ANGIOTENSIN-CONVERTING ENZYME: EFFECT OF ANTIENZYME ANTIBODY IN VIVO

Peter R. B. CALDWELL and H. Joachim WIGGER
Departments of Medicine and Pathology, College of Physicians and Surgeons, New York, N.Y. 10032, USA

and

Manjusri DAS and Richard L. SOFFER
Department of Molecular Biology, Division of Biological Sciences, Albert Einstein College of Medicine, Bronx, New York 10461, USA

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1. Introduction

Angiotensin-converting enzyme (EC 3.4.15.1) is a mammalian COOH-terminal dipeptidyl peptidase which catalyzes the release of His–Leu from angiotensin I to yield angiotensin II [1], the vasoactive agent of the renin–angiotensin system [2]. The same enzyme inactivates bradykinin, a vasodepressor nonapeptide, by cleavage of the COOH-terminal and adjacent dipeptides [3,4]. We have recently shown, using specific fluorescein-labeled antibody, that converting enzyme is present in the luminal cells of the vasculature of every organ examined, including lung, liver, kidney, pancreas, adrenal and spleen [5]. While extrapulmonary conversion [6–9] and converting activity [10,11] are well documented, the lung is thought to play an important role in both conversion of angiotensin I to II [12,13] and the degradation of bradykinin [14] in the circulation. This is because the lung is perfused by the entire circulating blood volume and has a very large capillary surface area. Further, enzymatically generated angiotensin II is not taken up by the lung [13,15] but delivered to the systemic circulation where it exerts its pressor effect. Recent immunohistochemical evidence has indicated that pulmonary converting enzyme may be localized on the luminal surface of the endothelial cells [16]. The possibility that converting enzyme is accessible to circulating antibodies prompted us to examine the effects in vivo of antibody with anticatalytic activity.

We found that goat antibodies specific for rabbit pulmonary converting enzyme produce a lethal reaction when administered intravenously in the rabbit. At lower dosage permitting limited survival, there is inhibition of the vasopressor response to angiotensin I and potentiation of the vasodepressor effect of bradykinin.

2. Materials and methods

Hippurylhistidylleucine (Hip–His–Leu) was from Research Plus and (1-Asp, 5-Ile) angiotensins I and II were from Schwarz/Mann. Bradykinin triacetate was a Sigma product. Pure solubilized rabbit lung converting enzyme was obtained as described [17]. Antibody was raised and maintained in goats by monthly intradermal injections of 150 μg of enzyme in 1 ml of 0.15 M NaCl emulsified with an equal volume of complete Freund's adjuvant. Anticatalytic activity was determined by preincubating antibody for 30 min at 37°C with pure enzyme in 10 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl and assaying residual enzyme activity with Hip–His–Leu as substrate [18]. Sera were heated at 56°C for 30 min and centrifuged for 60 min at 105 000 g. Globulins were obtained by addition of solid $(NH_4)_2SO_4$ to 50% saturation. The precipitate was dissolved and dialyzed extensively against Tris-NaCl buffer. Protein concentrations in serum and globulin fractions were estimated using $A_{280}^{1\%}$ values of 9.0 and 14.1, respectively.

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New Zealand white rabbits were prepared in a preliminary operation by insertion of indwelling Long-dwell 20 gauge 8 inch catheters (Becton Dickinson) into the right atrium for intravenous administration and aortic arch for blood pressure determinations. Catheters were exteriorized for later use in the awake, unanaesthetized animal and were occluded with a Teflon monofilament obturator. Prior to administration of vasoactive peptides, experimental animals received intravenous benadryl (1 mg/kg). Dose response curves were then performed with the vasoactive agents before and after a 15 min infusion of preimmune or immune goat globulin fractions. Systemic arterial blood pressure was continuously monitored using a Stathem transducer Model #P23Db and an Electronics for Medicine recorder. Mean arterial pressure was determined by graphic integration of the pressure tracing for several respiratory cycles during the control period and at the peak of response to the vasoactive agent.

