sin seems to indicate that it resembles much more the tropomyosin-troponin type of regulatory proteins found in skeletal muscle than the myosin-linked type present in the muscles of molluscs.

Zusammenfassung. Die Mg-ATPase des kontraktilen Systems von Blutplättchen, Thrombosthenin, wird durch Spuren von Calciumionen reguliert. Eine Regulationseiweisse enthaltende Fraktion kann von Thrombosthenin abgetrennt werden. Zugabe dieser Eiweissfraktion zu sogenanntem «desensibilisierten» Thrombosthenin aber auch zu «desensibilisiertem» Actomyosin aus Skelettmuskel vermag die Mg-ATPase beider Systeme wieder von Spuren von Calciumionen abhängig zu machen. Die Regulationseiweisse, d.h. der Tropomyosin-Troponin-Komplex aus Skelettmuskeln, vermögen ebenfalls «desensibilisiertes» Thrombosthenin wieder Calcium-abhängig zu machen.

S. Thorens, M. C. Schaub and E. F. Lüscher

Theodor Kocher Institut, Freiestrasse 1, CH–3012 Bern (Switzerland), and
Pharmakologisches Institut der Universität Zürich, Gloriastrasse 32, CH–8006 Zürich (Switzerland),
13 October 1972.

[15] R. S. Adelstein, J. E. Godfrey and W. W. Kielley, Biochem. biophys. Res. Commun. *12*, 34 (1963).

Immunoassay for Lysine^8-Vasopressin (LVP): Comparison of Biological and Immunological Activity of Lysine-Vasopressin and Some of its Synthetic Analogues

In radioimmunossay of vasopressin already reported (Klein[1]; Permutt[2]; Miller[3]; Edwards et al.[4]; Robertson[5]; Johnston[6]) no details about the biological activity of radioiodinated molecule and the role of the various amino-acid residues in the immunological reactivity are given. In this report, the antidiuretic activity of ^{125}I-LVP is measured and the cross reactivity of vasopressin analogues with a LVP antiserum studied with a view to identification of the antigenic sites of antidiuretic hormone and to comparing the structural requirements for its immunological and biological activity.

Materials and methods. Synthetic LVP (Sandoz) is conjugated to rabbit serum albumin by the method of Goodfriend et al.[7]. 0.5 ml of conjugate containing 100 U LVP is emulsified with an equal volume of Freund's adjuvant. About 0.4 ml is injected directly into rabbit spleen and the other part into the toe pads. Subsequent weekly i.m. and i.p. injections are administered, as well as toe pad injections (on the whole 100 U). 10 days after the 6th immunization, the serum is screened. Injections of similar materials are carried out at 3 week intervals.

Synthetic LVP (5 to 8 μg) is labelled according to Hunter and Greenwood[8] using 2 mC ^{125}I Na specific radioactivity higher than 14 mC/μg (Radiochemical Center, Amersham). ^{125}I-LVP is separated from free ^{125}I by passage through a 1 × 10 cm column (I) of DEAE cellulose developed with 0.02 *M* ammonium acetate buffer pH = 5.4. For a further purification, ^{125}I-LVP from column I (1 ml) is applied to a G15 Sephadex column

[1] L. A. Klein, J. Roth and M. J. Petersen, Surg. Forum *17*, 240 (1966).
[2] M. A. Permutt, C. W. Parker and R. D. Utiger, Endocrinology *78*, 809 (1966).
[3] A. Miller and A. M. Moses, Endocrinology *84*, 557 (1969).
[4] C. R. W. Edwards, T. Chard, M. J. Kitau and M. L. Forsling, J. Endocr. *48*, 11 (1970).
[5] G. L. Robertson, L. A. Klein and J. Roth, Proc. natn. Acad. Sci., USA *66*, 1298 (1970).
[6] C. I. Johnston, J. Endocr. *52*, 69 (1972).
[7] T. Goodfriend and L. Levine, Science *144*, 1344 (1964).
[8] W. M. Hunter and F. C. Greenwood, Nature, Lond *194*, 495 (1962).

