SYNTHESIS AND PROPERTIES OF OXYTOCIN ANALOGUES ACTING AS IRREVERSIBLE INHIBITORS OF THE UTEROTONIC RESPONSE TO OXYTOCIN*

Michal LEBL, Vera BOJANOVSKA, Tomislav BARTH and Karel JOŠT

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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A series of compounds containing reactive groups of different chemical character was synthetized from [4-glutamic acid]deamino-1-carba-oxytocin or from [2-O-methyltyrosine, 4-glutamic acid]deamino-1-carba-oxytocin. The analogues acted as specific irreversible inhibitors of the uterotonic response to oxytocin. Active esters of the initial compounds were the most effective inhibitors.

A number of inhibitors of oxytocin** (*Ia*) have been synthetized so far. However, most of them were competitive inhibitors, incapable of covalent binding with the receptor[2]. Specific irreversible inhibitors could be used for studying the action of the hormone at molecular level. In order to obtain compounds with these properties it is necessary to introduce a reactive group into the analogue molecule which would be capable of forming a covalent bond with the functional group or groups of the macromolecules of the receptor in the target tissue. On the other hand, no unspecific covalent bonds should be formed. It is practically impossible to prevent reactions with proteins or other polymers which are not part of the receptor (during transport in the vicinity of the receptor compartment). At best, we can hope that these "side" reactions will only decrease the quantity of the inhibitor that reaches the receptor and will not cause qualitative changes in the response of the tissue. In order to eliminate intra- or intermolecular reactions of the reactive groups with other functional groups of the molecule, several modifications of the structure of oxytocin (*Ia*) were performed. The α-amino group of cysteine in position 1 was removed, the disulfide bond was substituted by a thioether group and the hydroxyl group of tyrosine in position 2 was transformed into a methyl ether.

Irreversible inhibitors of peptide hormones described as yet were obtained, for example, by the acylation of the N-terminal amino acid of angiotensin *II* by *p*-[N,N-

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** The nomenclature and symbols of amino acids and peptides were used according to published suggestions[1]. The amino acids used in this work were of L-configuration.

-bis(2-chloroethyl)amino]phenylbutyric acid[3] or by substituting pyroglutamic acid of luliberin by the same reactive group[4]. The only irreversible inhibitor of neurohypophysial hormones studied so far was N-bromoacetyloxytocin[5] which irreversibly blocks adenylate cyclase of the frog bladder and rabbit kidney, but does not inhibit the uterotonic response[6]. Analogues that had the amino group of cysteine in position 1 acylated by a maleoylglycyl or maleoylundecanoyl group[6] did not have intrinsic activity or inhibitory properties. We obtained reversible inhibitors of oxytocin by acylation of [2-O-methyltyrosine]oxytocin (*Ib*) by a maleoylglycyl[7] or bromoacetyl[8] group. The diazotation of [2-*p*-aminophenylalanine]oxytocin also did not produce an irreversible inhibitor[9].

In our work* we concentrated on modifications in position 4 of the peptide chain, as it is known that they do not significantly hamper the biological activities of the resultant analogues (ref.[9,11,12]). The starting material of our experiments was [4-glutamic acid]deamino-1-carba-oxytocin (*Ic*), the synthesis of which was published earlier[13]. We have already described suitable method for the transformation of this analogue into the highly active deamino-1-carba-oxytocin[13] (*Id*) or into other analogues substituted in position 4 (*cf.*[11,12]). Due to the fact that the analogues described in this paper have a strongly reactive group in their molecule, it was not possible to isolate, purify and characterize them by the methods commonly applied to other analogues of neurohypophysial hormones and biologically active peptides. Purification was limited to gel filtration on Sephadex LH-20 in dimethylformamide or on Bio-Gel P–4 in diluted acetic acid, or to precipitation from a dimethylformamide or methanol solution by ether. The analogues were usually characterized by chromatography, spectroscopy or by their reaction with ammonia (resulting in the known compound *Id*). In this way it was difficult to eliminate the presence of traces of other compounds used in the reaction or of the products. Therefore, we checked that the solvents and reagents used (dimethylformamide, dicyclohexylcarbodiimide, 1-hydroxybenzotriazole and *p*-nitrophenol) did not influence the contractions of the isolated uterus under our experimental conditions.

