A Pressor Agent in Human Stable Plasma Protein Solutions

ABSTRACT
A pressor material in human stable plasma protein solutions is described, and evidence that this material may be angiotensin II is presented. When six samples of stable plasma protein solution were assayed against standard angiotensin in the sensitive, ganglioplegic, nephrectomized rat anesthetized with urethane, the levels of pressor activity ranged from 320 to 830 ng/100 ml, with a mean of 552 ng/100 ml. The solution gives a pressor response similar to that of angiotensin II, is heat stable, dialyzable, and destroyed by trypsin and α-chymotrypsin, and reacts with the antibody in a radio-immunoassay for angiotensin II. It is present in sufficient concentration to have significant effects on blood pressure, renal handling of sodium, and aldosterone secretion when infused into patients at rates commonly employed.

ADDITIONAL KEY WORDS human angiotensin II bioassay rat blood pressure immunoassay renin angiotensinase

In a modification (1) of the Boucher procedure (2, 3) for assay of plasma renin activity, bovine albumin solution is used as a carrier for angiotensin. This solution can be rapidly injected intravenously into sensitive rats in volumes up to 0.08 ml without effect on the blood pressure (1). Since bovine albumin is expensive, the possibility of using readily available human stable plasma protein solutions was investigated. This solution is prepared from outdated human blood, collected from donors for transfusion, by cold ethanol fractionation of the plasma and prolonged heat treatment to inactivate serum hepatitis virus (4).

The first step was to determine the effect of injecting stable plasma protein solution intravenously on the rat's blood pressure. In contrast to untreated human plasma, which does not raise the blood pressure, pressor activity was found in all six samples tested.

Further studies indicated that the pressor agent in stable plasma protein solution is angiotensin and that it is present in sufficient concentration to raise the blood pressure in a human subject during rapid infusion.

Methods and Results
Bioassay of Pressor Activity
Nephrectomized, ganglioplegic rats were used for the assay of pressor activity (1). Each unknown solution was assayed at least twice in each of two rats, and the results were expressed as mean values. The levels of pressor activity in six different samples of stable plasma protein solution (Commonwealth Serum Laboratories, Parkville, Vic.) are given in Table 1.

Radio-immunoassay of Pressor Activity
The ability of the pressor agent to combine with a specific antibody in a recently described radio-immunoassay for human angiotensin II (5) was tested.

Angiotensinase Activity, Renin Substrate Level, and Renin Concentration in Stable Plasma Protein Solution
To detect the presence of angiotensinases, 30 ng of standard angiotensin II (Hypertensin, Ciba) were added to two 1.7-ml aliquots of stable plasma protein solution and two 1.7-ml aliquots of normal plasma and incubated at pH 6.5 for 3 hours at 37°C. (The normal plasma was obtained from blood...
TABLE 1
Range of Pressor Activity in Stable Plasma Protein Solution

<table>
<thead>
<tr>
<th>Batch no.</th>
<th>Pressor activity (ng/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>690</td>
</tr>
<tr>
<td>2</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>830</td>
</tr>
<tr>
<td>4</td>
<td>540</td>
</tr>
<tr>
<td>5</td>
<td>430</td>
</tr>
<tr>
<td>6</td>
<td>590</td>
</tr>
<tr>
<td>Mean</td>
<td>552</td>
</tr>
</tbody>
</table>

collected from three normal subjects on liberal salt diets, using 0.1% ammonium EDTA as an anticoagulant. The plasma was quickly separated at 4°C, pooled, and stored at −10°C.) Angiotensin levels were measured after 10, 60, 120, and 180 minutes of incubation and compared in the two incubates. Whereas less than 30% of added angiotensin standard remained in normal plasma after 10 minutes at 37°C, and less than 15% after 3 hours, the survival of angiotensin added to stable plasma protein solution was 100% at all times of sampling.

The effect of prolonged incubation for 100 hours on the pressor activity of stable plasma protein solution was determined, seeking evidence of further generation of destruction of pressor material.

Incubation of batches 3 and 4 at 37°C for 100 hours did not significantly alter the level of pressor activity. Thus no evidence was found for the presence of any angiotensinase activity in stable plasma protein solution.

Renin substrate levels in five samples and renin concentration in three samples of stable plasma protein solution were determined by the method of Skinner (6) with the exception that the bioassay was performed as outlined above.

The renin substrate levels ranged from 75 to 200 ng/ml with a mean value of 103 ng/ml, compared with values for three plasma samples from normal males of 955, 1085, and 1055 ng/ml. No measurable renin concentration was detected in the three batches of stable plasma protein solution tested.

