Lysine-vasotocin, a Synthetic Analogue of the Posterior Pituitary Hormones Containing the Ring of Oxytocin and the Side Chain of Lysine-vasopressin*

RAYMOND D. KIMBROUGH, JR. AND VINCENT DU VIGNEAUD

From the Department of Biochemistry, Cornell University Medical College, New York, New York

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The synthesis of oxytocin (1), arginine-vasopressin (2), and lysine-vasopressin (3-5) has opened the way for the synthesis of analogues of the natural hormones, thus affording an opportunity to study the relationship between structure and biological activity in these hormones. One of the most interesting analogues thus far prepared is arginine-vasotocin, an octapeptide amide containing the ring of oxytocin and the side chain of arginine-vasopressin. Some time after the synthesis of this compound would represent the basis of pharmacological and chromatographic evidence.

Chauvet, Lenci, and Acher (10) in a preliminary communication have reported the isolation of a peptide from fowl neurohypophysial extracts which appears to be arginine-vasotocin. Thus pharmacological and chromatographic evidence.

Recent work in this laboratory (6), the arginine-vasotocin was tentatively identified as a naturally occurring hormone in fowl neurohypophysial extracts by Munsie, Sawyer, and van Dyke (7-9) on the basis of pharmacological and chromatographic evidence. Their evidence also indicates that it appears in the most primitive living vertebrates, the cyclostomes. The one elasmobranch (spiny dogfish) that they studied appeared to possess an active peptide unlike any that they had encountered elsewhere among the vertebrates. Pharmacological studies on the pituitaries of fish, fowl, and frogs by Pickering and Heller (11) also provided evidence for the presence of a peptide different from arginine-vasotocin in these species. Maetz et al. (12, 13) came to a similar conclusion with regard to cold-blooded vertebrates.

The present paper describes the synthesis and pharmacological properties of lysine-vasotocin, a compound similar to arginine-vasotocin but having the ring of oxytocin attached to the side chain of lysine vasopressin rather than arginine vasopressin. It may be noted that lysine-vasotocin bears a close relationship to both oxytocin and vasopressin, differing from oxytocin by replacement of the leucine residue with lysine, and from lysine-vasopressin by replacement of the phenylalanine residue with isoleucine. Thus lysine-vasotocin might equally well be called 8-lysine oxytocin or 3-isoleucine lysine-vasopressin.

While our work was in progress, the synthesis and pharmacological behavior of this substance were reported by Boissonnas and Huguenin (15). However, different pathways were used for the preparation of the required nonapeptide intermediate in the two laboratories. Furthermore, the potency of our preparation of lysine-vasotocin is considerably higher than that reported by Boissonnas and Huguenin.

In the present work the protected nonapeptide intermediate, S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparginyl-S-benzyl-L-cysteinyl-L-prolyl-N-tosyl-L-lysylglycinamide, was prepared by the stepwise attachment of L-isoleucine, L-tyrosine, and S-benzyl-N-carbobenzoxy-L-cysteine to L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-N-tosyl-L-lysylglycinamide (5) according to the nitrophenyl ester method as employed in the synthesis of long chain polypeptides (5, 16).

The lysine-vasotocin was prepared from the protected nonapeptide by reduction with sodium in liquid ammonia and subsequent oxidation to the cyclic disulfide form by aeration. The crude product was then desalted and the lysine-vasotocin was isolated by ion exchange chromatography (17). The lysine-vasotocin thus obtained traveled as a single spot upon paper chromatography in butanol-acetic acid-water (4:1:5). The amino acid analysis of an acid hydrolysate of the material showed the expected amino acid ratios. The material also gave the expected results on elementary analysis. It is of interest to note that lysine-vasopressin of this high degree of purity was obtained repeatedly in a yield of 65% from the protected nonapeptide.

