The Synthesis and Pharmacological Study of 4-Decarboxamido-oxytocin (4-\(\alpha\)-Aminobutyric Acid-oxytocin) and 5-Decarboxamido-oxytocin (5-Alanine-oxytocin)∗

VINCENT DU VIGNEAUD, GEORGE S. DENNING, JR., STEFANIA DRABAREK,† AND W. Y. CHAN

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

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Oxytocin, the main oxytocin-milk-ejecting, avian depressor principle of the posterior pituitary gland, the structure of which is shown in Fig. 1, possesses a number of chemical functional groups: a primary amino group in position 1, a phenolic hydroxyl group in position 2, three carboxamide groups in positions 4, 5, and 9, and a disulfide linkage in the 20-membered cyclic moiety. The significance of the presence of these functional groups to the possession of biological activity of the hormone has been investigated in this and other laboratories through the synthesis and study of various analogues of oxytocin. The importance of the phenolic hydroxyl group and the primary amino group to the biological activity of the hormone has already been examined by synthesis of analogues of the hormone lacking these groups, namely, deoxy-oxytocin (2-5), deamino-oxytocin (6, 7), and deamino-deoxy-oxytocin (8). These studies showed that the phenolic hydroxyl group in oxytocin is not essential for the production of the biological responses characteristic of the hormone, but its presence does contribute strongly to the avian depressor and oxytocic potencies of oxytocin. Deamino-oxytocin, in which the free amino group is replaced by hydrogen, has very high avian depressor and oxytocic activities in comparison with oxytocin, and exhibits an antidiuretic activity approximately 5 times that of oxytocin (6, 7). Thus the amino group of oxytocin is not required for the manifestation of biological activity. The activities of deamino-deoxy-oxytocin (8) are similar to those of deoxy oxytocin. When the disulfide linkage of oxytocin was replaced by hydrogen through the use of Raney nickel, the resulting dethio-oxytocin was found to be devoid of avian depressor activity (9) and oxytocic activity (10).

In a continuing effort to probe the importance of the functional groups to the production of pharmacological properties of oxytocin by replacement of the functional groups by hydrogen, two analogues, 4-decarboxamido-oxytocin and 5-decarboxamido-oxytocin, have been synthesized in which the CONH group in position 4 or in position 5 of the ring of the hormone is substituted by a hydrogen atom. 4-Decarboxamido-oxytocin (4-\(\alpha\)-aminobutyric acid-oxytocin) was obtained by replacing the glutamine residue in the peptide ring by an \(\alpha\)-aminobutyric acid residue. In 5-decarboxamido-oxytocin (5-alanine-oxytocin) the asparagine residue was substituted by an alanine residue.

The syntheses of the two analogues were carried out by use of the stepwise \(p\)-nitrophenyl ester procedure used for the synthesis of oxytocin (11, 12). In the preparation of the 4-decarboxamido-oxytocin, the protected pentapeptide, \(N\)-carbobenzoxy-\(L\)-asparaginyl-\(S\)-benzyl-\(L\)-cysteinyl-\(L\)-prolyl-\(L\)-leucylglycinamide (12), served as starting material. After removal of the amino-protecting group, the free base was coupled with \(p\)-nitrophenyl \(N\)-carbobenzoxy-\(L\)-\(\alpha\)-aminobutyrate to give the protected hexapeptide. The chain was then lengthened by the stepwise \(p\)-nitrophenyl ester method to give the protected nonapeptide, \(N\)-carbobenzoxy-\(S\)-benzyl-\(L\)-cysteinyl-\(L\)-tyrosyl-\(L\)-isoleucyl-\(L\)-\(\alpha\)-aminobutyryl-\(L\)-asparaginyl-\(S\)-benzyl-\(L\)-cysteinyl-\(L\)-prolyl-\(L\)-leucylglycinamide.