1. The characteristics of the blood pressure response curve of the rat following administration of stable plasma protein solution were examined. The similarity between the pressor responses to this solution and to standard angiotensin II is shown in Figure 1.

2. The pressor activity of stable plasma protein solution resembled that of standard angiotensin in that it was not destroyed by boiling.

3. Dialysis of stable plasma protein solution against 23 volumes of distilled water for 60 hours at 4°C resulted in complete loss of pressor activity, but this activity could be recovered in the dialysate which was freeze-
dried and reconstituted in 2.5% bovine albumin solution. When this solution was assayed in the rat bioassay system, a value of 800 ng/100 ml was obtained; in the radio-immunoassay system of Catt et al. (5), a result of 730 ng/100 ml was obtained.

4. Incubation of stable plasma protein solution with either α-chymotrypsin (Armour Pharmaceutical Co.) or trypsin (Trypure Novo, Nova industri A/S, Copenhagen) for 3 hours at pH 7.5 and 37°C resulted in complete loss of pressor activity.

Stable plasma protein solution thus differs from untreated human plasma in certain important respects. It has pressor activity in the rat, lacks renin and angiotensinase activity, and contains substrate in much smaller amounts than does normal plasma. The pressor agent which it contains shares several properties with angiotensin II.

Discussion

Attention is drawn to the presence of a pressor agent in stable human plasma protein solution, and several lines of evidence are presented which strongly suggest that it is angiotensin II. In the rat it produces a similar pressor response to standard angiotensin II, its stability on boiling is similar, and it is dialyzable. Like angiotensin II the pressor activity is destroyed by incubation with α-chymotrypsin and trypsin, and its ability to bind specific antibody in the radio-immunoassay for angiotensin II confirms its close similarity to, if not identity with, angiotensin II.

Standard angiotensin II is rapidly destroyed in normal plasma but retains full activity when added to stable plasma protein solution. This suggests that stable plasma protein solution is free from angiotensinase activity, and this is confirmed by survival of the pressor activity in the solution during incubation at 37°C for as long as 100 hours. Providing angiotensinases are inhibited, prolonged incubation of plasma will generate angiotensin in a linear fashion (6). Prolonged incubation of stable plasma protein solution failed to generate any further pressor activity, but this is not surprising in view of the undetectable renin concentration and low substrate levels.

Stable plasma protein solution is prepared from pooled human plasma collected in acid-citrate-dextrose anti-coagulant (4), and ethanol fractionation at low temperatures is carried out to remove unstable proteins. The final fraction is reconstituted and sterilized by Sertiz filtration followed by heating at 60°C and pH 7.5 for 10 hours to inactivate serum hepatitis virus. This last step would be expected to destroy renin activity since renin is unstable at temperatures above 56°C (7). Nevertheless it is conceivable that sufficient renin may remain early in the incubation to account for angiotensin formation. The complete freedom (in our assay systems) of stable plasma protein solution from angiotensinases and renin might make it a useful source of human renin substrate provided concentration were possible. Studies are in progress to further elucidate the mode of generation and survival of angiotensin in stable plasma protein solution.

Regarding the possible physiological significance of angiotensin in stable plasma protein solution, it is known that an infusion of 300 ng angiotensin II per minute into a normal individual on a liberal salt diet will raise diastolic blood pressure by 20 mm Hg (8). One hundred ml of stable plasma protein solution contained more than 300 ng of angiotensin in the six samples tested, and hence rapid infusion of this solution should lead to a rise of diastolic blood pressure by virtue of the angiotensin effect alone. The effect of angiotensin on blood pressure is known to be reduced in patients with high endogenous angiotensin levels. When stable plasma protein solution is given as a plasma expander, the endogenous angiotensin levels may be high and the direct pressor effect attenuated.

There are two other actions of angiotensin in man when infused in this range of concentration. Angiotensin has an effect on the kidney, causing sodium retention independent of any aldosterone effect (9). This is thought
to be due to a reduction in glomerular filtration. When infused into patients with essential hypertension (10) or high endogenous levels of angiotensin, for example cirrhotics with ascites (11), angiotensin has a natriuretic effect. Angiotensin also acts on the adrenal cortex, stimulating aldosterone secretion (12, 13). When infused at rates as low as 9 ng/hour into a normal subject on a liberal salt diet, a definite increase in aldosterone secretion occurred (12). Infusion at the rate of 100 ng/hour, a rate exceeded even during slow infusion of stable plasma protein solution, would be expected to result in a marked increase in aldosterone secretion.

These previously unsuspected properties of stable plasma protein solution are of physiological significance and should be considered when this material is used clinically.

Acknowledgments
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ROBERT VANDONGEN and RICHARD D. GORDON

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