Peptide *Ie* was prepared by the reaction of analogue *Ic* with S-benzylcysteine methyl ester, using dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The protective benzyl group was removed directly before performing the biological assay by using sodium in liquid ammonia. The freeze-dried reaction product *If* was dissolved in oxygen-free water and the solution was used for the tests. The medium in which the uterine strip was suspended was saturated with nitrogen instead of oxygen for 5 min prior to the addition of analogue *If* and during its presence in the bath. We made sure that this treatment did not influence the response of the uterus to oxytocin.

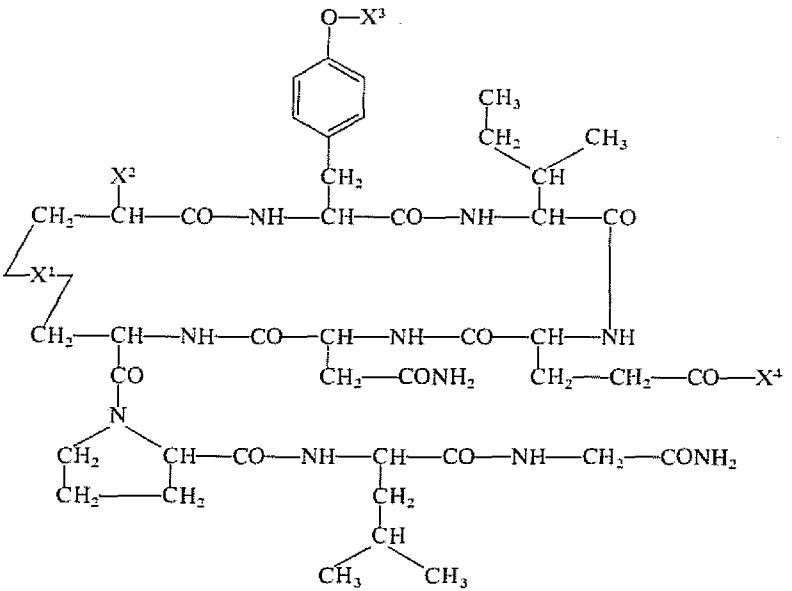
The mixed anhydride *Ig* was prepared by the reaction of compound *Ic* with sec-butyl chloroformate in the presence of N-ethylmorpholine. The structure of compound *Ig*

* Some of the results were published in a preliminary report[10].

was checked by IR spectroscopy and by its transformation into compound *Id*. The next three analogues had a common intermediary product, namely hydrazide *Ih* (ref.[11]). The reaction of *Ih* with maleoylglycine in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole resulted in compound *Ii* (characteristic band in the IR spectrum and amino-acid analysis). Even after two gel filtrations (Sephadex LH–20 and Bio-Gel P–4), compound *Ii* contained a second component (approximately 20% by TLC) which, as we assume, has the structure of a maleamic acid derivative (by analogy with maleoylglycyl-oxytocin[6]). The reaction of bromoacetyl bromide with hydrazide *Ih* produced compound *Ij*, the structure of which was verified by transforming the bromoacetyl residue into a glycine residue by the action of ammonia, and by amino-acid analysis. The treatment of hydrazide *Ih* by nitrite resulted in the formation of azide *Ik* which had a characteristic band in the IR spectrum; its reaction with ammonia gave compound *Id*.

