Table 2. Triacylglycerol (TG) content and incorporation of $^3H_2O$ into total lipid* in liver and carcass of male $A^{vy}/a$ mice**

| Treatment | N | Body weight (g) | μmoles TG/ g b.wt | Liver weight (g) | μmoles TG/ g liver | μmoles $^3H_2O$ incorporated into total lipid in carcass/g b.wt | μmoles $^3H_2O$ incorporated into total lipid in liver/g liver wt |
|---|---|---|---|---|---|---|---|
| Control | 10 | 40.0±1.7* | 278±25 | 1.62±0.09 | 39±10 | 3.5±0.3 | 31±3 |
| 5α-androstan-17-one 10 mg/kg, i.p. | 8 | 31.7±0.8*** | 144±15*** | 1.39±0.05*** | 12± 1*** | 3.0±0.1 | 33±1 |

*Mean±SE. **Both control and treated mice were 5–6 months of age at the time of sacrifice. ***$p<0.05$.

was used to facilitate the suspension of 5α-androstan-17-one in saline.

*Results and discussions.* Male and female $A^{vy}/a$ mice given 5α-androstan-17-one, 200 mg/kg, s.c., 3 times weekly were prevented from becoming obese (figure 1). Like dehydroepiandrosterone[2], the effect of treatment was reversible upon withdrawal of 5α-androstan-17-one injections (figure 1). At 10 mg/kg, i.p., the compound also controlled the weight gain of male $A^{vy}/a$ mice (figure 2).

The effect of 5α-androstan-17-one on the weight of $A^{vy}/a$ mice was not due to appetite suppression (table 1). Treated mice that were autopsied revealed no pathological changes that would account for the weight difference between treated and control mice[1]. The only finding that was significant was an increase of eosinophilic cells in the ovary of 4 of the 5 female $A^{vy}/a$ mice treated with the compound at 200 mg/kg, s.c. The meaning of this change is not clear to us.

The concentration of triacylglycerol in the carcass and livers of treated $A^{vy}/a$ mice was substantially less than that of age-matched control $A^{vy}/a$ mice (table 2). This difference in triacylglycerol content amounts to 63% of the difference in weight between control and treated $A^{vy}/a$ mice, suggesting that the compound acts on the metabolism of triacylglycerol. However, unlike dehydroepiandrosterone-treated mice, which had lower hepatic lipogenesis rates[2], the 5α-androstan-17-one-treated mice had lipogenesis rates not significantly different from those of control mice (table 2). This suggests that 5α-androstan-17-one prevents $A^{vy}/a$ mice from becoming obese through a mechanism other than the inhibition of lipogenesis.

Alternatively, it is possible that the inhibition of triacylglycerol synthesis by 5α-androstan-17-one is masked in younger mice. Previous studies on dehydroepiandrosterone[2] were done on older mice which probably had a higher rate of triacylglycerol synthesis than the younger mice used in the present study. Since we were measuring the incorporation of $^3H_2O$ into all lipid species, changes in the rate of triacylglycerol synthesis in older mice should be easier to detect because triacylglycerol synthesis represents a higher proportion of their total lipid synthesis.

Regardless of the mechanism, this study shows that in addition to dehydroepiandrosterone, a second, structurally similar mammalian glucose-6-phosphate dehydrogenase inhibitor, namely 5α-androstan-17-one, can also prevent $A^{vy}/a$ mice from becoming obese.

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## Oxytocin analogs effective as noncompetitive inhibitors in uterotonic test

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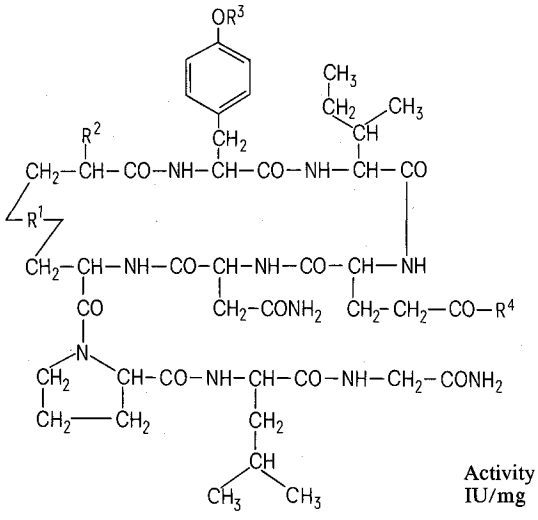
*Summary.* Deamino-l-carba-oxytocin analogs with a chemically reactive group in position 4 were demonstrated to act as noncompetitive oxytocin inhibitors in the assay on isolated rat uterus.