3. Results

Immune sera and globulin fractions showed a single precipitin band after immunodiffusion against the pure enzyme and exhibited anticatalytic activity in vitro. Initially a dose of antibody was employed which contained approx. twice the anticatalytic activity required to inhibit the total lung content (1.5 mg) of pure enzyme by 50% in vitro. Recipient rabbits given this dose invariably died of acute pulmonary edema, accompanied by hypotension, within 30 min after receiving antibody. Equivalent amounts of protein from preimmune sera, which exhibited no anticatalytic activity in vitro, were well tolerated and did not produce any change in the dose response of angiotensin I. To determine whether immune fractions demonstrated anticatalytic effects in vivo it was necessary to use the maximum amount of antibody which did not cause immediate death. The recipient rabbits given this lesser dose all died within 3–6 days and there was a progressive fall in their mean arterial pressure of about 15 mm Hg during this period. Autopsy in these animals revealed pulmonary congestion and hemorrhage. Within 30 minutes after administration of antibody the pressor effect of angiotensin I was decreased and the depressor action of bradykinin was potentiated as compared to the responses prior to antibody infusion. Typical results are shown in figs.1 and 2. Further, the pressor response to angiotensin II was not immediately altered. The

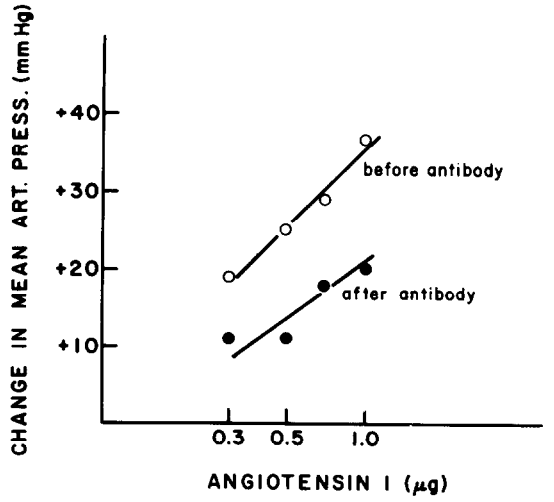

Fig.1. Effect of antibody on vasopressor response to angiotensin I. Dose responses were determined before and 30 min after infusion of 0.5 ml of immune globulin. The administered ammonium sulfate fraction contained 24 mg of protein and sufficient anticatalytic activity to inhibit the action of 1.4 mg of pure enzyme by 50% in vitro.

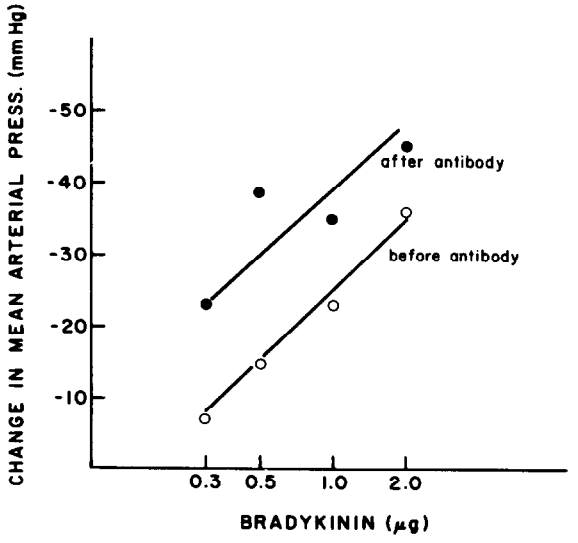

Fig.2. Effect of antibody on the vasodepressor response to bradykinin. The experiment was carried out as described for fig.1, except that the infused immune globulin solution contained 28 mg of protein and anticatalytic activity sufficient to inhibit 0.4 mg of pure enzyme by 50% in vitro.

decreased pressor response to angiotensin I persisted for as long as four days in some animals, although by this time the vasopressor effect of angiotensin II was also diminished to a lesser extent, perhaps because of the debilitated state of these animals.

4. Discussion

Angiotensin-converting enzyme is a surface glycoprotein which is thought to be directly accessible to the circulating blood and which can be solubilized and isolated as a pure, highly antigenic molecule. These characteristics render it unique as a model for studying specific surface immunologic phenomena in vivo and for exploring the possibility that enzyme activity can be regulated by exogenous antibody. The most striking result which we have observed is the immediate immune-specific lethality of antibody directed against the enzyme. The pulmonary injury is almost certainly not due to anticatalytic activity per se, since venom peptide inhibitors of the enzyme are remarkably nontoxic [19,20]. The injury may be the result of an immune reaction on the capillary endothelial cell surface, perhaps associated with the release of anaphylactic mediators. The predominance of lung pathology could be explained by the fact that the capillary bed of this organ is the first exposed to antibody after intravenous administration.

Those animals which temporarily survived the lower dose of antibody showed a diminished vasopressor response to angiotensin as compared with angiotensin II, whereas the vasodepressor response to bradykinin was potentiated. These results suggest that inhibition of enzyme activity by specific antibody may have occurred in vivo. The duration of antibody effect was considerably longer than that reported for peptide inhibitors which have been shown to prevent initiation of experimental, renin-dependent hypertension [19,20]. Clearly, however, dissociation of the lethal and anticatalytic actions of the antibody preparations will be required for the development of a useful regulatory immunologic reagent.

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