Most attention we paid to compounds of the active ester type. The reaction of compound *Ic* with dicyclohexylcarbodiimide and *p*-nitrophenol did not produce the active ester required; it seemed probable that a side reaction took place. In order to check whether another group of the analogue molecule apart from the free carboxyl was not affected, we tested the properties of deamino-1-carba-oxytocin (*Id*) in the presence of dicyclohexylcarbodiimide and *p*-nitrophenol or 1-hydroxybenzotriazole. We observed no changes in the properties of the analogue tested. The reaction of compound *Ic* with *p*-nitrophenol is probably very slow and we therefore prepared 1-hydroxybenzotriazole ester *Il* by using dicyclohexylcarbodiimide. The preparation of this compound is complicated by a concurrent reaction of the active ester formed with dimethylamine liberated from dimethylformamide used as a reaction medium[11]; it is therefore necessary to use dimethylformamide freshly distilled from picric acid[14]. The structure of compound *Il* was checked by IR spectroscopy which showed a band characteristic of this type of ester[15,16] (1730 cm^{-1}), by UV spectroscopy before and after the alkalinization of the solution (liberation of 1-hydroxybenzotriazole) and by transformation into the known analogue *Id* by means of ammonia. The compound originating from the reaction of analogue *Ic* with dicyclohexylcarbodiimide alone is probably N-acylurea, as it does not react with ammonia and it is not an irreversible inhibitor. The *p*-nitrophenyl ester *Im* was prepared by the reaction of analogue *Ic* with bis(*p*-nitrophenyl) sulphite in pyridine. The content of nitrophenyl ester was determined by the UV spectrum and by transformation into compound *Id*. Analogue *In* was prepared with the aim of studying the importance of the localization of the reactive group. The carbodiimide condensation of compound *Ic* and *p*-nitrophenyl ester of ε-aminocaproic acid, as well as the action of the same amino component on the mixed anhydride *Ig* results in a product which contains more than one equivalent of ε-aminocaproic acid after hydrolysis. It was therefore more suitable to treat [4-γ-glutamoyl-ε-aminocaproic acid]deamino-1-carba-oxytocin (prepared as described earlier[11]) with bis(*p*-nitrophenyl) sulphite.

We also prepared two analogues in which the hydroxyl group of the tyrosine residue was transformed into a methyl ether. Both active esters, *Io* and *Ip*, were



Ia, $X^1 = S—S$, $X^2 = NH_2$, $X^3 = H$, $X^4 = NH_2$
Ib, $X^1 = S—S$, $X^2 = H$, $X^3 = CH_3$, $X^4 = NH_2$
Ic, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = OH$
Id, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = NH_2$
Ie, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = NHCH(CH_2SC_7H_7)—OCH_3$
If, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = NHCH(CH_2SH)—OCH_3$
Ig, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = OCOOCH(CH_3)C_2H_5$
Ih, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = NHNH_2$
Ii, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = NHNHCOCH_2N$ (succinimido)
Ij, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = NHNHCOCH_2Br$
Ik, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = N_3$
Il, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = O—C_6H_4N_3$[a]
Im, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = O—C_6H_4—NO_2(p)$
In, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = H$, $X^4 = NH(CH_2)_5COO—C_6H_4—NO_2(p)$
Io, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = CH_3$, $X^4 = O—C_6H_4N_3$[a]
Ip, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = CH_3$, $X^4 = O—C_6H_4—NO_2(p)$
Iq, $X^1 = CH_2—S$, $X^2 = H$, $X^3 = CH_3$, $X^4 = OH$

[a] 1-Hydroxybenzotriazole residue.

synthetized from analogue *Iq* (prepared according to an earlier paper[12]) in a similar way as analogues *Il* and *Im*.

The action of the analogues prepared was tested on isolated strips of the rat uterus. The individual analogues had intrinsic activity and they also behaved as irreversible inhibitors of the action of oxytocin (*Ia*). This type of biological effect was observed in the case of analogues *If*, *Ig* and *Ii*–*Im*. Both active esters *Il* and *Im*, were very strong inhibitors; after their application the uterine strip completely stopped reacting to oxytocin doses. Analogues *Io* and *Ip* which have the hydroxyl group of tyrosine substituted by a methoxy group also had very strong inhibitory action but no intrinsic activity; this is in agreement with their expected properties[17,18].

