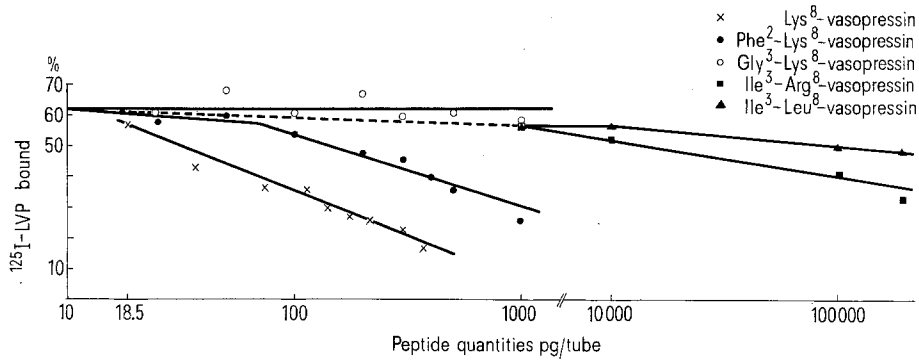

Fig. 3. Comparison of the competitive effect of LVP and other LVP analogues on the binding of ^{125}I-LVP to rabbit anti-LVP antiserum. Increasing amounts of different peptides are added to the incubation medium (0.5 ml) containing constant amount of antibody (1/3000 dilution) and ^{125}I-LVP (8000 to 10.000 cpm).

^{125}I-LVP peak contains monoiodinated LVP which has lost its antidiuretic activity. Similarly a diminution of biological activity of monoiodinated angiotensin is observed by LIN[13].

The substitution of an aliphatic amino-acid for a phenylalanine ring prevents this peptide from competing with ^{125}I-LVP for binding to antibody (Figure 3). This residue therefore seems essential to hormone antigenic activity. This finding agrees with VORHERR's[14] assumption. The hydroxyl group of tyrosine in position 2 looks important also since Phe^2-Lys^8-vasopressin is 5 to 7 times less inhibitory than LVP. When phenylalanine is present in position 3, this same anti-LVP serum has no specificity with regard to amino-acids in position 7 and 8 (Figure 4). However, inhibition observed with very high concentrations of analogues whose phenylalanine has been replaced (Figure 3) implies that other sites besides this last are immunoreactive, but their affinity for antibody is very weak. The difference in activity between Ile^3-Arg^8-vasopressin and Ile^3-Leu^8-vasopressin entitles one to suppose that residue 8 participates in the reaction.

Furthermore, there are no precise correlations between biological potency and antigenicity. Indeed, homoval$^{7/8}$-Lys-vasopressin and Arg^8-vasopressin have the same immunoreactivity but their biological activities are very different. The same is true for AVT which cross-reacts very poorly in the immunoassay but which has an antidiuretic effect comparable to that of LVP (Table II).

Résumé. Ces résultats suggèrent d'une part que ^{125}I-LVP monoiodée a peu d'activité antidiurétique, d'autre part que le noyau phénylalanine en position 3 est essentiel pour la liaison de l'antigène à l'anticorps et que les sites immunologiques et biologiques de l'hormone sont différents.

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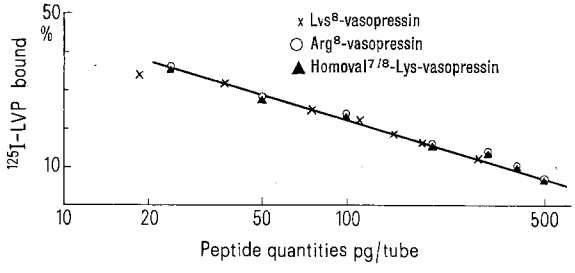

Fig. 4. Cross reacting of anti-LVP antiserum with Arg^8-vasopressin and Homoval$^{7/8}$-Lysine-vasopressin. Immunoassay is carried out by the addition of increments of synthetic peptide to a constant amount of antibody (1/3000 dilution) and ^{125}I-LVP (10.000 cpm) in a tota volume of 0.5 ml.

[16] Acknowledgments. We wish to thank Professor S. JARD (Collège de France, 11, place Marcellin Berthelot, Paris 5eme) for his valuable advice in connection with this work. LVP analogues were generously supplied by Dr. R. A. BOISSONNAS of Sandoz Pharmaceuticals.

Radioimmunoassay of Angiotensin II in Rat Plasma

Various experimental procedures for the radioimmunoassay of angiotensin II in human plasma have been described, but no information is available on plasma levels in the rat, the laboratory animal most widely used in studies on the pathophysiology of the renin-angiotensin system. In this report a sensitive and specific radioimmunological method is presented that is suitable for the determination of angiotensin II concentration in unextracted plasma.

Male white New Zealand rabbits were immunized with Asp^1-Ileu^5-angiotensin II (Schwarz/Mann) coupled by the carbodiimide method[1] to porcine γ-globulin. Iodination was performed according to GREENWOOD et al.[2]; the labelled material was purified on a DEAE Sephadex A 25 column[3].

The immunoassay tubes, containing antiserum in a 1:80,000 dilution, 4 pg of labelled angiotensin, and 0.05 ml

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of the plasma sample in a total volume of 0.45 ml *Tris*-HCl buffer, pH 7.5, were equilibrated for 40 h at 0–4 °C. Commercially available Asp[1]-Ileu[5]-angiotensin II served as standard and was adjusted to the International Research Standard A (Nr. 70/302, provided by the Medical Research Council, Mill Hill, London). Free and bound angiotensin were separated by means of 10% plasma-coated charcoal, the charcoal pellet being counted in a Packard well-type scintillation counter. A typical calibration curve is shown in Figure 1.