The lysine-vasotocin possessed 130 U.S.P. units per mg of pressor activity in the rat (18) in comparison to lysine-vasopressin which possesses somewhat more than twice this potency. The lysine-vasotocin was found to possess 190 U.S.P. units per

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mg of avian depressor activity upon assay by the method described in the Pharmacopeia (19). However, the chicken was prepared according to the procedure of Mirske, Sawyer, and van Dyke (9). This value is more than 4 times the potency of lysine-vasopressin but less than one-half that of oxytocin with respect to avian depressor activity. The material obtained by Bodanszky, Meienhofer, and du Vigneaud (5), was used for the preparation of the protected hexapeptide I. The protected hexapeptide had a melting point of 30 ml), and the melting point was raised to 224–226°. The mixture was allowed to stand for 3 days at room temperature. Then 2 ml of glacial acetic acid and 100 ml of water were added. The precipitate was filtered and washed with 50 ml of water, 10 ml of 1:1 acetone-ethyl acetate, and 10 ml of ethanol. The air-dried material weighed 0.95 g. This material was dissolved in 20 ml of dimethylformamide, precipitated with 200 ml of ether, filtered off, triturated with 50 ml of ethyl acetate, and air dried; weight, 3.1 g (82%), melting point, 220–223°, \([\alpha]_D^{20} = -38° (c 1, dimethylformamide). The sample for analysis was recrystallized from 85% ethanol (0.2 g in 50 ml) with no resultant change in the melting point.

**Experimental Procedure**

Carboxbenzoxyl-L-lysine-L-glutamyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Ne-tosyl-L-lysylglycinamide (I)—The protected hexapeptide, carboxbenzoxyl-L-glutaminal-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Ne-tosyl-L-lysylglycinamide, prepared by the procedure of Bodanszky, Meienhofer, and du Vigneaud (5), was used for the preparation of the protected heptapeptide I. The protected hexapeptide had a melting point of 195–199° (softening at 185°) and \([\alpha]_D^{20} = -40° (c 1, dimethylformamide). These values agree closely with the values reported by Bodanszky, Meienhofer, and du Vigneaud (5). Boissonnas and Huguenin (15) report a melting point of 155° (decomposition) and \([\alpha]_D^{20} = -36.3° (c 2, dimethylformamide) for this compound. The p-nitrophenyl carboxbenzoxyl-L-asparaginate, which was used in the preparation of the protected hexapeptide, was prepared by the procedure of Bodanszky and du Vigneaud (16) and purified by recrystallization from ethyl acetate, which we have found to be particularly effective for the purification of this compound.

To a solution of 3.3 g of the protected hexapeptide in 15 ml of glacial acetic acid were added 25 ml of 4 N hydrogen bromide in glacial acetic acid. The resulting solution was allowed to stand for 1 hour at room temperature. Absolute ether (150 ml) was added to the solution with swirling. The solid thus formed was filtered off and washed twice with 50 ml of absolute ether. The hydrobromide was dried in a vacuum over NaOH and CaCl₂. The hydrobromide was dissolved in 6 ml of warm dimethylformamide and cooled in ice. To this solution was added 1.5 ml of triethylamine followed by 0.40 g of p-nitrophenyl S-benzyl-L-carbobenzoxy-L-cysteinate (16). The mixture was allowed to stand for 3 days at room temperature. Then 2 ml of glacial acetic acid and 100 ml of water were added. The precipitate was filtered and washed with 50 ml of water, 10 ml of 1:1 acetone-ethyl acetate, 40 ml of warm ethyl acetate, and 10 ml of ethanol. The air-dried material weighed 0.95 g. This material was dissolved in 20 ml of dimethylformamide, precipitated with 200 ml of ether, filtered off, triturated with 50 ml of ethyl acetate, and air dried; weight, 0.81 g (75%), melting point, 224–227°, \([\alpha]_D^{20} = -38° (c 1, dimethylformamide). A sample (0.1 g) for analysis was dissolved in hot 85% ethanol (100 ml) and the solution was filtered and cooled, whereupon the material precipitated. The melting point was thus raised to 229–231°. The rotation was unchanged; literature (15) melting point, 230°, \([\alpha]_D^{20} = 32° (c 0.8, dimethylformamide).