The analogues prepared had the following uterotonic activities (values given in parenthesis are I.U./mg): *If* (1·8), *Ig* (16·0), *Ii* (5·0), *Ij* (2·0), *Ik* (0·6), *Il* (160), *Im* (5·0), *In* (not determined), *Io* (0), *Ip* (not determined). The fact that some of the inhibitors have intrinsic activity (although its determination is encumbered by significant error) strongly suggests that the analogues really react with the active site of the receptor or its immediate vicinity. The occurrence of irreversible inhibition is within certain limits independent on the chemical nature of the reactive group introduced into the molecule. It would therefore follow that the individual inhibitors react with different functional groups of receptors in the target tissue. The quantitative differences between the intrinsic activities and inhibitory potencies of the individual inhibitors could be caused by the higher or lower reactivity of the reactive groups with macromolecules during the transport and in the vicinity of the receptor compartment. On the other hand, the position of the reactive group in the inhibitor molecule is very important. The results of our earlier experiments showed[7,8] that, when the maleoylglycyl and bromoacetyl groups are bound to the amino group of cysteine in position 1, the inhibitory action of the compounds is reversible. However, the reactive groups placed in position 4 are capable of covalent binding only if they are not too far from the backbone of the molecule. When the active ester was shifted by inserting the ε-aminocaproic acid residue, as in the case of compound *In*, no inhibitory potency was observed. The inhibitory action is specific for the oxytocin receptor. The response to prostaglandin $F_2\alpha$ was not influenced by compound *Il*; the addition of prostaglandin increased the maximum response of the uterus which had been decreased by the presence of compound *Il*.

All the results obtained indicate that a covalent bond is formed between the analogue with inhibitory action and the oxytocin receptor in the rat uterus. The suitable labelling of these analogues would enable detailed studies of the receptor in the target tissue, its isolation and characterization.

EXPERIMENTAL

Thin-layer chromatography was performed on ready-for-use Silufol (Kavalier Glassworks-Votice, Czechoslovakia) silica gel sheets in the following solvent systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3) and pyridine–1-butanol–acetic acid–water (10 : 15 : : 3 : 6) (S4). Spots were detected by chlorination. Samples for amino-acid analysis were hydrolysed for 20 h at 105°C in 6M-HCl (in ampoules sealed at 150 Pa) and analyses were carried out on an automatic analyzer (Developmental Workshops, Czechoslovak Academy of Sciences, type 6020). The UV spectra were taken on a Specord UV VIS (Carl Zeiss, Jena) apparatus. The IR spectra were measured in KBr micropellets on a UR 10 (Carl Zeiss, Jena) apparatus. Gel filtration was carried out on Sephadex LH—20 (Pharmacia, Uppsala) in dimethylformamide or on Bio-Gel P—4 (Bio-Rad Laboratories, Richmond) in 3M acetic acid.

[4-γ-Glutamoyl-S-benzylcysteine Methyl Ester]deamino-1-carba-oxytocin (*Ie*)

To the solution of [4-glutamic acid]deamino-1-carba-oxytocin[13] (*Ic*) (10 mg) in dimethylformamide (500 μl) 1-hydroxybenzotriazole (27 mg), S-benzylcysteine methyl ester hydrochloride (26 mg), and N-ethylpiperidine (15 μl) were added. The mixture was cooled to 0°C and after the addition of dicyclohexylcarbodiimide (41 mg) it was left aside for 1 h at 0°C and for 20 h at room temperature. The solution was diluted with dimethylformamide (2·5 ml), dicyclohexylurea was filtered off and the solution was placed on a column of Sephadex LH—20 in dimethylformamide. The peak containing peptide material was evaporated at room temperature (150 Pa). The residue was dissolved in a mixture of methanol (0·2 ml) and 3M acetic acid and transferred to a column of Bio-Gel P—4. Lyophilization of the main peak afforded 8·2 mg of the compound with R_F 0·53 (S1), 0·43 (S2), 0·59 (S3), and 0·74 (S4). For $C_{55}H_{79}N_{11}O_{14}S_2.4\,H_2O$ (1254) calculated: 52·66% C, 6·99% H, 12·28% N; found: 52·40% C, 6·71% H, 11·96% N.

[4-γ-Glutamoyl-cysteine Methyl Ester]deamino-1-carba-oxytocin (*If*)

Protected peptide *Ie* (4·3 mg) was dissolved in liquid ammonia (10 ml) and reduced with a sodium rod until the blue color persisted for 60 s. Ammonium chloride was added and the ammonia was dried from the frozen state. The residue was taken up in oxygen-free water and used for the biological tests.

