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Oxytocin analogs effective as noncompetitive inhibitors in uterotonic test

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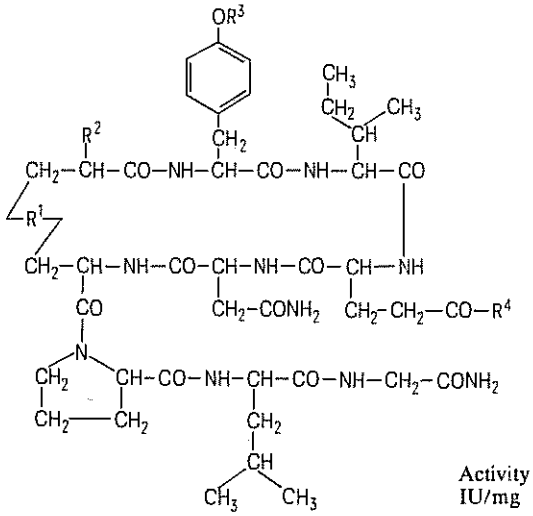
Summary. Deamino-l-carba-oxytocin analogs with a chemically reactive group in position 4 were demonstrated to act as noncompetitive oxytocin inhibitors in the assay on isolated rat uterus.

Even though numerous oxytocin (**Ia**) inhibitors are known[1], no compounds with a specific irreversible effect have so far been reported. Our efforts to obtain this type of compound by substitution of the primary amino group of the cysteine in position 1, have resulted merely in the preparation of products showing a competitive inhibitory effect[2,3].



	Activity in uterotonic assay (isolated rat uterus) IU/mg
Ia, $R^1 = S{-}S$, $R^2 = NH_2$, $R^3 = H$, $R^4 = NH_2$	500
Ib, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = H$, $R^4 = OH$	1
Ic, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = H$, $R^4 = NHCH(CH_2SH)COOCH_3$	1.8
Id, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = H$, $R^4 = OCOOCH(CH_3)C_2H_5$	16
Ie, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = H$, $R^4 = NHNHCOCH_2N$(CO–CH, CO–CH)	5
If, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = H$, $R^4 = NHNHCOCH_2Br$	2
Ig, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = H$, $R^4 = N_3$	0.6
Ih, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = H$, $R^4 = O{-}C_6H_4N_3$*	160
Ii, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = CH_3$, $R^4 = O{-}C_6H_4N_3$*	0
Ij, $R^1 = CH_2{-}S$, $R^2 = H$, $R^3 = CH_3$, $R^4 = OH$	0.1

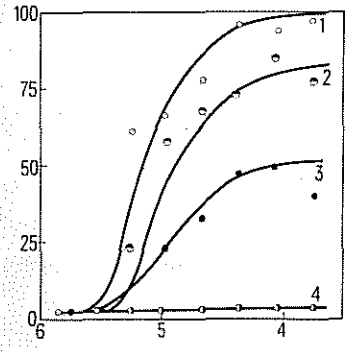
*N-Hydroxybenzotriazole.

In this study we have been able to synthesize a series of analogs derived from deamino-oxytocin[4] whose disulfide bond was replaced by a thioether group; these analogs have different reactive groups at the γ-carbon of the glutamic acid in position 4.