**Lysozine-vasoprotocin**—The nonapeptide derivative III (300 mg) was dissolved in 300 ml of liquid ammonia which had been distilled from sodium. Sodium was added to this solution, and the reduction was carried out according to the procedure described previously for the synthesis of oxytocin (1). Three drops of acetic acid were added to expel the blue color. The ammonia solution was allowed to evaporate down to a volume of about 20 ml, and the rest of the ammonia was removed in a vacuum. The contents of the flask were kept under reduced pressure for ½ hour. The residue was washed twice with 100 ml of ethyl acetate and then dissolved in 500 ml of water. The pH of the solution was adjusted from approximately 4.5 to approximately 7 with dilute ammonia. Air was bubbled through this solution, during which time crystallization usually occurred. Then 6 ml of glacial acetic acid were added, followed by 150 ml of water. The precipitate was filtered and washed with 25 ml of ethanol and 25 ml of warm ethyl acetate. The dried solid weighed 2.6 g (82%) and melted at 222–224°, \([\alpha]_D^{20} = -31° (c 1, dimethylformamide). The analytical sample was recrystallized from 85% ethanol (0.15 g in 30 ml), and the melting point was raised to 224–226°.
solution until the sodium nitroprusside test for sulphydryl groups was negative (1 hour). This solution contained about 18,000 units of rat pressor activity, based on comparison with the U.S.P. Posterior Pituitary Standard Powder (18). The pH was adjusted to approximately 4 with acetic acid, and the solution was poured onto a column of IRC-50 (XE-64) (1 by 38 cm) in the acid form for desalting (20). The column was washed with 0.25% acetic acid until the pH of the effluent reached about 3 (300 ml) and then with 25 ml of water. The material was eluted with 60 ml of 30% pyridine-4% acetic acid solution. The eluate was lyophilized to a product (210 mg) with 90 to 100 units of rat pressor activity per mg.

The crude lysine-vasotocin was purified by use of ion exchange chromatography. The 210 mg of product were dissolved in 1 ml of 0.5 M ammonium acetate buffer pH 6.3 and placed on an IRC-50 (XE-64) column (1 by 38 cm) which had been equilibrated with the buffer. The column was developed with the buffer at a flow rate of 3 to 6 ml per hour, and 3-ml fractions were collected. The fractions were analyzed by determination of the Folin-Lowry color reaction (21) and rat pressor activity, which indicated that the active material was in Fractions 32 to 62. The contents of these tubes were combined and acidified to a pH of approximately 4 with acetic acid. This solution was desalted on IRC-50 as already described. The lyophilized product weighed 130 mg and had a rat pressor activity of 130 U.S.P. units per mg and an avian depressor activity of 190 U.S.P. units per mg.

Paper chromatography with the system butanol-acetic acid-water (4:1:5) showed the material to travel as a single spot (Rf 0.14). The amino acid analyses were performed on a Beckman-Spinco amino acid analyzer according to the procedure of Spackman, Stein, and Moore (22). The product showed the following amino acid molar ratios (with the ratio for isoleucine taken as 1): isoleucine 1.0, tyrosine 0.9, proline 1.0, glutamic acid 1.1, aspartic acid 1.0, glycine 1.0, lysine 1.0, cystine 1.0, and ammonia 3.1.

For elementary analysis, the material was dried at 100° over P2O5 in a vacuum for 8 hours with a loss in weight of 13%. The analysis indicated that the dried material was the free base.

\[
\begin{align*}
\text{C}_{96}\text{H}_{162}\text{O}_{33}\text{N}_{12}\text{S}_{2} \\
\text{Calculated: C 50.52, H 6.61, N 17.82} \\
\text{Found: C 50.46, H 6.77, N 17.50} \\
\left[\alpha\right]^{D}_{D} -25^\circ (c 0.4 \text{ in } n \text{ acetic acid})
\end{align*}
\]

**SUMMARY**

Lysine-vasotocin, an analogue of the posterior pituitary hormones containing the ring of oxytocin and the side chain of lysine-vasopressin, has been synthesized from the appropriately protected nonapeptide intermediate by reduction with sodium in liquid ammonia and oxidation by aeration to the 20-membered disulfide ring. The required intermediate was prepared from 1-glutamyl L-asparaginyl L-benzyl-L-tyrosinyl L-prolyl N-acytosyl-L-lysylglycinamide by the stepwise attachment of L-isoleucine, L-tyrosine, and L-benzyl-N-carbobenzoxy-L-cysteine with the use of the nitrophenyl ester method. The pressor activity of lysine-vasotocin was found to be approximately one-half that of lysine-vasopressin. The avian depressor activity was found to be less than one-half of that of oxytocin but more than 4 times that of lysine-vasopressin.

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