Beiträge der Wirkstofforschung 24, 182-197, 1985

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MONO- AND DIIODO-DERIVATIVES OF NEUROHYPOPHYSIAL HORMONES AND THEIR ANALOGUES

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Introduction

The preparation of radioactively labelled biologically active peptides is of considerable importance for basic research. When dealing with neurohypophysial hormones and some of their synthetic analogues, the procedure usually includes tritiation of halogenated derivatives of the compounds concerned. In most cases, the halogenated derivative was prepared by substituting a halogen for the hydrogen atom in the tyrosine residue of the complete peptide chain (FLOURET et al. 1977, FRAKER and SPECK 1978, HUNTER and GREENWOOD 1962, Mc FARLANE 1958) rather than by using halogenated tyrosine in the synthesis of the peptide (KÉRI and TEPLAN 1978). Several procedures have been elaborated for the iodination of neurohypophysial hormones and their analogues; mono- as well as diiodo-derivatives have been thus obtained. In order to achieve high specific radioactivity of the resultant peptide derivative, it is necessary to use the purest possible precursors. The use of diiodo-derivatives is most suitable in this respect. In the present paper we compare several methods for preparing and partly characterizing mono- and diiodo-derivatives of neurohypophysial hormones and some analogues used in clinical practice or planned for clinical trials.

Materials

8-L-Arginine vasopressin, 8-L-lysine vasopressin, oxytocin and N-glycyl-glycyl-glycyl-8-L-lysine vasopressin, prepared in Léčiva, were purified by HPLC. [8-L-Arginine,9-desglycinamide]-vasopressin was prepared in the Institute of Organic Synthesis, Riga.

Results and Discussion

A comparison was made of three simple methods with regard to their efficacy to prepare diiodo-derivatives of neurohypophysial hormones and their analogues which are then used as precursors for catalytic tritiation. The following methods were used: iodination with elementary iodine (FLOURET et al. 1977), with iodide in the presence of Iodogen (FRAKER and SPECK 1978) and with iodide in the presence of Chloramine T (HUNTER and GREENWOOD 1962). The reaction products were analysed by means of reversed phase HPLC on a 0.4 x 25 cm column packed with 5 µm octadecyl silica (Separon SIX, Laboratory Instruments, Prague) using a VCM 300 model pump, universal LC injector and UVM 4 variable-wavelength UV monitor with a 10 µl flow-through cell (the apparatus was manufactured by Developmental Workshop of Czechoslovak Academy of Sciences). Elution was performed isocratically at room temperature using a methanol-water system (4:6) containing 0.1% trifluoroacetic acid at a flow-rate of 1 ml/min. The mobile phase was passed through a 0.5 µm membrane filter (Millipore EAWP) and degassed in vacuo.

All three iodination methods tested resulted in a mixture of the free hormone with its monoiodo- and diiodo-derivatives. Iodination in the presence of Chloramine T produced a number of unidentified oxidation by-products in addition to the monoiodo-derivative as the main product, even though the reaction time was short (40 s). Iodination using elementary iodine resulted mainly in the monoiodo-derivative, and only a small amount of by-products was formed. When more iodine was used or the reaction time was prolonged with the aim of increasing the yield

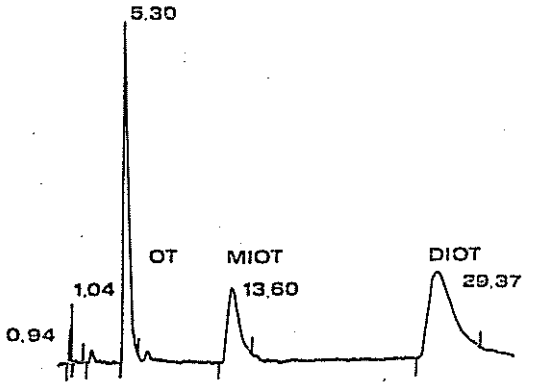

Figure 1. Chromatogram of the reaction mixture after iodination of oxytocin using Iodogen