[4-γ-Glutamic Sec-Butyl Carbonic Anhydride]deamino-1-carba-oxytocin (*Ig*)

To the solution of the compound *Ic* (4 mg) and N-ethylmorpholine (2 μl) in dimethylformamide (100 μl), cooled to —20°C, sec-butyl chloroformate (3 μl) was added. After standing at —20°C for 10 min the mixture was warmed to room temperature, the product was precipitated with ether and reprecipitated from dimethylformamide with ether. The product was dried *in vacuo* and used for the biological tests. IR spectrum: ν(CO) 1818, 1798 (infl.) and 1762 cm^{-1}. The reaction with ammonia gives the compound *Id* (40% according to TLC); the reaction mixture has uterotonic activity of about 400 I.U./mg.

[4-γ-Glutamoyl-(N′-maleoylglycine) Hydrazide]deamino-1-carba-oxytocin (*Ii*)

To the solution of hydrazide[11] *Ih* (3·5 mg) in dimethylformamide (250 μl), maleoylglycine (15·5 mg) and 1-hydroxybenzotriazole (13·5 mg) were added, the solution was cooled to 0°C and di-

cyclohexylcarbodiimide (20·6 mg) was added. After standing for 1 h at 0°C and 20 h at room temperature the mixture was diluted with dimethylformamide (2 ml), the solution was filtered and placed on a column of Sephadex LH—20. The pooled fractions containing peptide material were evaporated (room temperature, 150 Pa). The yield of the product was 2 mg; R_F 0·13 and 0·19 (S1), 0·00—0·30 (S2, diffuse) and 0·13 (S3). A part of this product was dissolved in 3M acetic acid (1 ml) and filtered through the column of Bio-Gel P—4; the compound obtained had the same chromatographic properties as above. IR spectrum: ν(CO) 1719 cm^{-1}. Amino-acid analysis: Asp 1·10, Glu 1·08, Pro 0·92, Gly 1·97, Ile 1·05, Leu 1·05, Tyr 0·90, Cys(C_3H_6COOH) 0·90 (hydrolysis was carried out for 3 h at 160°C in a mixture of concentrated HCl–propionic acid[19]).

[4-γ-Glutamoyl-N'-bromoacetohydrazide]deamino-1-carba-oxytocin (*Ij*)

To the solution of hydrazide[11] *Ih* (3 mg) in 1M-$NaHCO_3$ (0·3 ml), cooled to 0°C, the solution of bromoacetyl bromide (50 µl) in dioxane (0·3 ml) was added. The pH of the solution was maintained for 60 min on the value 8—9 by the addition of 1·5M-Na_2CO_3. The addition of acetic acid lowered the pH to 4, the solution was diluted with 3M acetic acid (total volume 3 ml) and transferred to a column of Bio-Gel P—4. Fractions containing peptide material (absorption at 280 nm) were pooled and dried from the frozen state. The yield was 1·9 mg of the product with R_F 0·29 (S1), 0·30 (S2), 0·32 (S3), and 0·70 (S4). A part of the product (0·4 mg) was dissolved in dimethylformamide (0·2 ml), the solution was bubbled through with ammonia for 30 min, set aside for 1 h, and the product was precipitated with ether. Amino acid analysis: Asp 1·06, Glu 0·96, Pro 1·00, Gly 1·78, Ile 1·00, Leu 1·04, Tyr 1·02, Cys(C_3H_6COOH) 0·93.

[4-γ-Glutamoyl Azide]deamino-1-carba-oxytocin (*Ik*)

To the solution of hydrazide[11] *Ih* (5·8 mg) in dimethylformamide (0·15 ml) 2M-HCl in ether (20 µl) was added, the solution was cooled to —30°C and 1-butyl nitrite (4 µl) was added. After standing at —30°C for 10 min, the temperature was raised to 0°C during 20 min and the product was precipitated with ether, washed with ether and dried *in vacuo*. The yield was 5·5 mg of the product with R_F 0·09 (S1) and 0·02 (S2). IR spectrum: $\nu(N_3)$ 2275 and 2145 cm^{-1}. The reaction with ammonia (analogously as in the case of the compound *Il*) gives in 50% yield a compound chromatographically identical with the analog *Id*.