In the synthesis of 5-decarboxamido-oxytocin, \(S\)-benzyl-\(L\)-cysteinyl-\(L\)-prolyl-\(L\)-leucylglycinamide obtained from the corresponding protected tetrapeptide was allowed to react with \(p\)-nitrophenyl \(N\)-carbobenzoxy-\(L\)-alaninate to give the required protected pentapeptide. The successive attachment to this peptide of \(L\)-glutamine, \(L\)-isoleucine, \(L\)-tyrosine, and \(L\)-cysteine residues by use of the \(p\)-nitrophenyl ester method resulted in the protected nonapeptide, \(N\)-carbobenzoxy-\(S\)-benzyl-\(L\)-cysteinyl-\(L\)-tyrosyl-\(L\)-isoleucyl-\(L\)-glutaminyl-\(L\)-alanyl-\(S\)-benzyl-\(L\)-cysteinyl-\(L\)-prolyl-\(L\)-leucylglycinamide.

In both syntheses from the tetrapeptide through the octapeptide stage, hydrobromic acid in glacial acetic acid was used for the removal of the carboxbenzoyl groups. The free bases were obtained from the hydrobromides by treatment with IRA-410 resin (in the OH cycle) and, in the case of the hydrobromides of the free octapeptides, by triethylamine. All the protecting groups of the protected nonapeptides were removed by treatment with sodium in liquid ammonia (13). The reduced materials so obtained were then oxidized at pH 6.5 to 7.0 by aeration followed by titration with a solution of potassium ferricyanide (6, 7).

The solutions of the analogues from the oxidation steps were assayed for avian depressor activity. After concentration to about 20 ml, they were purified by countercurrent distribution in

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the solvent system butanol:propanol:0.05 N acetic acid (2:1:3). The partition coefficient (K) for 4-decarboxamido-oxytocin was found to be approximately 0.63, and for 5-decarboxamido-oxytocin, approximately 0.53. The contents of the tubes containing the active materials were combined, concentrated, and lyophilized. The two analogues so obtained gave the expected amino acid and elementary analyses. Amino acid analysis of acid hydrolysates of the hormone analogues was carried out according to the method of Spackman, Stein, and Moore (14) in a Beckman Spinco amino acid analyzer. For 4-decarboxamido-oxytocin, the usual 50° system (15) was initially employed. No separate peak appeared on the chromatogram representing the α-aminoibutyric acid under these conditions, but the cystine peak was abnormally high, representing a molar ratio of 1.89 instead of 1.0. Hence it was assumed that the peaks of cystine and α-aminoibutyric acid had coincided.

The 4-decarboxamido-oxytocin and 5-decarboxamido-oxytocin were tested for the biological activities characteristic of oxytocin. Four-point assay was used in all the bioassays. Avian depressor assays were performed on conscious chickens according to the procedure employed by Munsick, Sawyer, and van Dyke (16). Oxytocin assays were performed on natural estrous rat uteri according to the method of Holton (17) as modified by Munsick (18), with the use of magnesium-free van Dyke-Hastings solution. Rat pressor assays were carried out on urethane-anesthetized male rats as described in the United States Pharmacopeia (19). Assays for antidiuretic activity were performed on male rats according to the method of Jeffers, Livezey, and Austin (20) as modified by Sawyer (21). All potencies were measured against the U.S.P. Posterior Pituitary Reference Standard. The potencies of the 4-decarboxamido-oxytocin and 5-decarboxamido-oxytocin are presented in Table I along with those of oxytocin and the analogues so far prepared in which chemical functional groups have been replaced by hydrogen.

EXPERIMENTAL PROCEDURE<sup>2</sup>

**N-Carbobenzyo-L-α-aminoibutyric acid**—Carbobenzoxy chloride, 10.8 g, was added dropwise to a solution of 5 g of L-α-aminoibutyric acid in 4 N sodium hydroxide. The mixture was then extracted with ether, and the water layer after acidification with HCl to pH 2 was again extracted with ether. The ether solution was evaporated and the residual colorless oil was dissolved in benzene-acetone (1:1). After removal of the solvent, the oily residue crystallized on being allowed to stand. The product recrystallized from ether-hexane (1:1) weighed 2.2 g. Analytical data: m.p. 79-80°, [α]<sub>D</sub> +12.1° (c, 2 in glacial acetic acid), [α]<sub>b</sub> +10.5° (c, 1 in absolute ethanol), and [α]<sub>b</sub> +9.8° (c, 1.2 in 95% ethanol); recorded value (24), [α]<sub>b</sub> +22° (c, 2.8 in ethanol).

