Synthesis and Biological Properties of 1-Desamino-8-lysine-vasopressin*

RAYMOND D. KIMBROUGH, JR., WILLIAM D. CASH, LUIS A. BRANDA,† W. Y. CHAN, AND VINCENT DU VIGNEAUD

From the Department of Biochemistry, Cornell University Medical College, New York 21, New York

(Received for publication, December 7, 1962)

Attention has been directed recently in this laboratory to a study of the role of the various chemical functional groups of the oxytocin molecule in the production of the biological effects associated with this hormone (1-7). One of the analogues of oxytocin synthesized as part of this study was desamino-oxytocin, the analogue in which the free amino group is replaced by a hydrogen atom. This polypeptide exhibited the typical biological activities of oxytocin (1, 2, 5, 7). In fact, it exhibited some of these activities to a higher degree than did oxytocin itself. These results demonstrated clearly that the free amino group of oxytocin is not essential for biological activity. Because of an interest in extending this particular modification in structure to the vasopressins, we have synthesized 1-desamino-8-lysine-vasopressin (1-β-mercaptopropionic acid-8-lysine-vasopressin) (Fig. 1). The synthesis and biological properties of this polypeptide are discussed in the present paper.

1-Desamino-8-lysine-vasopressin was synthesized by way of the protected octapeptide derivative, N-(S-benzyl-β-mercaptobenzoyl)-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-aspartaginyl-L-lysylglycinamide. The starting material was the protected octapeptide, O-benzyl-N-carbobenzoxy-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-lysylglycinamide, an intermediate previously used for the synthesis of 8-lysine-vasopressin by the p-nitrophenyl ester method (8). After removal of the blocking groups from the tyrosine residue with hydrogen bromide in glacial acetic acid, the octapeptide was allowed to react in the presence of triethylamine with p-nitrophenyl S-benzyl-P-mercaptopropionate (1, 5) to yield the desired protected β-mercaptobenzoyl octapeptide. 1-Desamino-8-lysine-vasopressin was prepared from this octapeptide derivative by first removing the tosyl and benzyl protecting groups with sodium in liquid ammonia (9), and then converting the resulting sulfhydryl peptide into the cyclic disulfide with the aid of potassium ferricyanide (5). The crude product was purified by ion exchange chromatography and countercurrent distribution. The purified material appeared to be homogeneous when subjected to paper chromatographic and paper electrophoretic analysis. Elementary analysis and analysis for amino acids and ammonia gave the expected results for 1-desamino-8-lysine-vasopressin.

The purified material and an authentic sample of 8-lysine-vasopressin were assayed for various biological activities exhibited by the posterior pituitary hormones. The results are presented in Table I along with the corresponding potencies of oxytocin and desamino-oxytocin. 1-Desamino-8-lysine-vasopressin exhibits to a high degree all of the biological effects of 8-lysine-vasopressin that were studied. Like desamino-oxytocin, the avian depressor, oxytocic, and antiuretic activities are greater than those of the parent compound whereas the pressor activity is less than that of the parent compound. However, the pressor activity is still approximately one-half that of 8-lysine-vasopressin. The antiuretic activity of 1-desamino-8-lysine-vasopressin is higher than that of any other known peptide, with the exception of 8-arginine-vasopressin. Furthermore, the ratio of antiuretic activity to pressor activity for 1-desamino-8-lysine-vasopressin is 2.4, in contrast to the value of 0.8 for 8-lysine-vasopressin.

The relatively high activities of 1-desamino-8-lysine-vasopressin in all of the assays listed in Table I show that the free α-amino group of 8-lysine-vasopressin is not essential for biological activity. On the other hand, blocking this group with an acetyl substituent leads to almost complete biological inactivation (10). Likewise, blocking the free amino group of oxytocin with an acetyl group causes a large decrease in biological activity (11). Thus, the role of this particular functional group in the production of the biological effects of oxytocin and 8-lysine-vasopressin cannot be evaluated by studying the effect of acetylation on biological activity. Similarly, the role of the phenolic hydroxyl group of oxytocin cannot be evaluated from the changes in biological activity caused by methylation of this group. 0-Methyl-oxytocin exhibits 1% or less of the avian depressor, oxytocic, and pressor activities of oxytocin (4, 12, 13) whereas desoxy-oxytocin exhibits a much larger fraction of the same activities of oxytocin (3, 11). Although the extent to which these observations apply to other blocking groups and to other polypeptides and proteins of biological interest remains to be established, they do suggest that changes in biological activity produced by the blocking of functional groups of polypeptides and proteins should be interpreted with caution.

