The Interaction of Angiotensin with a Binding Factor in Plasma

The presence of a non-dialyzable pressor substance has been reported in renal vein blood from renal hypertensive patients[1, 2]. Also, a non-filterable pressor substance has been found in rat plasma incubated with rat renin[3]. That these pressor substances are non-dialyzable or non-filterable indicates that they are either of a larger molecular species than angiotensin, or are angiotensin bound to some plasma protein.

In the development of a method for the immunoassay of angiotensin, we found that normal plasma showed some evidence of angiotensin binding. Similar results have been reported by POULSEN[4].

On that basis, the binding of angiotensin by plasma proteins using the method of equilibrium dialysis was studied. A dialysis system was employed in which 1 ml of various test solutions within cellulose tubing were suspended in 20 ml of different types of external solutions usually containing 400–600 ng asp^1-val^5 angiotensin II-amide (Hypertensin-CIBA). The entire system was agitated by means of a magnetic stirrer or by shaking for 18 to 24 h, usually at 4°C. Following dialysis, both the test solution inside the cellulose bag and the external solution were assayed in the rat[5]. To inhibit angiotensinases, all plasma and protein solutions were treated according to the method of PICKENS et al.[6] and the pH adjusted to 6 prior to use. When the angiotensin concentration of the test solution inside the cellulose bag was greater than the angiotensin concentration of the external solution following dialysis, it was interpreted as binding of angiotensin by the test solution. Binding capacity, expressed as a whole number, was the result of the difference between the angiotensin concentration of 1 ml of the test solution minus the concentration of 1 ml of the external solution.

When plasma obtained from normal and nephrectomized dogs, normal and nephrectomized rats, and normal or nephrectomized humans was dialyzed against an external solution of distilled water containing angiotensin, appreciable binding of angiotensin by the test solution was consistently observed (Figure 1). The normal rat plasma had the highest binding capacity, while normal human plasma had the lowest. There was no binding of angiotensin in control systems in which rabbit albumin, Dextran, water, or 0.2 *M* phosphate buffer pH 7.5 were used as test solutions within the cellulose tubing. To insure that the large concentration of angiotensin in the test plasmas was not the result of angiotensin generated during dialysis, controls were run in which each plasma was dialyzed against distilled water with no added angiotensin. Under these conditions, no angiotensin was found in either the test solution or the external solution.

No binding effect was evident when physiological conditions were approximated by dialyzing each of the 3 species of plasma against Ringer solution containing angiotensin at 37°C, or when saline, citrate-phosphate buffer, or *tris* buffer were employed as external solutions, with dialysis at 4°C. Consequently, to study the effect of pH on the binding capacity of plasma, distilled water was used as the external solution. The pH of both the plasma and the distilled water were adjusted prior to dialysis and measured afterwards. Optimum binding capacity was found to be between pH 5.5 and 6.0 (Figure 2).

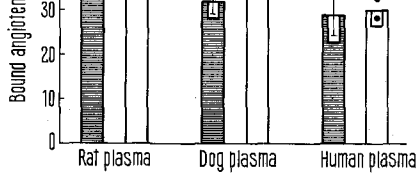

Fig. 1. Angiotensin II binding capacity of plasma from normal and nephrectomized rats, dogs, and humans, with distilled water as the external solution in the dialysis system. Each column represents a mean of 10 determinations except the last, which is the mean of 2 determinations. Vertical lines represent SEM values.

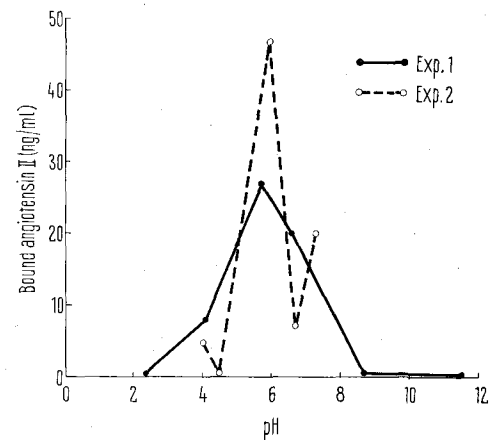

Fig. 2. Effect of pH on angiotensin binding capacity of nephrectomized dog plasma. The results of 2 experiments are shown here.