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].

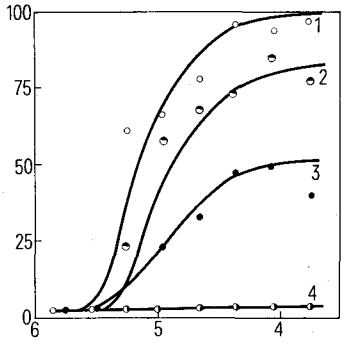
In this study we have been able to synthesize a series of analogs derived from deamino-oxytocin[4] whose disulfide bond was replaced by a thioether group; these analogs have different reactive groups at the γ-carbon of the glutamic acid in position 4.

*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



| | 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.




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.

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.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## The synovial fluid hyaluronic acid in rheumatoid arthritis[1]


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*Summary.* The intrinsic viscosity of hyaluronic acid in synovial fluid decreases significatively in mild and severe arthritis (24% and 37% respectively). Variation in hyaluronic acid concentration parallels the above results. Chondroitin-6-sulfate can be detected in about 30% of the arthritic fluids.


Chemically, the principal acidic glycosaminoglycen (GAG) in human synovial fluid is hyaluronic acid, although the presence of other GAG has been described[3–5]. It is presumably formed in the periarticular connective tissue by the same cells which produce hyaluronic acid in all other connective tissues. Most of the studies have been carried out in larger animals, because of the minute amounts of fluid that can be obtained from the largest human joint, the knee joint, in vivo. It has been shown to contain up to 2 ml of fluid.

This report presents further data on hyaluronic acid in normal human synovial fluid obtained in vivo and from human joints with mild and severe rheumatoid arthritis[6], in order to get further insight into the hyaluronic acid alteration in this disease.

*Material and methods.* The fluids were obtained from 27 donors (male, age 18–40 years) with clinically normal joints; from 6 patients with severe rheumatoid arthritis (male, age 24–38 years) and from 18 cases of mild rheumatoid arthritis (male, age 21–32 years) by puncturing the knee joint as described by Balasz et al.[7]. All patients were not under corticoid treatment when samples were taken. As much fluid as possible was obtained from each normal jount (0.4–1.0 ml). Owing to the small amount of fluid obtained from the controls, the fluids of 3 joints were pooled. The fluid volume obtained from the pathological joints varied from 1.5 to 5.2 ml and was studied individually. Prior to analysis, all samples were centrifuged at 75,000 × g to remove the cells, and stored at −20 °C. Intrinsic viscosity of hyaluronic acid was determined as described by Sundblad[8]. The GAG were precipitated by the addition of cetylpiridinium chloride to final concentration of 0.2% and incubated at 37 °C for 1 h. The centrifuged crude GAG were purified by dissolving in 1.25 M magnesium chloride. The resulting GAG was then precipitated with 3 vol. of 2% sodium acetate in ethanol 95% for 24 h. Further purification was obtained by redissolving the GAG in 5% potassium acetate and precipitating with 3 vol. of ethanol 95% for 12 h. Purified GAG thus obtained was dissolved in 0.75 M magnesium chloride for further analy-

Table 1. Intrinsic viscosity of hyaluronic acid in normal and arthritic synovial fluids

| | Cases | Intrinsic viscosity* | |
|---|---|---|---|
| Normal fluid | 27 | 3850 | |
| Arthritic fluid, mild | 18 | 2920 | −24% |
| Arthritic fluid, severe | 6 | 2340 | −37% |

*(cc/g) mean. The measurements were made at velocity gradients of 500 $sec^{-1}$.

Table 2. Concentrations of acid glycosaminoglycans in normal and pathological synovial fluid

| | Normal fluid* | Pathological fluid | |
|---|---|---|---|
| | | *mild* | *severe* |
| Total GAG | 251.4 ± 18.3 | 121.3 ± 8.3 | 88.1 ± 5.6 |
| Hyaluronic acid | 228.3 ± 14.3 | 98.2 ± 5.8 | 68.7 ± 4.8 |
| Chondroitin-4-sulfate | 5.3 ± 0.3 | 2.8 ± 0.4 | 2.7 ± 0.2 |
| Chondroitin-6-sulfate | 2.6 ± 0.1 | 2.6 ± 0.1 | 2.1 ± 0.1 |
| Recovery (by addition) | 236.2 | 103.6 | 73.5 |

*Average of 9 pooled samples. Concentration of GAG was based on 40% uronic acid (carbazol) content. Figures are expressed as mg of GAG/100 ml of fluid ± SE. For statistic analysis see the text.