[4-Glutamic Acid γ-1-Hydroxybenzotriazole Ester]deamino-1-carba-oxytocin (*Il*)

To the solution of the analog *Ic* (ref.[13]) (5 mg), *p*-nitrophenol (13 mg), and 1-hydroxybenzotriazole (13·5 mg) in dimethylformamide (0·25 ml), cooled to 0°C, dicyclohexylcarbodiimide (20·6 mg) was added. The mixture was set aside for 1 h at 0°C, 20 h at room temperature; after dilution with dimethylformamide (2 ml) and filtration, the solution was transferred to a column of Sephadex LH—20. Fractions containing peptide material (280 nm) were evaporated, the residue was precipitated from dimethylformamide with ether and dried *in vacuo*. In an alternative working up of the mixture, the product was precipitated with ether, three times reprecipitated from dimethylformamide with ether and thoroughly washed with ether. The product decomposes during TLC (analogously as in the case of compound *Im*). IR spectrum: ν(CO) 1730 cm^{-1} (ref.[15] gives for this type of ester the value of about 1730 cm^{-1}). UV spectrum: 278 (max), 312 (sh); after alkalinization: 244 nm (max), 300 nm (max). The solution of the product (1 mg) was bubbled through with ammonia for 30 min and then left aside for another 30 min. The precipitation with ether gave a mixture of four compounds; the one with the same chromato-

graphic properties as analogue *Id* prevailed (60% according to TLC). The uterotonic activity of the mixture was 450 I.U./mg.

[4-Glutamic Acid γ-*p*-Nitrophenyl Ester]deamino-1-carba-oxytocin (*Im*)

The compound *Ic* (ref.[13]) (5 mg) and bis(*p*-nitrophenyl) sulphite (13 mg) were dissolved in pyridine (1 ml) and the solution was warmed for 4·5 h at 50°C. The product was precipitated with ether, thoroughly washed with ether and dried. The product decomposed during TLC. The proof was obtained in the following way: The title compound was subjected to preparative chromatography on the plate with silica gel (S1), a zone containing *p*-nitrophenol grouping (yellow-colored after detection with NaOH) was isolated and this compound again gave several spots on TLC, as was found for the compound before chromatographical purification. The R_F values of the compound *Im*: 0·50 (S1), 0·13 (S2), 0·55 (S3), 0·76 (S4). IR spectrum: 1751 cm^{-1} (slight). UV spectrum: 241 nm (min), 252 nm (sh), 257 nm (max), 263 nm (sh), 280 nm (sh); after alkalinization: 257 and 263 nm (sh), 278 nm (min), 295 and 410 nm (max). The content of *p*-nitrophenyl ester was estimated from this last maximum: 87%. The reaction of the compound *Im* with ammonia in dimethylformamide solution afforded a result comparable with that of compound *Il*. The treatment with 1M-NaOH gave a product chromatographically identical with the acid *Ic*.

[4-γ-Glutamoyl-ε-aminocaproic Acid *p*-Nitrophenyl Ester]deamino-1-carba-oxytocin (*In*)

a) To the solution of the compound *Ic* (5 mg), ε-aminocaproic acid *p*-nitrophenyl ester hydrobromide (prepared immediately before reaction from its benzyloxycarbonyl derivative with HBr in acetic acid) and N-ethylpiperidine (8 μl) in dimethylformamide (0·5 ml), cooled to 0°C, dicyclohexylcarbodiimide (20·6 mg) was added. After standing for 1 h at 0°C and 20 h at room temperature the product was precipitated with ether, dissolved in 3M acetic acid (3 ml) and transferred to a column with Bio-Gel P—4. The peptide material was recovered by lyophilization. Amino acid analysis: Asp 1·04, Glu 1·00, Pro 0·99, Gly 1·06, Ile 1·00, Leu 1·03, Tyr 0·97, Cys .(C_3H_6COOH) 0·92, ε-Aca 5·11.

b) To the solution of the anhydride *Ig* (prepared from 5 mg of the compound *Ic*) in dimethylformamide (0·2 ml), ε-aminocaproic acid *p*-nitrophenyl ester hydrobromide (20 mg) and N-ethylmorpholine (4 μl) were added. After 1 h standing at room temperature the mixture was worked up as described under *a*). The product obtained again contained about 4 equivalents of ε-aminocaproic acid.

