ROLE OF EXTRAPULMONARY CONVERSION IN MEDIATING THE SYSTEMIC PRESSOR ACTIVITY OF ANGIOTENSIN I*

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Subsequent to the demonstration by Ng and Vane (1, 2) that angiotensin I (AI)[1] is rapidly converted to the active form, angiotensin II (AII), in the canine lung, evidence obtained by infusing AI into various regional circulations in the dog (3–6) has indicated that some conversion also occurs at extrapulmonary sites. In the rat, conflicting results have been reported. Whereas Barrett and Sambhi (7) concluded that AI, at physiological concentrations, is converted almost quantitatively to AII in a single passage through the rat lung, converting enzyme activity has been demonstrated in homogenates of many rat tissues (8), and Freer and Stewart (9) have recently reported that the pulmonary vasculature in this species does not appear to be involved in the conversion of AI.

Circulating antibodies directed against AII have been much used in analyzing the pressor function of the renin-angiotensin system in rat renal-clip hypertension (10–13). However, the interpretation of such studies requires an understanding of the relative extent to which the pressor activity of renin, in the intact animal, is dependent upon free-circulating AII generated in the lungs, as distinct from AII released locally from AI at extrapulmonary sites, or intrinsic activity of the decapeptide (14–16).

Accordingly, we have investigated the dose-response characteristics of AI administered intra-arterially and intravenously, both before and after AII blockade by specific antibody, to determine whether AI can elicit a systemic pressor response before lung transit. An attempt was also made to distinguish between a direct action of AI and one mediated by peripheral formation of AII.

Materials and Methods

Four groups of male Wistar rats (250–450 g) were anesthetized with Inactin (100 mg/kg, i.p.; Promonta, Hamburg), tracheotomized, vagotomized, and injected with atropine sulfate (1.5 mg/kg, i.m.) and pentolinium tartrate (25 mg/kg, i.m.). Two polyethylene catheters (PE 10) were inserted through the right jugular vein into the superior vena cava.

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[1] *Abbreviations used in this paper:* AI, angiotensin I; AII, angiotensin II.

Groups 1 and 2.—The right common carotid artery was catheterized (PE 50) and connected via a Statham pressure transducer to a Sanborn recorder for continuous monitoring of arterial pressure. Dose-response curves were determined for intravenous AII (1-Asn-5-Val-angiotensin II, Hypertensin, Ciba), and AI (5-Ile-angiotensin I, Schwarz Bio Research Inc., Orangeburg, N. Y.), by measuring peak pressor responses to a series of 4–8 injections of each peptide, given alternately. Doses of AI were expressed as ng of an equimolar amount of AII (i.e., ng of AI ÷ 1.25).

Group 1 (five animals); effect of control plasma on intravenous AII and AI responses: Dose-response curves for intravenous AII and AI were repeated after intravenous administration of 0.3 ml of pooled normal rabbit plasma.

Group 2 (eight animals); effect of anti-AII plasma on intravenous AII and AI responses: Dose-response curves were repeated after intravenous administration of 0.3 ml of rabbit anti-AII plasma. Larger doses of both peptides were required to obtain a similar range of responses.

Anti-AII plasma used in the experiments in Groups 2–4 was collected from three rabbits immunized by repeated injections, over 10 mo, of AII (1 mg/rabbit) coupled to bovine serum albumin and emulsified in Freund's adjuvant (13). By methods of immunologic evaluation previously described (13), the plasma was found to have a very high anti-AII titer of 1/233,000 and cross-reactivity of 4.4% with AI.

Groups 3 and 4.—In these animals one femoral artery was catheterized (PE 50) for arterial pressure measurement. The right common carotid artery was also catheterized (polyethylene, ID 0.35 mm, OD 1.05 mm) and the catheter tip advanced until contact with the base of the left ventricle was detected by pulsation transmitted through the catheter; it was then withdrawn 3 mm. At the end of each experiment the animal was sacrificed, and the catheter tip shown to lie freely within the ventricular cavity, with no damage to aortic valve cusps or ventricular muscle. Injections via the left ventricular catheter are described as "intra-arterial", since it was assumed that they would pass, well mixed with blood, into the systemic arterial circulation.

Group 3 (eight animals); effect of anti-AII plasma on intravenous and intra-arterial AI responses: Dose-response curves for both intravenous and intra-arterial AI were determined before and after infusion of 0.3 ml of anti-II plasma.