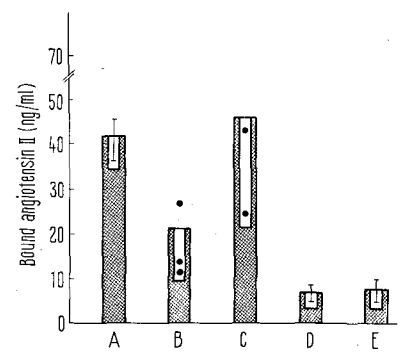

Fig. 3. Angiotensin II binding capacity of plasma from nephrectomized dog in: A, the native state; B, after denaturation by boiling, pH not adjusted; C, boiling and digestion with trypsin, pH not adjusted; D adjusting pH to 4.9 and boiling; E, adjusting pH to 4.9, boiling, and digestion with trypsin. Vertical lines represent SEM values. Dots represent individual experiments.

[1] O. M. HELMER and W. E. JUDSON, *Hypertension* (A.H.A. Publication 1960), vol. 8, p. 38.
[2] A. GROLLMAN, Clin. Pharmac. Ther. *10*, 755 (1969).
[3] D. B. GORDON and C. E. LEE, Physiologist *13*, 209 (1970).
[4] K. POULSEN, Scand. J. clin. Lab. Invest. *24*, 285 (1969).
[5] O. A. CARRETERO and J. A. HOULE, Am. J. Physiol. *218*, 689 (1970).
[6] P. T. PICKENS, F. M. BUMPUS, A. M. LLOYD, R. R. SMEBY and I. H. PAGE, Circulation Res. *17*, 438 (1965).

Boiling of nephrectomized dog plasma at pH 4.9 for 10 min prior to dialysis decreased the binding capacity by 83% (Figure 3). There was no change in the binding capacity of nephrectomized rat plasma following dialysis at 5° to 50°C.

Seven commercially available Cohn fractions of normal human plasma showed some capacity to bind angiotensin (Figure 4). Since each fraction was tested at a concentration equal to the total protein concentration of the dialyzed plasma of nephrectomized dogs (40 mg per ml), the binding capacity of these fractions, excepting fraction IV-4 (α-β-globulin) and fraction V (albumin), can be considered insignificant when compared to the binding capacity of whole plasma. These 7 Cohn fractions were also studied in a concentration of 28 mg/ml and incubated with 2.5 pg angiotensin I labeled with I^{125} for 2 h, at 37°C, in the presence of *tris* buffer with lysozyme (100 mg/100 ml. Free angiotensin was then separated from the bound by charcoal absorption. Three of the fractions showed capacity to bind angiotensin: human fraction IV-1 (α-globulin) bound 46% of the radioactive material, human fraction III (β-globulin) bound 30% and, human fraction III-0 (β-lipoprotein) bound 28%. Similar results were obtained when radioactive angiotensin II was used. The difference in the results obtained by these 2 methods is confusing, but may be due to the difference in the molecular conformation between angiotensin labeled with I^{125} and unlabeled angiotensin. Furthermore, we were unable to displace the radioactive angiotensin I or II bound to the α-globulin proteins by adding unlabeled angiotensin, even using amounts as high as 1 ng.

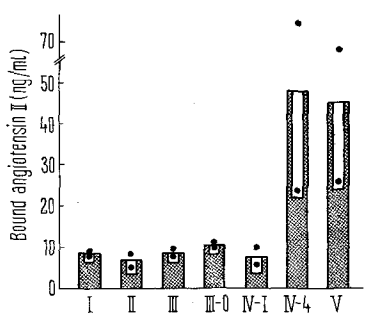

Fig. 4. Angiotensin II binding capacity of Cohn's fractions of human plasma. The protein composition of each fraction is: I, fibrinogen; II, γ-globulin; III, β-globulin; III-0, β-lipoprotein; IV-1, α-globulin; IV-4, α-and β-globulin; V, albumin.