That the difference between the values for optical rotation obtained by us and by Waley (24) was not due to racemization under the conditions used for the preparation was shown by removal of the protecting group from the N-carbobenzyo-L-α-aminoibutyric acid by catalytic hydrogenation. The resulting α-aminoibutyric acid had a rotation of [α]<sub>b</sub> +8.7° (c, 2 in water), [α]<sub>b</sub> +20.6° (c, 1 in 5 N HCl); recorded values (25), [α]<sub>b</sub> +21.2° (c, 1 in 5 N HCl).

<sup>2</sup> All melting points are corrected, capillary melting points.

FIG. 1. Oxytocin

![Diagram of Oxytocin](image)

**TABLE I**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean activities and standard errors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depressor (mg)</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Oxytocin*</td>
<td>567 ± 15</td>
</tr>
<tr>
<td>Deamino-oxytocin</td>
<td>735 ± 23</td>
</tr>
<tr>
<td>Deoxy-oxytocin</td>
<td>60 ± 30</td>
</tr>
<tr>
<td>Deoxy-deoxy-oxytocin</td>
<td>108 ± 5</td>
</tr>
<tr>
<td>4-Deoxycarboxamido-oxytocin</td>
<td>0.2-0.3</td>
</tr>
<tr>
<td>Dethio-oxytocin</td>
<td>None</td>
</tr>
</tbody>
</table>

* All of the values in the table, with the exceptions noted, were determined in this laboratory.

* These values are the mean values from assays on uteri from a large number of rats taken at random without regard to the stage of the estrous cycle. The relative response of deamino-oxytocin to that of oxytocin has been determined at each stage of estrus (23).

* See (3-5).

* See (10).

p-Nitrophenyl N-Carbobenzyo-L-α-aminoibutyrate—Three grams of N-carbobenzyo-L-α-aminoibutyric acid and 2.1 g of p-nitrophenol were dissolved in 45 ml of ethyl acetate, and the solution was cooled to 0° and stirred while 2.61 g of dicyclohexylcarbodiimide were added.

N,N'-Dicyclohexylurea began to separate out immediately. The stirring was continued for 31/2 hours at 0°. Five drops of glacial acetic acid were added and the urea was filtered off and washed with 12.5 ml of ethyl acetate. The filtrate and washing were combined and concentrated. The oily residue with some crystals present was taken up in 10 ml of absolute ethanol, filtered to remove additional free urea, and diluted with 50 ml of hexane. On being allowed to stand at -15°, the product separated in crystalline form. Recrystallization from the same solvent system (33% ethanol-hexane) gave 2.3 g of a product with a melting point of 60.5-61.5° and [α]<sub>b</sub> +34.9° (c, 2 in dimethylformamide).
N-Carbobenzyoxy-l-α-aminobutyryl-l-asparaginyl-S-benzyl-l-cysteinyl-l-proplyl-l-leucylglycinamide—Finely powdered N-carbobenzyoxy-l-asparaginyl-S-benzyl-l-cysteinyl-l-proplyl-l-leucylglycinamide, 5.5 g, was dissolved in 22 ml of glacial acetic acid and treated with 22 ml of HBr in glacial acetic acid (32%, weight for weight). After 2 hours at room temperature, the solution was poured into 400 ml of cold, dry ether. When the hydrobromide of the free pentapeptide had settled, the supernatant liquid was decanted and the precipitate was washed with three 150-ml portions of ether, each washing being followed by decantation. After being dried in a vacuum over potassium hydroxide and calcium chloride for 3 hours, the hydrobromide was dissolved in 80 ml of dry methanol and passed through a column of ion exchange IRA-410 resin (in the OH cycle). The column was washed with 100 ml of methanol. The eluate and washing were combined and evaporated in a vacuum to a white solid. This was dissolved in 14 ml of dimethylformamide, 2.98 g of p-nitrophenyl N-carbobenzyoxy-l-α-aminobutyrate were added, and after 2 days the solids were precipitated by ethyl acetate, collected, and washed with 100 ml of ethyl acetate, 100 ml of ethanol, and again with 50 ml of ethyl acetate. The substance was dried to constant weight over P2O5, in a vacuum; weight 5.33 g, m.p. 243-245°, [α]D +56.1° (c, 1 in dimethylformamide).