Group 4 (two animals); effect of converting enzyme inhibitor on AI responses: These experiments were carried out as for Group 3, with the additional step that, after the second pair of dose-response curves had been completed, the rats were injected intravenously with 0.4 mg of SQ 20,881 (Squibb Institute for Medical Research), a synthetic nonapeptide that inhibits enzymatic conversion of AI to AII, both in vivo and vitro, leaving responses to AII unaltered (6). Dose-response curves for both intravenous and intra-arterial AI were immediately repeated.

RESULTS

In each rat studied, the log dose/response relationships for AI and AII were linear in the range 5-25 mm Hg. The potency of AI relative to AII, and the potency of intra-arterial AI relative to that of intravenous AI, did not vary significantly throughout the dose-response ranges tested. Thus, to compare potencies, the regression equation of each dose-response curve was solved for a pressure rise of 15 mm Hg (the midpoint of the range tested), and the mean dose required to elicit this response was determined for each group. These doses, hereafter called reference doses, are shown in Table I.

Groups 1 and 2.—Before infusion of plasma, in all experiments, AII was slightly more potent than AI, as evidenced by the higher doses of AI required to produce an equipressor effect (Table I). In Group 1, administration of control rabbit plasma did not change this relationship, and had no effect on basal blood

pressure readings. Nor, in Group 2, did infusion of anti-AII plasma change basal blood pressure. However, it resulted in a 36-fold increase in the reference dose of AII, while that of AI increased only 10-fold, to a value less than that of AII. The dose-response curves obtained in a typical Group 2 experiment are shown in Fig. 1, first panel. It is clear that, after administration of anti-AII plasma, the pressor potency of AI greatly exceeded that of AII, and that the relative positions of the curves were reversed. A similar reversal was observed in every other experiment in this group. The AII antibody was only 29 ± 3 (SE)% as

TABLE I

Mean Dose (± SE) of Angiotensin (ng) Required to Elicit a 15 mm Hg Rise in Arterial Pressure, Before and After Infusion of Normal Plasma (in Group 1), or Anti-AII Plasma (in Groups 2 and 3)*

Group	No. animals	Peptide	Route	Before plasma (B)	After plasma (A)	Factor of increase in dosage (A/B)
1	Five	AII	i.v.	1.7 ± 0.2	1.7 ± 0.2	1.0
		AI	i.v.	2.1 ± 0.2	2.1 ± 0.2	1.0
2	Eight	AII	i.v.	2.0 ± 0.4	71.6 ± 15.3	35.8
		AI	i.v.	3.4 ± 0.6	35.2 ± 7.7	10.3
3	Eight	AI	i.v.	3.0 ± 0.4	30.4 ± 3.8	10.1
		AI	i.a.	4.3 ± 0.5	19.6 ± 2.5	4.6

* Calculated by solution of log-dose response regressions.

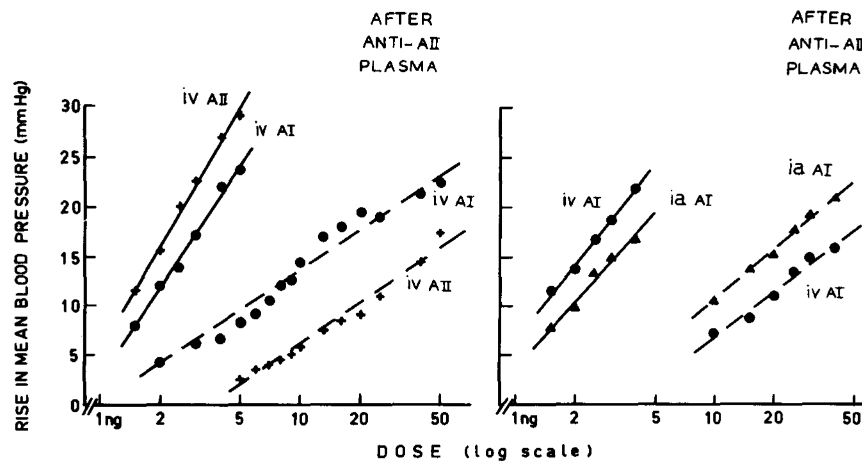

FIG. 1. Dose-response curves before and after anti-AII plasma. Typical dose-response curves before immunization (continuous lines), and after infusing anti-AII plasma (interrupted lines), in a bioassay rat from Group 2 (first panel), and another from Group 3 (second panel), determined by injection of intravenous AII (i.v. AII), intravenous AI (i.v. AI), or intra-arterial AI (i.a. AI). Doses of AI are expressed as ng of an equimolar amount of AII (i.e., ng of AI ÷ 1.25).

effective in blocking AI given intravenously as it was in blocking AII injected by the same route.