The amount of angiotensin used in the equilibrium dialysis study was far above the physiological level. Concentrations of 20 to 100 pg of angiotensin per ml of plasma have been reported for normal human plasma[7-9]. The amount of binding of angiotensin which we are observing, 30 to 50 ng/ml, is incredibly great when compared to these normal circulating levels. Although binding of radioactive angiotensin by the human α-globulin fraction was observable in an ionic environment, the fact that the bound angiotensin could not be displaced indicates that the binding is not specific. These results, together with the observation that with equilibrium dialysis no measurable binding by the assay used was detectable in the presence of saline or buffer, precludes the postulation of any definite physiological significance for this binding. However, since dialysis against distilled water is commonly used for the elimination of angiotensin from plasma, our observations may have some technical significance. The non-dialyzability of the pressor substance found in renal vein blood plasma by HELMER et al.[1] and GROLLMAN[2] and which seems to be pharmacologically similar to angiotensin, may be due to this binding phenomenon. This possibility has been suggested by Schweikert et al.[10]. Since boiling at pH 4.9 destroys most of the binding activity of plasma, and pH has an influence on the degree of binding, leads us to think that plasma proteins are the factors that bind angiotensin.

Résumé. La liaison de l'angiotensine par les protéines plasmatiques est démontrée par dialyse équilibrée. L'angiotensine radioactive liée ne put être déplacée par l'angiotensine non radioactive, ce qui diminue la signification physiologique de cette liaison.

J. A. HOULE, ALEXANDRA PIWONSKA and O. A. CARRETERO[11]

Department of Medicine, Henry Ford Hospital, 2799 West Grand Blvd., Detroit (Michigan 48202, USA), 7 June 1971.

[7] R. BOUCHER, R. VEYRAT, J. DECHAMPLAIN and J. GENEST: Can. med. Ass. J. *90*, 194 (1964).
[8] P. J. MULROW, Can. med. Ass. J. *90*, 277 (1964).
[9] M. B. VALLOTTON, L. B. PAGE and E. HABER, Nature, Lond. *215*, 714 (1967).
[10] J. R. SCHWEIKERT, R. M. CAREY and G. W. LIDDLE, Clin. Res. *19*, 32 (1971).
[11] Supported in part by the Michigan Heart Association.

Spontaneous Transmitter Release from Nerve Endings and Contractile Properties in the Soleus and Diaphragm Muscles of Senile Rats

Senile atrophy proceeds at a different rate in different muscles[1,2]. This might be due to secondary changes in the skeletal muscle related to associated disturbances, especially joint diseases, differences in androgen sensitivity as indicated by the marked atrophy of the levator ani muscle after castration[3], or to differences in degree of motor activity. In the senile levator ani and extensor digitorum muscles of the rat, both fast muscles, a considerable reduction of spontaneous transmitter release and prolongation of contraction time[4,5], apparently related to decrease of myosin ATPase activity[6], has been observed. Marked reduction of motor activity is also very conspicuous in man, as demonstrated by decreased frequency discharge in motor units[7] and the obvious decrease in force of muscle contraction in the elderly age group[8].

The diaphragm muscle is forced to continuous regular neuromuscular function connected with the respiratory act, and it appeared of interest to compare the changes in spontaneous transmitter release and contractile behaviour in the diaphragm and the soleus muscle of old rats.

Materials and methods. For recordings of the frequency of miniature endplate potentials (MEPP), muscles of young and old rats were dissected and placed in a flat chamber, submersed in LILEY's solution[9]. 3–4 animals were used in each series and MEPP from 32 and 51 muscle cells from the diaphragm and 30 and 51 muscle cells from