C15H26O8N8S

Calculated: C 57.8, H 6.71, N 13.8
Found: C 57.7, H 6.76, N 13.8

N-Carbobenzyoxy-l-isoleucyl-l-α-aminobutyryl-l-asparaginyl-S-benzyl-l-cysteinyl-l-proplyl-l-leucylglycinamide—Two and one-half grams of the protected hexapeptide described in the preceding section were dissolved in 14 ml of dry acetic acid and treated with 14 ml of 327, HBr in glacial acetic acid according to the procedure already described. After 2 hours at room temperature, the hydrobromide of the free peptide was isolated, dried, and dissolved in 50 ml of dry methanol. The solution was passed through a column of IRA-410 resin (OH cycle) and the column was washed with 100 ml of ethyl acetate, 100 ml of ethanol, and again with 50 ml of ethyl acetate. The substance was dried to constant weight over P2O5 in a vacuum; weight 5.83 g, m.p. 243-245°, [α]D +56.1° (c, 1 in dimethylformamide).

C15H26O8N8S

Calculated: C 57.8, H 6.76, N 13.8
Found: C 57.7, H 6.76, N 13.8

N-Carbobenzyoxy-O-benzyl-l-tyrosyl-l-isoleucyl-l-α-aminobutyryl-l-asparaginyl-S-benzyl-l-cysteinyl-l-proplyl-l-leucylglycinamide—The protected heptapeptide described in the preceding section, 1.8 g, was dissolved in 10 ml of dry acetic acid and treated with 10 ml of 32% HBr in glacial acetic acid according to the procedure already described. The hydrobromide of the free heptapeptide was precipitated with ether, dried, and dissolved in 50 ml of dry methanol. The hydrobromide was removed by means of IRA-410 resin in the OH cycle. After removal of the solvent, the resulting solid was dissolved in 5 ml of dimethylformamide and filtered to remove some insoluble material. p-Nitrophenyl N-carbobenzyoxy-O-benzyl-l-tyrosinate, 1.1 g, was then added to the solution. After 24 hours at room temperature, ethyl acetate was added to the thick mass. The precipitate was filtered off and triturated with 50 ml of ethyl acetate and 100 ml of ethanol. It was dried in a vacuum over P2O5 at 56°; weight 1.9 g, m.p. 241-243°, [α]D +42.8° (c, 1 in dimethylformamide).

C16H28O3NN6S

Calculated: C 61.9, H 6.91, N 11.8
Found: C 62.0, H 6.86, N 11.9
lyophilized to give 52 mg of the desired analogue. The solutions from tubes 125 to 150, containing 4-decarboxamidooxytocin from the central portion of the peak, were combined, concentrated to a small volume, and lyophilized to give 52 mg of the desired analogue.

For elementary analysis, a sample was dried at 100° over P2O5 in a vacuum until it reached a constant weight, a loss in weight of 7% being observed; [α]D=0 -30.7° (c, 1 in n acetic acid). A sample was hydrolyzed in 6 N hydrochloric acid at 110° for 17 hours and then analyzed in the 80 °C system of the Beclain/Spinco amino acid analyzer. The following expected molar ratios of amino acids and ammonia were obtained (with the value of glycine taken as 1.0): aspartic acid, 1.0; proline, 1.0; glycine, 1.0; α-amino butyric acid, 1.0; cystine, 1.0; isoleucine, 1.0; leucine, 1.0; tyrosine, 0.92; and ammonia, 2.1.