EXPERIMENTAL PROCEDURE

O-Benzyl-N-carbobenzoxy-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-lysylglycinamide (8), 0.50 g, was dissolved in 3 ml of glacial acetic acid. Then 3 ml of 4 N hydrogen bromide, and ammonia gave the expected results for 1-desamino-8-lysine-vasopressin.

The purified material and an authentic sample of 8-lysine-vasopressin were assayed for various biological activities exhibited by the posterior pituitary hormones. The results are presented in Table I along with the corresponding potencies of oxytocin and desamino-oxytocin. 1-Desamino-8-lysine-vasopressin exhibits to a high degree all of the biological effects of 8-lysine-vasopressin that were studied. Like desamino-oxytocin, the avian depressor, oxytocic, and antiuretic activities are greater than those of the parent compound whereas the pressor activity is less than that of the parent compound. However, the pressor activity is still approximately one-half that of 8-lysine-vasopressin. The antiuretic activity of 1-desamino-8-lysine-vasopressin is higher than that of any other known peptide, with the exception of 8-arginine-vasopressin. Furthermore, the ratio of antiuretic activity to pressor activity for 1-desamino-8-lysine-vasopressin is 2.4, in contrast to the value of 0.8 for 8-lysine-vasopressin.

The relatively high activities of 1-desamino-8-lysine-vasopressin in all of the assays listed in Table I show that the free α-amino group of 8-lysine-vasopressin is not essential for biological activity. On the other hand, blocking this group with an acetyl substituent leads to almost complete biological inactivation (10). Likewise, blocking the free amino group of oxytocin with an acetyl group causes a large decrease in biological activity (11). Thus, the role of this particular functional group in the production of the biological effects of oxytocin and 8-lysine-vasopressin cannot be evaluated by studying the effect of acetylation on biological activity. Similarly, the role of the phenolic hydroxyl group of oxytocin cannot be evaluated from the changes in biological activity caused by methylation of this group. 0-Methyl-oxytocin exhibits 1% or less of the avian depressor, oxytocic, and pressor activities of oxytocin (4, 12, 13) whereas desoxy-oxytocin exhibits a much larger fraction of the same activities of oxytocin (3, 11). Although the extent to which these observations apply to other blocking groups and to other polypeptides and proteins of biological interest remains to be established, they do suggest that changes in biological activity produced by the blocking of functional groups of polypeptides and proteins should be interpreted with caution.

EXPERIMENTAL PROCEDURE

O-Benzyl-N-carbobenzoxy-L-tyrosyl-L-phenylalanyl-L-glutaminyl-N-carbobenzoxy-L-lysylglycinamide (8), 0.50 g, was dissolved in 3 ml of glacial acetic acid.

1 All melting points were determined in capillary tubes and are corrected.
were added. The precipitated solid was filtered and washed twice with 20 ml portions of ethanol; weight, 0.40 g; m.p., 227-230° for 3 days. Then 1 ml of glacial acetic acid and 50 ml of water were added. The resulting solution was allowed to stand at room temperature for 1 hour, and then 50 ml of anhydrous ether were added. The resulting solution was allowed to stand at room temperature for 15 minutes. In order to remove the ferriyanide and ferrocyanide ions, the solution was passed through a column, 0.9 X 17 cm, of AG 3-X4 anion exchange resin (100 to 200 mesh) in the chloride form. The column then was washed with 20 ml of water, and the wash was combined with the previous eluate. Assay of an aliquot of the combined solutions for pressor activity in the rat indicated a total activity of approximately 5600 units. The main portion of the solution was acidified by the addition of 1 ml of glacial acetic acid.

