANDERSON, Clin. Sci. *25*, 123 (1963).

[3] J. J. BROWN, D. L. DAVIES, A. F. LEVER and J. I. S. ROBERTSON, Postgrad. med. J. *42*, 153 (1966).

[4] R. L. HODGE, K. K. F. NG and J. R. VANE, Nature *215*, 138 (1967).

[5] G. W. BOYD, J. LANDON and W. S. PEART, Lançet *2*, 1002 (1967).

[6] S. L. SKINNER, Circulation Res. *20*, 391 (1967).

[7] G. D. LUBASH, E. C. HAMMEL and R. J. MEARLES, Clin. chim. Acta *18*, 439 (1967).

[8] The inverse correlation refers to a relative change in plasma angiotensin. When angiotensin II amide was given at the fastest rate, 2.0 μg/min, plasma peptide values rose but less so for subjects with the greatest blood pressure response and vice versa.

To test this, 5 of the same subjects underwent a similar infusion study after diuretic therapy and mild dietary salt restriction which increased plasma renin activity 3–10-fold and plasma angiotensin II concentration >5 to 15-fold. It was reasoned that if decreased receptor site uptake was a factor in determining the lesser blood pressure response and a relatively higher plasma angiotensin, then with higher circulating plasma angiotensin levels initially, the anticipated lesser blood pressure change during incremental infusion would be associated with a faster rise in plasma angiotensin. A more rapid rise in plasma angiotensin during the second infusion is shown by Figure 3 where the data are expressed as the curves drawn from the least squares equation.

Angiotensin II amide half-life correlated directly with plasma angiotensin II values for the 2 higher infusion rates ($r = 0.52, 0.76$; $p < 0.05$ at 2.0 µg/min) and inversely with change in mean blood pressure for all 3 infusion rates ($r = -0.59, -0.76, -0.52$; $p < 0.05$ at 1.0 µg/min). Increased serum 'angiotensinase activity' was thus associated with lower plasma angiotensin II levels[9] and with a greater change in mean blood pressure, and the reverse also applied.

Since there was no systematic change in 'angiotensinase activity' following angiotensin infusion, it may be inferred that substrate elevation does not induce a comparable rise in enzyme activity.

During incremental infusion of angiotensin II amide in normal volunteers, the anticipated direct relationship between blood pressure response and log dose of peptide was found. Also, as expected, pressor responses related directly to serum sodium concentration, and when plasma renin activity and angiotensin II were increased prior to infusion, inversely with those factors[3].

Plasma peptide levels rose along with blood pressure as the rate of angiotensin II amide infusion was increased, but a relative negative correlation of these values with blood pressure change at the highest rate of infusion suggested that other factors contributed to pressor response.

Plasma angiotensin II values during angiotensin II amide infusions in 7 normal subjects on a regular diet

Angiotensin infusion	Mean plasma angiotensin II pg/ml	SD	SE
Control	<13.0		
0.5 µg/min	51.7	22.1	8.4
1.0 µg/min	74.3	34.5	13.0
2.0 µg/min	169.1	109.9	41.5

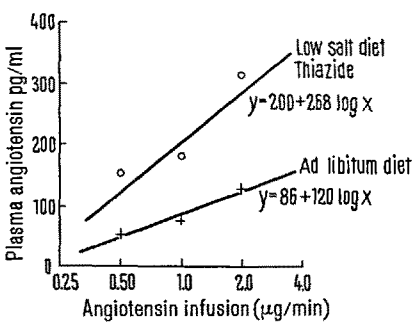

Fig. 3. Plasma angiotensin values during incremental infusion of angiotensin II amide in 5 volunteers on an ad libitum diet and again after salt depletion.

Serum 'angiotensinase activity' is probably a misnomer since studies have failed to show any relationship of this enzyme activity to renin-angiotensin system activity or to hypertension[7]. Much of the reported work has been based on indirect in vitro assays using substrates other than natural angiotensin. Current evidence indicates that circulating angiotensin is inactivated in tissue beds rather than in serum[4]. In this study, though the assay system was an in vitro one, it did not suffer from the use of improper substrate since the same synthetic peptide was both infused and assayed. The finding that enzyme activity was associated inversely with plasma angiotensin and directly with blood pressure change may be interpreted to mean that enzyme activity was important at tissue sites and influenced the turnover of angiotensin and the resultant pressor response. When plasma rich in 'angiotensinase activity' bathed receptor sites, angiotensin at receptor sites was metabolized, thus permitting additional plasma angiotensin to bind. In the process, relatively lower plasma levels and increased blood pressure response resulted. When plasma poor in such enzyme activity bathed receptor sites, the reverse occurred.

This concept is consistent with that of angiotensin tachyphylaxis and its reversal in vitro suggested by KHAIRALLAH et al.[10]. These investigators found that a free C-terminal carboxyl group of angiotensin was necessary for binding with receptor sites in animal blood vessels and for development of tachyphylaxis. They found that tachyphylaxis seemed to represent saturation of receptor sites and that it could be reversed by plasma fractions rich in angiotensinase A, possibly by metabolizing the N-terminal part of angiotensin directly from the bound state[11].

Résumé. Des doses croissantes d'angiotensine II amide furent injectées à des volontaires normaux. La réponse de la pression artérielle fut en relation directe avec le log de la dose de peptide injectée, avec la concentration sérique en sodium et avec l'activité sérique «angiotensinase», mais en relation inverse avec l'augmentation de la concentration plasmatique en angiotensine. Ces résultats suggèrent que la réponse de pression est déterminée, au moins en partie, par le «turnover» du peptide au niveau des récepteurs et qu'à ce niveau angiotensinase influence le «turnover» et la concentration plasmatique du peptide.

G. D. LUBASH, G. E. MUIESAN[12],
C. L. ALICANDRI, D. J. GARFINKEL,
E. C. SIEKIERSKI and C. K. MCCONNAUGHEY

Department of Medicine,
University of Maryland School of Medicine,
Baltimore (Maryland 21201, USA), 9 July 1970.

[9] As before, the inverse correlation refers to a relative change, i.e., a lesser positive response.

[10] P. A. KHAIRALLAH, I. H. PAGE, F. M. BUMPUS and R. K. TÜRKER, Circulation Res. *19*, 247 (1966).

[11] Supported by U.S.P.H.S. Grant No. HE 11691-02, NIH; the American and Maryland Heart Associations; and the Kidney Foundation of Maryland. Thanks are due Dr. J. R. HEBEL for statistical analyses and Miss D. S. BAKER for secretarial assistance.

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