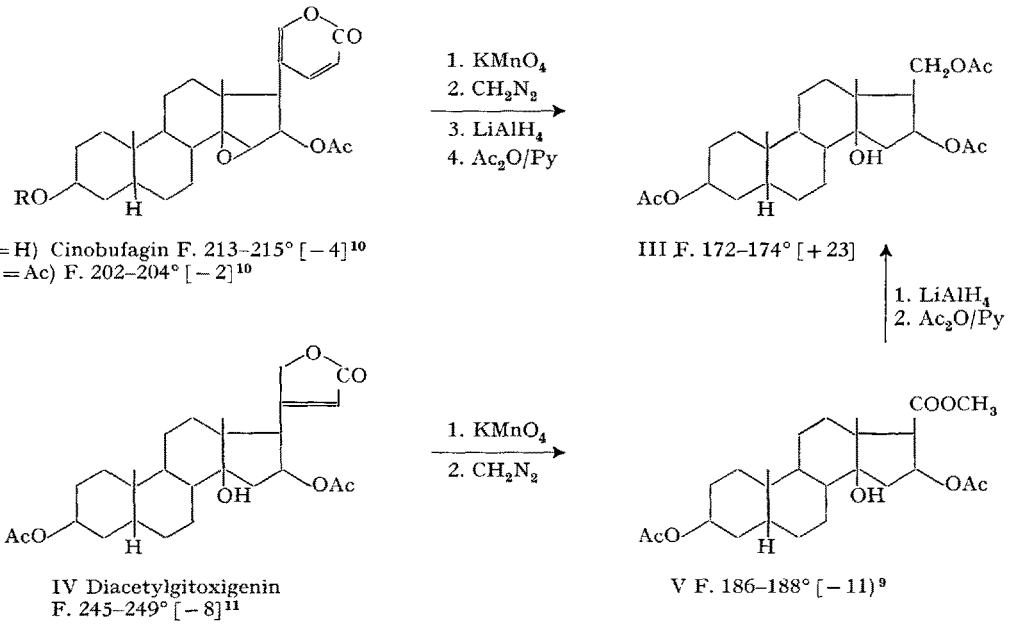


I (R=H) Cinobufagin F. 213–215° [–4][10]
II (R=Ac) F. 202–204° [–2][10]

III F. 172–174° [+23]

IV Diacetylgitoxigenin
F. 245–249° [–8][11]

V F. 186–188° [–11][9]

Ac = $CH_3CO$–. Die Zahlen in eckigen Klammern geben die auf ganze Grade auf- oder abgerundete spez. Drehung für Na-Licht in Chloroform an.

(IV)] und Acetylierung des dabei gebildeten Tetrols gewonnen worden.

Cinobufagin ist durch die hier geschilderte chemische Verknüpfung mit Gitoxigenin in seiner Konstitution praktisch völlig aufgeklärt. Es fehlt lediglich noch der Beweis für die zweite Haftstelle des an C–14 fixierten Oxydsauerstoffs. Da dieser in einer sekundär-tertiären Epoxygruppe (IR.-Spektrum, siehe oben) enthalten ist, kommt als andere Verknüpfungsstelle nur C–15 in Frage. Aus sterischen Gründen muss die von C–15 ausgehende Bindung dieselbe räumliche Lage einnehmen wie diejenige an C–14. Das Epoxyd ist also 14$\beta$,15$\beta$-ständig angeordnet. Cinobufagin ist chemisch somit als 3$\beta$-Hydroxy-14,15$\beta$-epoxy-16$\beta$-acetoxy-5$\beta$-bufa-20,22-dienolid (I) zu bezeichnen.

P. HOFER, H. LINDE und K. MEYER

*Pharmazeutische Anstalt der Universität Basel, den 9. Juni 1959.*

*Summary*

The structure of Cinobufagin is shown to be 3$\beta$-Hydroxy-14,15$\beta$-epoxy-16$\beta$-acetoxy-5$\beta$-bufa-20,22-dienolide.

[10] Literaturangaben siehe bei [4].

[11] Literaturangaben siehe bei H. JÄGER, O. SCHINDLER und T. REICHSTEIN, Helv. chim. Acta *42*, 977 (1959).

## Inhibition of Oxytocin Inactivation by Some Peptides

The peptide hormones are, as has been known for some time, inactivated by certain tissues in the organism. This process is undoubtedly of considerable importance in regulating the effects of these highly active compounds[1]. The elucidation of the chemical structure of a number of peptide hormones now makes it possible to study the mechanism of their biological inactivation in more detail; and this in turn should open up new possibilities for acting upon the neurohumoral regulating mechanisms of higher organisms.