of the diiodo-derivative, the opposite was achieved and finally no absorbance at 280 nm was noticable. Iodination in the presence of Iodogen produced almost no by-products and the yield of the diiodo-derivative could be increased by prolonging the reaction time. This method therefore proved to be most suitable. However, it was not possible to plate Iodogen in sufficient amounts onto the walls of the reaction vessel, because only a thin film adhered to the walls. We therefore performed further iodination experiments with Iodogen in aqueous suspension according to the following scheme: 3 mg of peptide were dissolved in 1 ml of 0.1 M Tris-HCl buffer, pH 7.5, 1.3 mg of Iodogen was added and the mixture agitated, 1.8 mg of NaI dissolved in 200 µl of water was added and the mixture was stirred occasionally in the course of 10 min. The suspension was centrifuged for 5 min at 10 000 RPM and the supernatant was fractionated by HPLC. The results of this procedure can be seen in the recording of the separation of the mixture obtained by the iodination of oxytocin (Fig. 1).

The identity of the individual peaks was checked by measuring the UV spectra (Specord, Zeiss, Jena), the iodo-derivatives having a characteristic bathochromic shift (oxytocin:

$\lambda_{max}^{w,pH\ 6}$=274.5, log ε=3.15; monoiodo-oxytocin: $\lambda_{max}^{w,pH\ 6}$=282, log ε =3.37, $\lambda_{max}^{w,pH\ 12.2}$=305, log ε =3.61; diiodo-oxytocin: $\lambda_{max}^{w,pH\ 6}$ = = 286, log ε =3.34, $\lambda_{max}^{w,pH\ 12.2}$=311, log ε =3.76). As can be seen in Fig. 1, the method is highly effective (the yield being 53% of DIOT, 16% of MIOT and 21% of OT) and produces almost no by--products under the mild conditions used. It is also obvious that reversed phase HPLC is convenient for separating iodo-derivatives of neurohypophysial hormones and their analogues. The introduction of the iodine atom into the molecule leads to a significant prolongation of the retention time. Table 1 states the capacity factors for all the peptides studied.

We also investigated the influence of iodination on the biological activity of neurohypophysial hormones. In the case of 8-lysine vasopressin, we determined binding and activation of adenylate cyclase in the membrane fraction of the rat kidney medulla (BUTLEN et al. 1983) and binding to the membrane fraction of rat liver (BARTH et al. 1983) (Tab. 2, 3). The introduction of one or two iodine atoms into the ortho position of tyrosine significantly decreased the binding affinity of the peptide in both membrane systems by approximately three orders of ten. The biological activity of the iodo-derivatives of oxytocin was determined using the rat uterus in vitro (MUNSICK 1960).

Table 1. Retention of neurohypophysial hormones, their analogues and iodo derivatives on Separon SIX C 18

Peptide	Capacity Factor		
		monoiodo	diiodo
Oxytocin	4.64	13.47	30.24
[8-Lysine] vasopressin	1.28	3.80	8.98
[8-Arginine] vasopressin	1.02	1.54	4.62
[8-Arginine,9-desglycinamide vasopressin	1.60	5.46	+
N-Glycyl-glycyl-glycyl-[8-lysine] vasopressin	1.21	3.81	7.00

+ Elution was not achieved by 40% methanol.

Table 2. Activation of adenylate cyclase by iodo-derivatives of LVP and their binding to the rat kidney membrane system

Peptide	Adenylate cyclase pK_A	Binding pK_D
[8-Lysine] vasopressin	8.35	8.32
[2-(3′-Iodotyrosine),8-lysine] - vasopressin	5.30	5.59
[2-(3′,5′-Diiodotyrosine,8-lysine] - vasopressin	5.10	4.96

Table 3. Binding of iodo-derivatives of LVP to the rat liver membrane system

Peptide	Binding pK_D
[8-Lysine] vasopressin	8.33
[2-(3′-Iodotyrosine),8-lysine] vasopressin	6.22
[2-(3′,5′-Diiodotyrosine),8-lysine] vasopressin	5.32

Monoiodo- and diiodo-oxytocin were found to be mild competitive inhibitors of the uterotonic action of oxytocin (pA_2=7.2 and 6.3, respectively; pD_2 for oxytocin was 9.3).

Although the iodinated derivatives of neurohypophysial hormones interact with the receptors concerned and the specific activity of ^{125}I is higher than that of ^{3}H, their affinity is too low for them to be suitable radioactive ligands in binding studies. In our hands the diiodo-derivatives have appeared to be stable for a long period and can so well serve for the immediate preparation of the tritiated hormones.

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