The crude product was desalted and subjected to two purifications on an Amberlite IRC-50 column by the procedures employed for the isolation and purification of 1-acetyl-8-lysine-vasopressin (10). In the first purification, a small amount of peptide material emerged from the column ahead of the main peak. In the second purification, this first peak was not detectable. After removal of water and ammonium acetate by repeated lyophilization, 31 mg of a white solid were obtained. The pressor activity of this material in the rat was approximately 83 units per mg.

![Fig. 1. 1-Desamino-8-lysine-vasopressin](image_url)

**Table I**

<table>
<thead>
<tr>
<th>Polypeptide</th>
<th>Activity (mean ± standard error)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antidiuretic (rat)</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>S-Lysine-vasopressin†</td>
<td>203 ± 7</td>
</tr>
<tr>
<td>1-Desamino-8-lysine-vasopressin†</td>
<td>301 ± 11</td>
</tr>
<tr>
<td>Oxytocin†</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Desamino-oxytocin†</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

* Uteri from rats in estrus were used for the assay of S-lysine-vasopressin and 1-desamino-8-lysine-vasopressin, whereas uteri from rats taken without regard to the stage of estrus were used for the assay of oxytocin and desamino-oxytocin. For a discussion of the oxytocic activities of oxytocin and desamino-oxytocin at different stages of estrus, see (7).

† The values for oxytocin and desamino-oxytocin are those reported recently from this laboratory (2, 5).

Acid were added. The resulting solution was allowed to stand at room temperature for 1 hour, and then 50 ml of anhydrous ether were added. The precipitated solid was filtered and washed twice with 30 ml portions of ether. It was allowed to stand for a short time over sodium hydroxide and calcium chloride in an evacuated desiccator, and then was dissolved in 3 ml of dimethylformamide. Triethylamine, 0.70 ml, and 0.20 g of p-nitrophenyl S-benzyl-$\beta$-mercaptopenionate (1, 5) were added to the resulting solution. The mixture was allowed to stand at room temperature for 3 days. Then 1 ml of glacial acetic acid and 50 ml of water were added. The precipitated solid was filtered and washed twice with 20 ml portions of ethanol; weight, 0.40 g; m.p., 227-230°.

A 100 mg portion of this material was dissolved in 70 ml of boiling anhydrous liquid ammonia. Very small pieces of sodium were added one at a time until the reaction mixture developed a blue color that persisted for 10 minutes. Ammonium chloride, 90 mg, was added, and ammonia was removed under reduced pressure from the frozen state. The residue was dissolved in 170 ml of water, and the pH of the resulting solution was adjusted to approximately 4.5 by the addition of a few drops of dilute acetic acid. This solution was extracted five times with 50 ml portions of ether, the ether extracts being discarded. The pH of the aqueous solution then was adjusted to approximately 6.7 with dilute ammonium hydroxide, and 10.0 ml of 0.011 N potassium ferricyanide were added dropwise to the solution with stirring. The pH was readjusted to about 6.5 with dilute ammonium hydroxide, and the solution was allowed to stand at room temperature for 15 minutes. In order to remove the ferriyanide and ferrocyanide ions, the solution was passed through a column, 0.9 X 17 cm, of AG 3-X4 anion exchange resin (100 to 200 mesh) in the chloride form. The column then was washed with 20 ml of water, and the wash was combined with the previous eluate. Assay of an aliquot of the combined solutions for pressor activity in the rat indicated a total activity of approximately 5600 units. The main portion of the solution was acidified by the addition of 1 ml of glacial acetic acid.

The crude product was desalted and subjected to two purifications on an Amberlite IRC-50 column by the procedures employed for the isolation and purification of 1-acetyl-8-lysine-vasopressin (10). In the first purification, a small amount of peptide material emerged from the column ahead of the main peak. In the second purification, this first peak was not detectable. After removal of water and ammonium acetate by repeated lyophilization, 31 mg of a white solid were obtained. The pressor activity of this material in the rat was approximately 83 units per mg.