Oxytocin is enzymatically inactivated in the mammalian organism by what has been called 'oxytocinase'[2]. Very little is so far known about this enzyme, or enzymes; it has merely been recorded that such activity is found in some tissues, e.g. liver or pancreas[3,4], and, at a high level, in human pregnancy plasma[2,3,5].

We wish here to present some preliminary results of work aimed at controlled intervention in the function of oxytocin and oxytocinase. We have been able to find, in certain simple peptides[6,7] corresponding to structural fragments of the hormone, specific inhibitors of the enzymes inactivating oxytocin in the organism.

Oxytocic activity was measured by the method of PAGE[5] with minor modifications[4]. As sources of the enzymes, we used normal human pregnancy serum[3,5], mouse pancreas and liver slices, and mouse liver cell sap[4]. Since all the tissue preparations showed the same behaviour to inhibitors, we shall refer to them in the sequel collectively as 'tissue oxytocinase' preparations.

The samples were incubated in a Warburg apparatus in Krebs' Medium III at 37°C in an atmosphere of oxygen for 30–60 min. The medium contained 2 i.u. of oxytocin, the inhibitor at the concentration stated, and the serum (30–40 mg of protein), tissue slices (5–7 mg of protein), or cell sap (2–4 mg of protein). The results of a number of experiments are recorded in the Table. Chromatographic

[1] I. A. MIRSKY, Recent Progr. Hormone Res. *13*, 429 (1957).

[2] K. v. FEKETE, Endokrinologie *7*, 364 (1930); *10*, 16 (1932).

[3] E. WERLE and K. SEMM, Arch. Gynäkol. *187*, 449 (1955).

[4] S. LÉBLOVÁ, I. RYCHLÍK, and F. ŠORM, Coll. Czechosl. chem. Comm., in press.

[5] E. W. PAGE, Amer. J. Obstet. Gynec. *52*, 1014 (1946); Science *105*, 292 (1947).

[6] M. ZAORAL and J. RUDINGER, Chem. listy *49*, 745 (1955); Coll. Czechosl. chem. Comm. *20*, 1183 (1955).

[7] J. RUDINGER, J. HONZL, and M. ZAORAL, Chem. listy *50*, 288 (1956); Coll. Czechosl. chem. Comm. *21*, 202 (1956).

Inhibition of Oxytocinase

| Compound | Concentration millimolar | Oxytocinase activity[a] | | | |
|---|---|---|---|---|---|
| | | Pregnancy serum | Pancreas slices | Liver slices | Liver cell-sap |
| – | – | 100 | 100 | 100 | 100 |
| Component amino-acids of oxytocin | (0·1%) | 100 | 1·7 | – | – |
| The same, without cystine | (0·1%) | 100 | 59 | – | 100 |
| Cystine | 0·87 | >100 | 1·5 | 13·7 | 2·5 |
| Carbobenzoxy-cystine | 0·87 | – | – | – | 34 |
| S-Benzylcysteine | 1·5 | >100 | 37 | 60 | 61 |
| Carbobenzoxy-S-benzylcysteine | 1·5 | – | – | – | 70 |
| Prolyl-leucyl-glycine amide | 1·5 | 116 | 90 | 86 | 113 |
| Carbobenzoxy-prolyl-leucyl-glycine amide | 1·5 | 100 | – | – | 100 |
| S-Benzylcysteinyl-prolyl-leucyl-glycine amide | 1·5 | 11 | 19·2 | 33 | 13·5 |
| Carbobenzoxy-S-benzylcysteinyl-prolyl-leucyl-glycine amide | 0·75 | >100 | – | – | 77 |
| Cystinyl-*bis*-(prolyl-leucyl-glycine amide) | 1·5[b] | 2 | 1·4 | 1·8 | 2 |
| Glutaminyl-asparaginyl-S-benzylcysteinyl-prolyl-leucyl-glycine amide | 1·5 | 28 | 1·4 | 33 | 3 |
| Glutaminyl-asparagine | 1·5 | >100 | – | – | 60 |
| S-Benzylcysteinyl-tyrosine | 1·5 | 44 | 1·8 | 1·5 | 6·8 |
| S-Benzylcysteinyl-tyrosine amide | 0·75 | 40 | – | – | – |
| Carbobenzoxy-S-benzylcysteinyl-tyrosine | 1·5 | 60 | – | – | 45 |
| S-Benzylcysteinyl-glycine | 1·5 | >100 | 1·8 | 1·5 | 6 |
| Glutathione | 1·5 | >100 | 100 | 100 | 100 |

[a] Expressed as percentage of control calculated on the basis of first-order rate constants[5].