\[ C_{24}H_{43}O_{11}N_{8} \]
Calculated: C 52.3, H 6.75, N 16.0
Found: C 52.0, H 6.90, N 15.6

p-Nitrophenyl N-Carbobenzoxy-L-alanine—N-Carbobenzoxy-L-alanine, 9.15 g, was dissolved in 140 ml of ethyl acetate and the solution was cooled. p-Nitrophenol, 6.85 g, and 8.45 g of di-cyclohexylcarbodiimide were added and the mixture was stirred at 0° for 30 minutes and then at room temperature for another 12 hours. After addition of 1 ml of glacial acetic acid, the N,N'-dicyclohexylurea was filtered off. On evaporation of the solvent a yellow oil remained. It was dissolved in hot ethanol, 70 ml, and allowed to crystallize. After recrystallization from the same solvent the product weighed 7.6 g, m.p. 79-79.5° [α]D=0 -42.0° (c, 2 in dimethylformamide). The values reported for the compound obtained by using tris-(p-nitrophenoxy)phosphine in pyridine (27) are: m.p. 79-79.5° [α]D=0 -81.0° (c, 1.4 in ethanol).

\[ C_{19}H_{18}O_2N_2 \]
Calculated: C 59.3, H 4.68, N 8.14
Found: C 59.3, H 4.72, N 8.09

N-Carbobenzyox-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide—N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide, 6.11 g, was dissolved in 25 ml of warm acetic acid, cooled, and treated with 25 ml of HBr in glacial acetic acid (20%, weight for weight) in the usual manner. The hydrobromide of the free base was precipitated with dry ether, dried, and dissolved in 75 ml of methanol. The solution was passed through a column of IRA-410 resin in the OH cycle. Removal of the solvent from the eluate left a crystalline residue which was taken up in 15 ml of dimethylformamide and coupled with 3.78 g of p-nitrophenyl N-carbobenzoxy-L-alanine. The reaction was allowed to proceed for 18 hours and then 200 ml of ethyl acetate were added. No precipitate was formed, so the solution was evaporated and the residue was treated with 120 ml of ethyl acetate. On being allowed to stand, the product precipitated. After being washed twice with 20-ml portions of ethyl acetate, the protected pentapeptide was dried in a vacuum over P2O5; weight 6.08 g, m.p. 160-161.5°.

For analysis, 0.5 g of the compound was recrystallized from 75 ml of boiling ethyl acetate to give 0.45 g of crystalline product, m.p. 161-163°; [α]D=0 -59.3° (c, 1 in dimethylformamide).

\[ C_{26}H_{41}O_4N_3S \]
Calculated: C 59.8, H 6.79, N 12.3
Found: C 59.8, H 6.80, N 12.4

N-Carbobenzyox-L-glutamyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide—The preceding protected pentapeptide, 5.49 g, was treated in 25 ml of dry acetic acid with 23 ml of HBr in glacial acetic acid (20%, weight for weight), and the hydrobromide of the free base was isolated as already described. It was dissolved in 75 ml of methanol and the hydrobromic acid was removed by means of IRA-410 resin in the OH cycle. The residue which remained on evaporation of the solvent was dissolved in 15 ml of dimethylformamide followed by the addition of 3.54 g of p-nitrophenyl N-carbobenzyox-L-glutaminamide. The solution soon solidified into a hard cake. After 24 hours the mass was triturated with 175 ml of ethyl acetate; the solid was collected, washed with 100 ml of ethyl acetate and 100 ml of ethanol, and dried in a vacuum at 56°; weight 6.0 g, m.p. 225-227°; [α]D=0 -56.3° (c, 1 in dimethylformamide).

\[ C_{28}H_{42}O_4N_3S \]
Calculated: C 57.8, H 6.71, N 13.8
Found: C 57.6, H 6.76, N 13.8

N-Carbobenzyox-L-isoleucyl-L-glutamyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide—The preceding protected hexapeptide, 5.46 g, was suspended in 60 ml of acetic acid, and 35 ml of HBr in acetic acid (30%) were added. After 2 hours at room temperature, 350 ml of dry ether were added. The precipitate was dried over KOH and CaCl2 and then dissolved in 75 ml of methanol and passed down a column of IRA-410 resin in the OH cycle. The resin was rinsed twice with 50-ml portions of boiling methanol. The eluate and washings were evaporated to dryness, leaving a residue which in turn was dissolved in 15 ml of dimethylformamide, and 2.84 g of p-nitrophenyl N-carbobenzyox-L-isoleucinate were added to the solution. After several hours at room temperature the semisolid mass was diluted with 400 ml of ethyl acetate. The solid was collected, washed with 200 ml of absolute ethanol and 100 ml of ethyl acetate, and dried in a vacuum over P2O5 at 100°; weight 5.45 g, m.p. 222-224°; [α]D=0 55.1° (c, 1 in dimethylformamide).