An 85 mg portion of material at this stage of purification was subjected to countercurrent distribution in the solvent system butanol-ethanol-0.05% acetic acid (4:1:5). After 532 transfers, analysis by the Folin-Lowry color reaction (14) indicated a main peak contained in tubes 95 to 145, the $K$ value being approximately 0.26. A smaller peak that represented approximately 4% of the amount of peptide material in the main peak was present in tubes 75 to 95. Aliquots from selected tubes in the main peak were assayed for pressor activity in the rat. The curve obtained by plotting pressor activity coincided closely with the Folin-Lowry color curve. The contents of tubes 95 to 145 were collected, combined, and concentrated in a rotary evaporator to a volume of approximately 40 ml. The concentrated solution was lyophilized to a white, fluffy solid; weight, 65 mg; $[\alpha]_{D}^{20} -58.4^\circ$ (c, 0.50 in n acetic acid).}

2 Analytical grade Dowex 3 anion exchange resin supplied by Bio-Rad Laboratories, Richmond, California.

The specific rotation of S-lysine-vasopressin under the same condition was $[\alpha]_{D}^{20} -38.4^\circ$ (c, 0.50 in n acetic acid).
Portions of the purified product were applied to strips of Whatman No. 1 paper and subjected to ascending chromatography in butanol-acetic acid-water (4:1:5). The chromatograms were developed with a modified ninhydrin reagent (15) and also with a platinum reagent for the detection of sulfur-containing compounds (16). The peptide traveled as a single spot ($R_F$ 0.47 to 0.49). The $R_F$ value of an authentic sample of 8-lysine-vasopressin under the same experimental conditions was 0.32 to 0.34. Paper chromatographic analysis also was carried out in pyridine-acetic acid-water (50:35:15), the chromatograms being developed with the bromphenol blue-mercuric chloride reagent (17). The product traveled as a single spot ($R_F$ 0.66). The $R_F$ value of 8-lysine-vasopressin under identical conditions was 0.55.

A portion of the purified product was applied to a strip of Whatman No. 1 paper and subjected to electrophoresis at 5° for 16 hours at 400 volts in pyridine acetate buffer at pH 5.1. The paper was developed with the bromphenol blue-mercuric chloride reagent. The peptide traveled a distance of 12.4 cm as a single spot toward the cathode. Under these conditions, a sample of 8-lysine-vasopressin travelled a distance of 19.5 cm in the same direction.

A sample of the purified peptide was hydrolyzed in 6 N hydrochloric acid at 110° for 17 hours. The hydrolysate was analyzed for amino acids and ammonia (18, 19). The following molar ratios were obtained (with the value for lysine taken as 1.0): lysine, 1.0; aspartic acid, 1.0; glutamic acid, 1.0; proline, 1.0; glycine, 1.0; cystine, 0.25; tyrosine, 1.0; phenylalanine, 1.0; the mixed disulfide of $\beta$-mercaptothiophenyl propionic acid and cysteine, 0.5, and ammonia, 2.0.

For elementary analysis, a sample of the purified product was heated for 5 hours at 100° in a high vacuum over phosphorus pentoxide. A loss in weight of 9.5% was observed. The results of the analysis agreed with the calculated values for the acetate of 1-desamino-8-lysine-vasopressin.

$$\text{CdH}_{n}\text{O}_{x}\text{N}_{y}\text{S}_{z}\cdot \text{C}_{m}\text{H}_{n}\text{O}_{p}$$

Calculated: C 52.4, H 6.22, N 15.3
Found: C 52.7, H 6.28, N 15.6

The biological activities listed in Table I were determined by assaying the purified product and an authentic sample of 8-lysine-vasopressin against the U.S.P. Posterior Pituitary Reference Standard. Pressor activity (20) and antidiuretic activity (21, 22) were determined on anesthetized male rats; avian depressor activity was approximately one-half that of the parent compound, whereas the pressor activity was approximately one-half that of the parent compound. These results demonstrate that the free amino group on the terminal half-cystine residue of 8-lysine-vasopressin is not essential for the production of the biological effects of this polypeptide hormone studied thus far.

**REFERENCES**


Synthesis and Biological Properties of 1-Desamino-8-lysine-vasopressin
Raymond D. Kimbrough, Jr., William D. Cash, Luis A. Branda, W. Y. Chan and
Vincent du Vigneaud