Groups 3 and 4.—In all Group 3 experiments, before administration of AII antibody, AI was less potent when given by intra-arterial injection than when given intravenously (i.a./i.v. reference doses for equipressor effects = 143% ± 8 SE; $P < 0.001$). There was however, no detectable difference between the intra-arterial route and the intravenous route with respect to the time taken for AI to produce the peak pressure rise (21 sec ± 1 SE from the start of injection of intra-arterial AI, and 22 ± 1 for intravenous AI, over the response range 10–25 mm Hg; $P > 0.3$).

After the injection of anti-AII plasma, the reference dose of intravenous AI increased 10-fold, as found in Group 2, while the reference dose of intra-arterial AI increased only five-fold, to a value 64% of that of the intravenous AI ($P < 0.05$). There was, again, no significant difference between the two routes of injection with respect to the time taken from the start of injection to the peak of the pressor response (intra-arterial AI, 20 sec ± 2 SE; intravenous 21 sec ± 1; $P > 0.7$). In every experiment, however, the relative positions of the intravenous and intra-arterial AI curves were reversed after AII antibody, as shown in Fig. 1, second panel. The AII antibody was only 45 ± 4 (SE) % as effective in blocking intra-arterial AI as in blocking intravenous AI.

In Group 4 experiments, reversal of the relative positions of the intravenous and intra-arterial AI curves occurred in the presence of AII antibody, as in Group 3. Following injection of converting enzyme inhibitor (SQ 20, 881), blood pressure fell by 6 mm Hg, but returned to the original basal level in less than 2 min. Immediate repetition of the dose-response curves revealed that all AI responses had been abolished. No responses were elicited by doses of AI as high as 100 ng, whether given by the intravenous or the intra-arterial route.

DISCUSSION

The finding that anti-AII plasma neutralized AII more effectively than it did AI, when the peptides were given intravenously (Group 2), is consistent with our previous finding in rats actively immunized against AII (13). There are several possible explanations for this result:

(*a*) The 5-Ile-angiotensin II, liberated by conversion from the decapeptide injected, may not have as great an affinity for the antibody as the 1-Asn-5-Val-angiotensin II used for comparison. However, some anti-II antibodies do not discriminate between 5-Val-AII and 5-Ile-AII (17). (b) AI may have intrinsic activity that remains unimpaired by specific AII antibody. (*c*) Injection of intravenous AI may result in the liberation of AII (whether in the pulmonary bed, or at extrapulmonary sites even closer to tissue receptors) nearer its site of action than the intravenous bolus of AII with which it was compared. This would minimize exposure of the newly generated AII to the circulating AII antibody, and so increase its relative potency.

The Group 3 and 4 experiments were designed to overcome the multiple interpretations to which those of Group 2 were subject, and to make possible a distinction between the alternative explanations. Firstly, since 5-Ile-angiotensin I was used throughout, differing affinities of the liberated AII for the antibody cannot explain the changed relationship between intravenous and intra-arterial responses. Secondly, though recent work has suggested that angiotensin I may have a significant central vasomotor action in the vertebral artery territory (16), and a marked direct stimulatory effect on the adrenal medulla (15), the fact that injection of converting enzyme inhibitor (Group 4) abolished both intravenous and intra-arterial AI responses over the entire dose range studied, makes it extremely unlikely that any direct intrinsic action of AI was contributing to the rapid systemic pressor responses measured in Groups 2–4.

The finding that, in the presence of AII antibody, intra-arterial AI became more potent than intravenous AI, can, then, only be explained in terms of conversion of the decapeptide to AII. There was no delay in the pressor peak resulting from intra-arterial AI injection such as would be expected if its activity resulted from recirculation and conversion in the lung. Moreover, if AI was completely dependent upon pulmonary conversion for its activation, being inert before lung transit, it would be impossible for the potency of intra-arterial AI to exceed that of intravenous AI, as found in all Group 3 experiments. Indeed, since intra-arterially injected AI would have to recirculate, being exposed to inactivation by angiotensinases, tissue uptake, and cross-reaction with the circulating AII antibody along its path, before reaching a position equivalent to the intravenous injection site, lower potency would be expected.