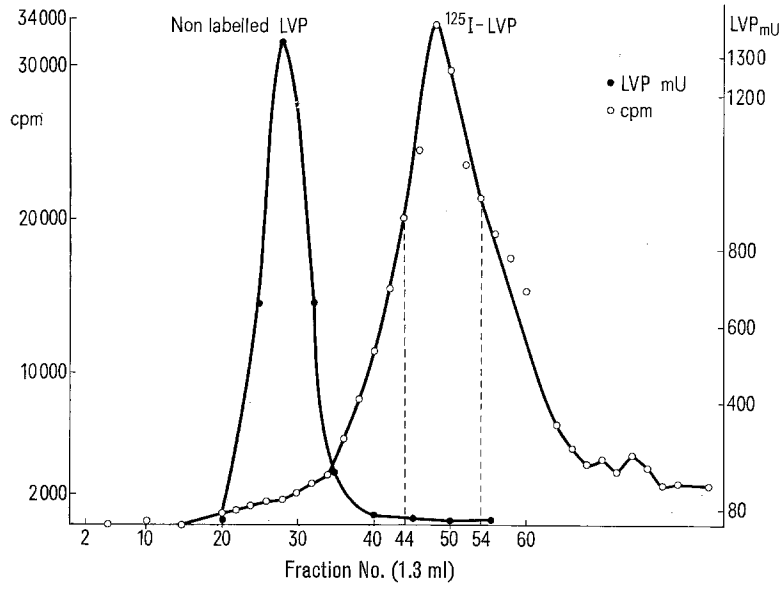

Fig. 1. Comparison between gel filtration of labellen ^{125}I-LVP (○—○) and antidiuretic activity of non labelled LVP (●—●) on a G15 Sephadex columd (30 × 0.9 cm).

Table I. Biological activity, immunoreactivity, specific radioactivity and iodination level of ^{125}I-LVP after different stages of purification

^{125}I-LVP after different purifications	LVP evaluated by bioassay	LVP evaluated by radioimmunoassay	Specific radioactivity	Level of iodination
After DEAE cellulose	90 mU/ml	92 mU/ml	470 μC/n*M*	0.23
After G15 Sephadex	0.63 mU	7.48 mU	1580 μC/n*M*	0.79
(17 ml of peak are together)	1.12 mU (in the peak)	7.75 mU (in the peak)	1660 μC/n*M*	0.83
After G15 Sephadex (13 ml of peak are together)	0.73 mU (in the peak)	5.46 mU (in the peak)	1800 μC/n*M*	0.90

In each experiment, 1 ml of ^{125}I-LVP from the DEAE cellulose column is chromatographed on G15 Sephadex gel.

(0.9 × 30 cm) and is eluated with 0.02 *M* ammonium formiate buffer pH = 4.5. All these operations are carried out at 4 °C. For the radioimmunoassay, 50 μl ^{125}I labelled LVP (10,000 cpm) and 50 μl of the antibody at the final concentration 1/3000 are incubated at 4 °C for 18 to 24 h with 100 μl of standard LVP dilutions (10 pg to 400 pg) or dilutions of other vasopressin analogues (25 pg 1 μg) in buffer (0.01 *M* phosphate- 0.15 *M* NaCl- 0.3% lysozyme) to give a total volume of 500 μl. 0.1 ml of a suspension of 10% charcoal and 1% dextran in equal parts is added to each reaction mixture tube, in order to separate bound from free LVP. 0.3 ml of the supernatants

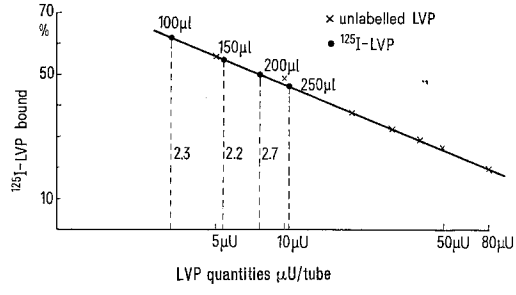

Fig. 2. Comparison of the antigenic reactivity of ^{125}I-LVP with standard unlabelled LVP. Each increment of labelled LVP to the system reduces the percentage of ^{125}I-LVP bound to antibody. The experimental points fall on the standard curve. This indicates that the ^{125}I-LVP reacts with antibody in the same way as synthetic Lys^8-vasopressin.

are counted. When control tubes without antibody are set up, the radioactivity measured is less than 3% of the total counts. The lowest dose detected is 2 μU/tube.

Specific activity is measured by the method of GOCKE et al.[9]. The method of JEFFERS[10] modified by GHARIB[11] is used for estimation of antidiuretic activity.

Results and discussion. Figure 1 shows that it is possible to separate non-iodinated LVP from ^{125}I-LVP through a G15 Sephadex column. Measurements of specific activity show that, after passage on DEAE cellulose or further purification by filtration on G15 Sephadex, ^{125}I-LVP reacts with the antibody in a manner indistinguishable from that of unlabelled LVP (Figure 2). It is thus possible to determine the concentration of ^{125}I-LVP and consequently its specific radioactivity (Table I). After gel filtration, the iodination level is 0.79 to 0.90 moles of ^{125}iodine per mole of LVP. The antidiuretic activity evaluated in various fractions after the passage of 10 IU LVP denotes that only 2% chromatographed LVP are found in 44–54 fractions corresponding to maximum ^{125}I-LVP peak (13 ml). This fact agrees with the observation of LEGROS et al.[12]. These results suggest that the

[9] D. J. COCKE, J. GERTEN, L. M. SHERWOOD and J. H. LARAGH, Circulation Res. *14*, 131 (1969).

[10] W. A. JEFFERS, M. M. LIVEZEY and J. H. AUSTIN, Proc. Soc. exp. Biol. Med. *50*, 184 (1942).