\[ C_{29}H_{43}O_5N_3S \]
Calculated: C 58.5, H 7.09, N 13.6
Found: C 58.3, H 7.20, N 13.7

N-Carbobenzyox-O-benzyl-L-tyrosyl-L-isoleucyl-L-glutamyl-L-alanyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide—Protected heptapeptide, 2.49 g, was suspended in 20 ml of acetic acid and treated with 20 ml of HBr in acetic acid (20%). After 2 hours at room temperature, dry ether was added and the precipitate was washed with ether. After being dried over CaCl2 and KOH, the solid was dissolved in 50 ml of methanol and treated with IRA-410 resin in the OH cycle. Removal of the solvent left a residue, which was dissolved in 30 ml of dimethylformamide, and 1.56 g of p-nitrophenyl N-carbobenzyox-O-benzyl-L-tyrosinate were added. When the mixture was shaken, some solid soon started to separate. After 23 hours, 400 ml of ethyl acetate were added with thorough mixing. On the next day the solid was collected and washed with 50 ml of ethyl acetate, 200 ml of absolute ethanol, and again with 75 ml of ethyl acetate, and dried in a vacuum over CaCl2 and then over P2O5.
at 100° for 2 hours; weight 2.73 g, m.p. 238°-239°, [α]D +15.3° (c, 1 in dimethylformamide).

\[ \text{C}_{4}H_{14}O_{2}N_{1}S_{3} \]

Calculated: C 62.2, H 6.85, N 11.9
Found: C 62.1, H 6.86, N 11.7

5-Decarboxamido-oxytocin (5-Alanine-oxytocin)—The preceding octapeptide, 1.4 g, was suspended in 10 ml of acetic acid and treated with 5 ml of HBr in acetic acid (30%). After 2 hours at room temperature, 150 ml of dry ether were added and the precipitated hydrobromide was filtered off and washed with ether. After being dried in vacuum over KOH and CaCl₂, the hydrobromide was dissolved in 15 ml of dimethylformamide, and triethylamine was added to pH 7, followed by 0.61 g of p-nitrophenyl N-carbobenzyloxy-S-benzyl-L-cysteinate. After 1 day at room temperature, the reaction mixture was mixed with 150 ml of ethyl acetate. The precipitate was washed on the filter with more ethyl acetate, 50 ml of ethanol, and 50 ml of ethyl acetate, dried in vacuum over CaCl₂, and then over P₂O₅ at 100° for 2 hours; weight 1.28 g, m.p. 231°-232°, [α]D -53.2° (c, 1 in dimethylformamide).

This protected nonapeptide, 277 mg, was dissolved in 250 ml of boiling ammonia, freshly distilled from sodium, and treated with sodium until a faint blue color enveloped the solution. Glacial acetic acid, 0.1 ml, was added, and most of the ammonia was removed in a vacuum, the last 30 ml being evaporated from the frozen state. The residue was dissolved in 200 ml of 0.1% acetic acid, and after adjustment of the pH to 6.5 to 7.0 the solution was aerated for 4 hours. The oxidation was completed by titration with 0.02 N potassium ferricyanide solution. The solution was deionized by passage through a column of ion exchange resin, AG3X4, in the chloride cycle. This solution was found to possess a total of 28 units of avian depressor activity. It was evaporated in a flash evaporator to a volume of 16 ml, and for further purification this solution was placed in the first 2 tubes of a 200-tube countercurrent machine and distributed in the solvent system butanol-propanol-0.05% acetic acid (4:1:5). Initially the crude 5-decarboxamido-oxytocin was purified in the solvent system butanol-ethanol-0.05% acetic acid (2:1:3).

The determination of the Folin-Lowry (26) color values in the solvent system butanol-propanol-0.05% acetic acid (4:1:5). However, in this system the separation of analogue from by-products was very unsatisfactory, and with the increased amount of transfers the curve became more irregular. After 400 transfers, the partition coefficient for the main peak was 0.74, but after 900 transfers, the value changed to 0.88.