The results therefore make it clear that AI cannot be completely dependent upon pulmonary conversion for its activity, and that intra-arterial AI must, in fact, be converted to AII at extrapulmonary sites to cause rapid systemic pressor responses at least of sufficient magnitude to account for the differences between the intra-arterial and intravenous curves obtained after AII antibody in the Group 3 experiments.

Significance of Extrapulmonary Angiotensin Conversion.—Having invoked extrapulmonary conversion of AI to AII to explain the findings in Groups 3 and 4, it then becomes apparent why the potency of intra-arterially injected AI actually exceeded that of intravenous AI in the presence of specific AII antibody. AII generated from AI in the arteriolar vascular tree, nearer the tissue receptor sites, was clearly less exposed to neutralization by the circulating AII antibody than was the AII released in the pulmonary circulation from AI injected intravenously. This, and the finding that specific antisera to AII neutralize intravenously injected AII to a far greater extent than they do intra-arterially injected AII (Oates and Stokes, unpublished observations), support the concept that the extent to which circulating AII antibody neutralizes a given dose of angiotensin is largely dependent on the site at which AII is released into the circulation, and hence upon the degree of exposure of the AII to the antibody.

The significance of these findings in vivo is as follows. Since AI is released by endogenous renin from renin substrate throughout the entire circulation, it would be expected that a significant proportion of the AI would be liberated within the arterial tree, to be converted to AII intramurally, close to receptor sites. Our Group 3 results show that AII antibody does not neutralize such locally converted AII as efficiently as it does lung-generated AII. It would thus be possible for endogenous AI, converted intramurally, in the region of arteriolar receptors, to completely escape neutralization by high titers of circulating AII antibody. Such an occurrence would, on the other hand, be less likely in the presence of lower molecular weight blockers of the renin-angiotensin system, such as inhibitors of converting enzyme (5, 6), or specific antagonists of AII which compete for AII at the receptor sites (18).

The results may thus offer a partial explanation for the success of renin pre-inhibitor (19), angiotensin converting enzyme inhibitor (20), and specific competitive antagonists of AII (21) in reducing blood pressure of 2-kidney Goldblatt renal-clip hypertensive rats, where studies relying on AII antibodies have failed (10–12). However, the failure of circulating AI antibodies to ameliorate renal-clip hypertension (13) is less readily explained, unless all steps in the reaction, including the generation of AI by renin, can occur intramurally (22).

Finally, though our results make it clear that extrapulmonary conversion to AII contributes to the total systemic pressor activity of AI released on the arterial side of the circulation, they do not support the conclusion of Freer and Stewart (9) that, in contrast to most species, the pulmonary vasculature of the rat appears not to be involved at all in the conversion of AI. In every experiment in Groups 3 and 4, before AII antibody administration, intra-arterial AI gave a smaller response than the equimolar dose of intravenous AI ($P < 0.001$).

It would thus appear that the lungs are the most effective site for activation of AI, but that extrapulmonary local tissue conversion of AI to AII occurs to a sufficient extent to render AII immunity an inefficient means of blocking the systemic pressor activity of the renin-angiotensin system.

SUMMARY

The effect of antibodies against angiotensin II (AII) on systemic pressor responses to intravenously injected AII and angiotensin I (AI) was studied in a group of bioassay rats. AII antibody was only 29% as effective in neutralizing AI given intravenously as it was in neutralizing AII injected by the same route. Control plasma caused no change in the relative potencies of AI and AII.

In a further series of experiments, AII antibody was significantly less effective in blocking intra-arterial AI than in blocking intravenous AI. The potency of intra-arterial AI, initially less than that of intravenous AI, became nearly twice that of intravenous AI after antibody administration, a result which could not occur if AI were inactive before lung transit. Thus, AI can elicit systemic

pressor activity independently of pulmonary conversion to AII. However, since the intra-arterial AI responses were abolished by an inhibitor of angiotensin-converting enzyme, the activity would appear to be mediated by peripheral conversion to AII rather than by an intrinsic action of the decapeptide.

Both series of experiments suggest that the efficacy of AII antibody in abolishing the systemic pressor activity of AI is highly dependent on the site of conversion of the AI to AII. The occurrence of localized intramural conversion of AI to AII near arteriolar receptors in vivo may so minimize exposure of the liberated AII to circulating antibody as to render AII immunization an inefficient means of blocking endogenous pressor activity of the renin-angiotensin system.

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