c) A solution of [4-γ-glutamoyl-ε-caproic acid]deamino-1-carba-oxytocin[11] (5 mg) and bis(*p*-nitrophenyl) sulfite (12 mg) in pyridine (1 ml) was warmed for 5 h to 50°C and the product was precipitated with ether. After reprecipitation from methanol and ether the product had R_F values: 0·34 (S1), 0·20 (S2), 0·40 (S3), 0·67 (S4). The content of *p*-nitrophenyl ester determined photometrically[16], was 83%.

[2-O-Methyltyrosine, 4-Glutamic Acid γ-1-Hydroxybenzotriazole Ester]deamino-1-carba-oxytocin (*Io*)

The preparation of the title compound was the same as described for the compound *Il*. From 5 mg of the compound *Iq* (ref.[12]) 2·8 mg of the product was obtained, with R_F values 0·42 (S1) 0·37 (S2), 0·39 (S3), 0·70 (S4). The treatment with 1M-NaOH afforded a compound chromatographically identical with the initial acid *Iq*. UV spectrum: 258 nm (min), 276 nm (max), 283 nm (sh). Alkalinization shifted the minimum to 251 nm and absorption was enhanced at 290—320 nm and diminished at 320—345 nm.

[2-O-Methyltyrosine, 4-Glutamic Acid γ-*p*-Nitrophenyl Ester]deamino-1-carba-oxytocin (*Ip*)

From the compound *Iq* (2·5 mg) the *p*-nitrophenyl ester was obtained using the same procedure as in the case of the compound *Im*. The content of *p*-nitrophenyl ester was 76%.

Model Reaction of [4-Glutamic Acid]deamino-1-carba-oxytocin with Dicyclohexylcarbodiimide

To the solution of the compound *Ic* (3·3 mg) in dimethylformamide (0·2 ml), cooled to 0°C, dicyclohexylcarbodiimide (30 mg) was added. After 16 h at room temperature the filtered solution was transferred to a Sephadex LH-20 column, the fractions containing the peptide material were pooled and evaporated; R_F values: 0·55 (S1), 0·48 (S2), 0·56 (S3), 0·80 (S4). The product was devoid of the properties of an irreversible inhibitor. Its dimethylformamide solution was bubbled through with ammonia (30 min) and the product was precipitated with ether. The chromatographical mobility did not change.

Pharmacological Methods

The analogues prepared were tested for their uterotonic activity and for their ability to inhibit the response to oxytocin. The assay was performed using an isolated rat uterus[20,21]. All the analogues tested had intrinsic activity which necessitated the following experimental arrangement. The response of the uterus to oxytocin was determined before and after the action of the inhibitor. First, the dependence of the response on the log of oxytocin doses (cumulative doses) was determined until the maximum response was achieved. Oxytocin was then removed from the bath by repeated washing and the compound that was expected to have inhibitory properties was added in a dose of 10—100 μg/6 ml (bath volume). After 5 min the uterine strip was thoroughly washed until all contractions stopped. Afterwards, the dependence of the response of the uterus on the log of oxytocin doses was determined again. All the reactive analogues prepared, with the exception of compound *In*, were found to be irreversible inhibitors of the action of oxytocin; compounds *Il*, *Im*, *Io* and *Ip* were most effective. The other analogues, even when tested in higher concentrations (by one order of ten), decreased the maximum responses to oxytocin by 40—60%. The inhibitory action was specific for oxytocin, which was documented by the fact that the addition of prostaglandin $F_2\alpha$ (250 μg) increased the maximum response that had previously been decreased by the presence of compound *Il*.

Certain other compounds, namely dimethylformamide, dicyclohexylcarbodiimide, 1-hydroxybenzotriazole and *p*-nitrophenol, were tested under the same experimental conditions as the inhibitors. These compounds were applied in molar concentrations ten times higher than those of the inhibitors, but did not influence the dependence of the uterotonic response on the log of oxytocin doses applied cumulatively.

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