Determination of the Folin-Lowry (26) color values in the solvent system butanol-propanol-0.05% acetic acid after 2 transfers indicated two peaks with distribution coefficients of approximately 0.31 and 0.53. After 400 transfers, the pattern remained practically unchanged but a new peak appeared with a K value of 0.16. Avian depressor activity was present in tubes 125 to 160, which corresponded to the peak with a K value of 0.53. The contents of tubes 130 to 150 were pooled, evaporated in a flash evaporator to approximately 50 ml, and lyophilized; weight 49 mg, [α]D +13.3° (c, 1.5 in n acetic acid). A sample was hydrolyzed in 0 N HCl at 100° and analyzed in the Beckman/Spincor amino acid analyzer. The following molar ratios of amino acids were obtained with the value for glycine taken as 1: glutamic acid, 1.0; proline, 1.3; glycine, 1.0; alanine, 1.0; cystine, 1.0; isoleucine, 1.0; leucine, 1.0; tyrosine, 1.0; and ammonia, 2.0.

The data presented in Table I summarize the biological effects observed thus far from the replacement of various chemical functional groups of oxytocin by hydrogen. The striking potencies of the deaminooxytocin demonstrate that the amino group is not essential for the manifestation of the characteristic biological activities of oxytocin. Furthermore, the data show that the presence of the phenolic hydroxyl group is also not essential for the exhibition of activity, although this group does contribute strongly to the activities of the hormone. It is also of interest that the replacement of the amino group of deoxy- oxytocin with hydrogen does not lower the avian depressor and oxytocic potencies, since deaminodeoxy-oxytocin has approximately the same avian depressor and oxytocic activities as the deoxy analogue. On the other hand, there is a 10-fold decrease in pressor activity when the amino group of deoxy oxytocin is replaced by hydrogen. The same effect on pressor activity is encountered on going from oxytocin to deaminooxytocin, although in the latter case the change is less marked. From the standpoint of antidiuretic activity, there is little change in the potency of deaminodeoxy-oxytocin as compared with that of deoxy-oxytocin. However, it is noteworthy that the replacement of the amino group of oxytocin with hydrogen enhances the antidiuretic potency 5-fold.

The first studies in our laboratory involving the carboxamide groups of oxytocin were in connection with the synthesis of 4-isoglutaminooxytocin (28, 29) and 5-isoadasparagine-oxytocin (30). These two analogues were originally synthesized to test the specificity of the glutamine and asparagine residues in the 4- and 5-positions for the activities of the hormone. Both of these analogues, in which the proximity and spatial relationship of the carboxamide groups to the disulfide ring as well as the size of the ring itself are changed from that found in oxytocin, were found to be biologically inactive. The carboxamide group in the 4-isoglutaminooxytocin is linked directly to the ring instead of being separated from the ring by 2 methylene units as in oxytocin, and the size of the ring is increased from 20 to 22 atoms. In the case of the 5-isoadasparagine-oxytocin, the carboxamide group is attached directly to the ring and the ring size is increased from 20 to 21 atoms. Although the results with these two analogues demonstrated that neither the glutamine nor asparagine residue in oxytocin could be replaced by hydrogen, the isomeric form without loss of biological activity, no assessment could be made as to whether this loss of activity was due to the change in ring size, to the change in the spatial relationship of the carboxamide groups to the rest of the molecule, or to both of these structural changes.

The current studies of the two decarboxamido analogues deal directly with the importance of the presence of the carboxamide groups at positions 4 and 5 in the hormone. The striking differences in the activities of the 4-decarboxamido-oxytocin (4-L-α-amino butyric acid-oxytocin) and 5-decarboxamido-oxytocin (5-L-alanine-oxytocin) clearly demonstrate that the presence of the carboxamide group of the asparagine residue at position 5 is vital for the exhibition of appreciable biological activity, in contrast to the carboxamide group of the glutamine residue at position 4. As shown by the data in Table I, the 4-decarbox-
Gated.