Angiotensin II concentration (pg/ml) in arterial plasma of rats

Group	n	mean ± S.D.	p
Controls	29	86.8 ± 24.8	< 0.001
DOCA/salt treated	9	29.2 ± 10.2	< 0.001
Low-sodium diet	10	1938.5 ± 714.2	

DOCA 12.5 mg/kg daily in microcrystalline suspension and 1% NaCl as drinking fluid for 10 days. Low-sodium diet containing 0.2 mEq Na^+/kg and demineralized water given for 3 weeks.

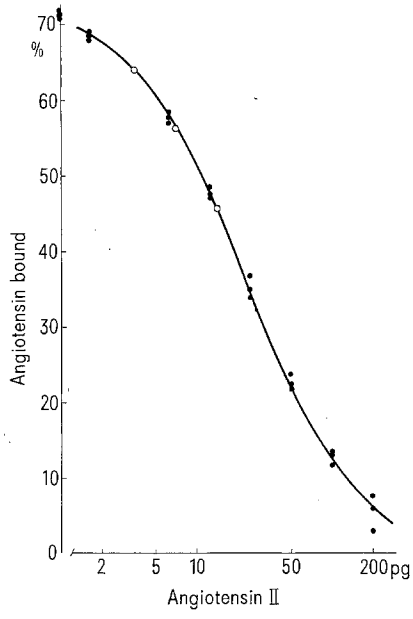

Fig. 1. Calibration curve with increasing amounts of angiotensin II (abscissa) and % angiotensin bound to antibody (ordinate); serial dilution of plasma extract (circles).

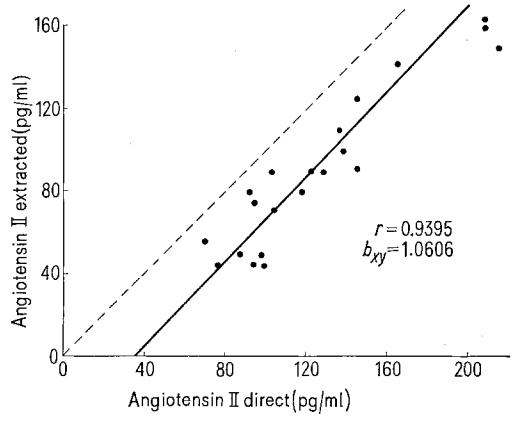

Fig. 2. Correlation between angiotensin II concentration assayed in extracted plasma samples (ordinate) and directly assayed samples (abscissa).

Male Sprague Dawley rats, weighing between 180 and 250 g, were kept on a standard diet (ssniff) and tap water. Blood samples were taken from the tail artery under light ether anaesthesia in 50µl 0.125 *M* Na_2EDTA and 0.025 *M* *o*-phenanthroline per ml blood[4].

The main objection against the use of unextracted plasma in the radioimmunoassay of angiotensin is the nonspecific interference of plasma proteins with the binding of angiotensin to its antibody, which may result in incorrect high values. If, for a significant range of the standard curve, this effect corresponded to a constant amount of angiotensin, it could be subtracted from the actual readings as a 'plasma blank'. That such an assumption is correct is evident from Figure 2. The 'apparent' angiotensin II concentration of plasma samples is plotted against the 'true' angiotensin II concentration obtained by extraction of an aliquot of the same plasma sample, to which a cation exchange resin was added[4]. All extraction figures are corrected for the recovery of unlabelled angiotensin (81.9 ± 10.7%, $n = 17$). The extraction blank (water) was 0. Serial dilutions of plasma extracts are on the calibration curve (circles in Figure 1). Figure 2 shows a good correlation between the values obtained by both methods. There is a parallel shift of the regression line to the right from the theoretical 1:1 ratio with an intercept at 36 pg on the abscissa. This value, which corresponds to 1.8 pg/0.05 ml, was subsequently subtracted as a blank in plasma samples that were directly assayed for angiotensin II.

By means of this method the normal range for angiotensin II in arterial rat plasma was found to be between 51.9 and 142.4 pg/ml. The 'within assay' reliability, expressed as the coefficient of variation of duplicates ranging throughout the standard curve, was 5.4%, the 'between assay' reproducibility was 12.9%. To assess the application of the method, two experimental conditions known to alter the activity of the renin-angiotensin system were studied (Table). Low-sodium diet[5] caused a marked elevation of angiotensin II levels (range 955–2947 pg/ml), whereas, after 10 days of DOCA and salt treatment, plasma angiotensin concentration was markedly reduced (9.6–39.9 pg/ml), with no overlap to the normal group[6].

Zusammenfassung. Eine radioimmunologische Methode zur direkten Bestimmung von Angiotensin II aus 50 µl arteriellem Rattenplasma wird beschrieben. Der Normalwert beträgt 86.8 pg/ml ± 24.8 S.D. ($n = 29$). Nach kochsalzarmer Ernährung nahm die Angiotensin-II-Konzentration im Plasma stark zu, während sie nach DOCA und Salzgabe deutlich abfiel ($p < 0.001$).

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[6] Supported by the Deutsche Forschungsgemeinschaft.

[7] Acknowledgment. We wish to thank Mr. H. DICK for his skilful technical assistance.