[11] G. GHARIB, Revue fr. Etud, clin. biol. *12*, 398 (1967).

[12] J. J. LEGROS and P. FRANCHIMONT, in *Radioimmunoassay Methods* (Eds. K. E. KIRKHAM and W. H. HUNTER, Churchill Publishers, Livingstone 1971), p. 40.

Table II. Cross reactivity studies using peptides containing one or two amino-acids different in composition from LVP

Analogue (18 pg to 1000 pg)	1	2	3	4	5	6	7	8	9		Cross reactivity	Antidiuretic activity (%)
Lys^8-vasopressin	Cys	Tyr	Phe	Glu	Asn	Cys	Pro	Lys	Gly-NH$_2$		100%	100[a]
Phe^2-Lys^8-vasopressin		Phe									15% to 20%	8[a]
Gly^3-Lys^8-vasopressin			Gly								0	~ 41
Ile^3-Lys^8-vasopressin			Ile								0	9,6[a]
Homoval$^{7/8}$-Lys-vasopressin							Val	Val	Lys	Gly-NH$_2$	100%	~ 1,5
Arg^8-vasopressin								Arg			100%	167[a]
Ile^3-Arg^8-vasopressin			Ile					Arg			0	100[a]
Ile^3-Leu^8-vasopressin			Ile					Leu			0	1,9[a]
Leu^3-Leu^8-vasopressin			Leu					Leu			0	~ 0,4[a]

All data are calculated on the basis of 100% activity for Lys-vasopressin. [a] Data for biological activity (rat antidiuresis) of these analogues are taken from BERDE and BOISSONNAS[15].

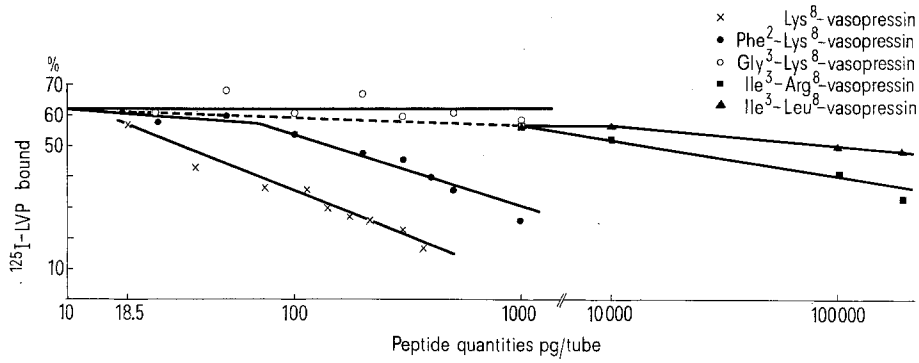

Fig. 3. Comparison of the competitive effect of LVP and other LVP analogues on the binding of ^{125}I-LVP to rabbit anti-LVP antiserum. Increasing amounts of different peptides are added to the incubation medium (0.5 ml) containing constant amount of antibody (1/3000 dilution) and ^{125}I-LVP (8000 to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

J. MARCHETTI[16]

Laboratoire de Physiologie,
U.E.R. de Sciences Médicales B de l'Université,
30, rue Lionnois, F–54000 Nancy (France),
4 August 1972.

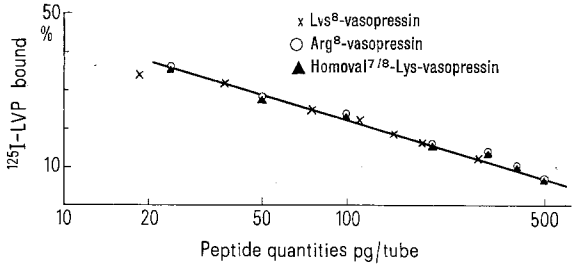

Fig. 4. Cross reacting of anti-LVP antiserum with Arg^8-vasopressin and Homoval$^{7/8}$-Lysine-vasopressin. Immunoassay is carried out by the addition of increments of synthetic peptide to a constant amount of antibody (1/3000 dilution) and ^{125}I-LVP (10.000 cpm) in a tota volume of 0.5 ml.

[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

[1] T. GOODFRIEND and L. LEVINE, Science *144*, 1344 (1964).
[2] F. C. GREENWOOD, W. M. HUNTER and J. S. GLOVER, Biochem. J. *89*, 114 (1963).
[3] G. DÜSTERDIECK and G. MCELWEE, in *Radioimmunoassay Methods* (Eds. K. E. KIRKHAM and W. M. HUNTER; Churchill, Livingstone, Edinburgh 1971), p. 24.

[13] S. Y. LIN and H. ELLIS, Biochem. Pharmac. *19*, 651 (1970).
[14] H. VORHERR and R. A. MUNSICK, J. clin. Invest. *49*, 828 (1970).
[15] B. BERDE and R. A. BOISSONNAS, in *Handbook of Experimental Pharmacology* (Ed. B. BERDE, Springer Verlag, Berlin 1968), vol. 23, p. 802.