approximately one-fifth of the avian depressor activity and one-

have been replaced by hydrogen. The 4-decarboxamido-oxyto-

carboxamide group in the glycinamide residue in position 9, still

awaits evaluation as part of the systematic replacement of chemi-

cal functional groups of oxytocin by hydrogen. This aspect of

the relation of structure to biological activity is being investi-

ment of the hormone to its receptor sites or for bringing about a

possibility that the carboxamide group of the asparagine residue

is of special significance to the activity of oxytocin. The pres-

amido-oxytocin possesses approximately 108 units per mg of

avian depressor activity and 75 units per mg of oxytocic activ-

ity, whereas the 5-decarboxamido-oxytocin has an extremely low

order of both these activities. With respect to pressor and an-

tiduretic activity, the absence of the carboxamide in position 4

results in a considerable decrease in both activities. The absence

of the carboxamide in position 5 has an even more drastic ef-

fect; the 5-decarboxamido-oxytocin has practically no

detectable pressor or antidiuretic activity.

In connection with these results on the biological activities

of 4-decarboxamido-oxytocin, it is significant that the new hor-

mone, isotocin, isolated by Acher et al. (31) from the posterior

pituitary of teleost fishes, lacks a carboxamide group in position

4. The structure proposed by them, 4-serine-8-isoleucine-oxyto-

cin, has been confirmed by synthesis (32, 33).

The three analogues of oxytocin prepared by Boissonnas et al.

(34, 35) have afforded a series of analogues of particular signifi-

cance to this question of the carboxamide groups. In this series,

the glutaminyl-asparaginyl sequence was replaced by glutaminyl-

glutaminyl (34), asparaginyl-asparaginyl, and asparaginyl-gluta-

minyl (35). Of these three analogues, the only one to show a

high degree of oxytocic or avian depressor activity was the one

with the asparaginyl-asparaginyl sequence possessing 108 units

of oxytocic activity and 202 units of avian depressor activity per

mg (36). The other two analogues possessed 1 unit or less of

these activities per mg. The results with these three analo-

gues indicated that the spatial relationship of the 5-carbox-

amide group to the rest of the molecule is extremely important to

activity.

Thus our studies involving the presence or absence of these

carboxamide groups and those just discussed on the spatial rela-

tionship of these groups to the rest of the molecule point to the

possibility that the carboxamide group of the asparagine residue

is of special significance to the activity of oxytocin. The pres-

ence of this carboxamide group may be required for the attach-

ment of the hormone to its receptor sites or for bringing about a

particular conformation of the molecule which is a prerequisite

for appreciable biological activity.

As shown in Table I, replacement of the disulfide moiety by

hydrogen, giving rise to dithio-oxytocin, results in complete loss

of activity. The remaining chemical functional group, the

carboxamide group in the glycaminamide residue in position 9, still

awaits evaluation as part of the systematic replacement of chemi-

cal functional groups of oxytocin by hydrogen. This aspect of

the relation of structure to biological activity is being investi-

ated.

SUMMARY

Two analogues of oxytocin in which the glutamine and aspara-

gine residues have been replaced by L-α-amino butyric acid and

L-alanine residues, respectively, have been prepared by total

synthesis according to the stepwise p-nitrophenyl ester method.

The carboxamide groups in positions 4 and 5 of the hormone

have been replaced by hydrogen. The 4-decarboxamido-oxyto-

cin (4-L-α-amino butyric acid-oxytocin) was found to possess

approximately one-fifth of the avian depressor activity and one-

sixth of the oxytocic activity of oxytocin, whereas these activities

in 5-decarboxamido-oxytocin (5-L-alanine-oxytocin) were of the

order of 0.2 to 0.3 unit per mg.

These striking differences in the activities of the 4- and 5-

decarboxamido analogues suggest an important role for the

carboxamide group of asparagine in determining the biological

effects of oxytocin.

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The Synthesis and Pharmacological Study of 4-Decarboxamido-oxytocin (4-α-Aminobutyric Acid-oxytocin) and 5-Decarboxamido-oxytocin (5-Alanine-oxytocin)
Vincent Du Vigneaud, George S. Denning, Jr., Stefania Drabarek and W. Y. Chan


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