Materials and methods. As starting material [4-glutamic acid] deamino-l-carba-oxytocin[5] (**Ib**) was used. Analog **Ic** was prepared by condensation of the latter with S-benzyl-cysteine methyl ester, followed by removal of the protecting group by sodium in liquid ammonia. Compound **Id** was obtained by the reaction of analog **Ib** with sec-butyl chloroformate in the presence of N-ethylmorpholine. The common intermediary product for the preparation of 3 other analogs was [4-glutamic acid-γ-hydrazide]deamino-l-carba-oxytocin prepared by carbodiimide condensation of compound **Ib** with Boc-hydrazine and subsequent removal of the tert-butyloxy-carbonyl group in trifluoroacetic acid. The reaction of this hydrazide with maleoylglycine affected by dicyclohexylcarbodiimide in the presence of N-hydroxybenzotriazole afforded analog **Ie.** The treatment of the hydrazide with bromoacetylbromide afforded analog **If,** while treatment with n-butyl nitrite and hydrogen chloride gave analog **Ig.** Compound **Ih** was prepared from analog **Ib** in dimethylformamide solution by carbodiimide condensation in the presence of N-hydroxybenzotriazole. An analogous treatment of product **Ij** afforded analog **Ii.** The analogs synthesized were purified by gel filtration on a column of Bio-Gel P-4 in 3 M acetic acid (**If**) or on Sephadex LH-20 in dimethylformamide (**Ie, Ih, Ii**); analogs **Id** and **Ig** were precipitated by the addition of ether to their dimethylformamide solutions. Their purity was checked by thin-layer chromatography on silica gel. A detailed account of the syntheses and characteristics of the compounds prepared will be given in the Collection of Czechoslovak Chemical Communications.

Results and discussion. The analogs synthesized were tested for the effect on isolated rat uterus[6,7] and for inhibitory activity against the uterotonic effect of oxytocin. The activity values are given in the table and a typical record of the decrease of maximal biological activity against oxytocin after the action of analog **Ih** is shown in the figure. All the analogs synthesized in this study behaved as typical irreversible oxytocin inhibitors. The strongest inhibitors under the experimental conditions chosen (see legend to the figure) were analogs **Ih** and **Ii**; the remaining products, tested at concentrations higher by 1 order, decreased maximal response to oxytocin by 40–60%. The inhibitory effects of the compounds tested should be attributed to the analogs themselves, since the presence (if any) of the reaction components (dimethylformamide, dicyclohexylcarbodiimide, N-hydroxybenzotriazole) is under the reaction conditions given without effect on uterus contractions induced by oxytocin. The inhibitory effect is specific of oxytocin; the addition of prostaglandin $F_{2\alpha}$ increased the maximal response, which has been decreased by analog **Ih,** to the original maximal value.

The modifications of the oxytocin molecule left intact its characteristic structural features which are necessary for its binding to the oxytocin receptor in the uterus. This is evidenced by the activity of all analogs (with the exception of analog **Ii**) which, to a rough approximation, can be correlated with their inhibitory power. The strongest inhibitor of all the products synthesized is analog **Ih** which completely eliminates the sensitivity of the uterus to oxytocin (figure). This is the result of a specific effect of analog **Ih** since the action of prostaglandin $F_{2\alpha}$ was not affected. The examination of oxytocin effect as a function of the dose after the application of the inhibitor and its washing off the bath clearly indicate the noncompetitive nature of the inhibition[8] characterized by a decrease of the maximal response to oxytocin. The properties of an irreversible inhibitor require the proper location of the reactive groups in the peptide molecule[2,3], the chemical character of the groups is of lesser importance. The fact that all the analogs tested in this study are irreversible inhibitors leads us to believe that analogs **Ic–Ii** are covalently bonding to various groups of the receptor in the target tissue (or its immediate neighbourhood) rather than to react with 1 functional group only. We cannot, however, disregard the possibility that the testing of these analogs is paralleled by nonspecific bonding which manifests itself by differences in quantitative parameters (such as activity itself and inhibitory power). An answer to these questions could provide the isolation of the receptor macromolecules with covalently bonded inhibitors, which in this particular case can be regarded as affinity label compounds.



Noncompetitive inhibitory effect of analog **Ih** on contraction of rat uterus in vitro produced by oxytocin. The uterotonic test was carried out according to Holton[6] using the modification of Munsick[7]; the uterus contractions were recorded by a magnetoelectric scanner. Ordinate: uterus contraction in mm, abscissa: -logarithm of oxytocin concentration (mM). Curve 1: effect as function of cumulated oxytocin doses, 2: effect as function of cumulated oxytocin doses after 5-min treatment with 2 μM solution of analog **Ih** and its washing off, 3: repetition of experiment under the conditions described for 2, 4: effect as function of cumulated doses after 5-min treatment with 10 μM solution of analog **Ih** and its washing off.

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