[b] Concentration approximate; the peptide was not isolated in a pure state[12].

analyses were carried out, after deproteinisation by boiling, in the solvent system methyl-ethyl-ketone-pyridine-water (70:15:15)[8].

In addition to the compounds listed in the Table, the following peptides were examined[9]: glycyl-glutamine, tyrosyl-glycine, carbobenzoxy-α-glutamyl-tyrosine, and carbobenzoxy-tyrosyl-glycyl-glycine; none of these, however, inhibited oxytocinase, and some caused slight stimulation. The effect of cysteine itself was rather variable, probably owing to the varying degree of oxidation undergone by it during the aerobic incubation.

Plasma oxytocinase was inhibited only by peptides representing structural fragments of the oxytocin molecule, and containing a cystine or S-benzylcysteine residue. Inhibition by these peptides appears to be rather specific: S-benzyl-cysteinyl-glycine, which is not a fragment of the oxytocin structure, was without effect as an inhibitor. Substitution of the free amino group in S-benzylcysteinyl-tyrosine by a carbobenzoxy group sharply lowers the inhibitory effect; in other analogous cases, the inhibitor activity disappears altogether.

The pronounced specificity of oxytocinase is also borne out by the results of chromatographic experiments, which showed S-benzylcysteinyl-tyrosine amide to be split rapidly at the peptide bond by pregnancy serum while S-benzylcysteinyl-prolyl-leucin-glycine amide and S-benzylcysteinyl-glycine were practically unattacked under the same conditions. It is therefore likely that S-benzylcysteinyl-tyrosine amide inhibits the inactivation of oxytocin as a competitive substrate, whereas S-benzylcysteinyl-prolyl-leucyl-glycine amide acts as a specific inhibitor. The fission of S-benzyl-cysteinyl-tyrosine amide to S-benzylcysteine and tyrosine amide by the pregnancy serum is presumably responsible for the decreased effectiveness of this peptide in inhibiting the inactivation of oxytocin, as compared with the activity of S-benzylcysteinyl-prolyl-leucyl-glycine amide. Our results are in good agreement with the findings of TUPPY and NESVADBA[10], who showed the oxytocin molecule to be split between the hemicystine and tyrosine residues by human pregnancy serum.

In the case of tissue oxytocinase, the situation is somewhat more complicated. Unlike plasma oxytocinase, this enzyme is very strongly inhibited by free cystine. This effect cannot be accounted for merely by a non-specific action on sulphydryl-disulphide equilibria of the substrate or enzyme, since S-benzylcysteine, which cannot act by a redox mechanism, also causes marked inhibition. The effect of peptide-bound S-benzylcysteine is several times greater than that of the free amino-acid; the structural requirements for inhibition of the tissue enzyme are, however, less rigorous than in the case of plasma oxytocinase. The presence of a cystine or S-benzylcysteine residue again appears to be required, but peptides not corresponding to any sequence in the oxytocin molecule, such as S-benzylcysteinyl-glycine, are also effective. Substitution of the cysteine amino group by carbobenzoxy lowers the activity considerably. Chromatographic analysis showed that the peptides which strongly inhibit the inactivation of oxytocin by tissue preparations are themselves broken down to the individual amino-acids by these same preparations.

These results indicate that the oxytocin-inactivating enzymes are most probably peptidases; though the rigid requirement for the presence of sulphur suggests that the possibility of a exocystine desulphydrase type of activity be kept in mind in the case of the tissue enzyme[11].

Details of this work, together with further experiments, will be published in due course in the Collection of Czechoslovak Chemical Communications.

The peptides used were prepared by J. RUDINGER, M. ZAORAL, and K. JOŠT, of the Department of Organic Synthesis of this Institute.

Z. BERÁNKOVÁ, I. RYCHLÍK, and F. ŠORM

*Department of Biochemistry, Institute of Chemistry, Czechoslovak Academy of Science, Prague, April 27, 1959.*

*Zusammenfassung*

Es wird festgestellt, dass einige peptidische Fragmente des Oxytocins dessen Abbau durch Schwangerenserum, Mäusepankreas und Mäuseleber hemmen.

[8] I. M. HAIS and K. MACEK, *Handbuch der Papierchromatographie* (Gustav Fischer Verlag, Jena 1958).

[9] All amino acids (except glycine) were of the L configuration.

[10] H. TUPPY and H. NESVADBA, Mh. Chem. *88*, 977 (1957).

[11] J. P. GREENSTEIN and F. M. LEUTHARDT, J. nat. Cancer Inst. *5*, 209 (1944).

[12] D. W. WOOLLEY, R. B. MERRIFIELD, C. RESSLER, and V. DU VIGNEAUD, Proc. Soc. exp. Biol. Med., N.Y. *89*, 669 (1955).