Synthesis of Tritium-labelled Oxytocin and Lysine-vasopressin

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The high biological activity of the hormones of the posterior pituitary gland, oxytocin and vasopressin, necessitates the use of isotopically labelled hormones for the study *in vivo* of the physiology and biochemistry of these peptide hormones. In many respects, the labelling of peptides with tritium is convenient for this purpose, and some papers discussing the tritium labelling of oxytocin and vasopressin have appeared.[1-4] However, the methods hitherto applied have given too low specific radioactivities of the peptides to allow them to be studied under physiological conditions. A brief account is given in this paper of the total synthesis of specifically tritium-labelled oxytocin and vasopressin, which can be used in animal and human studies of the properties of these hormones *in vivo*.

The method used is depicted in Fig. 1.* It is based on the hydrogenolysis of 3-iodotyrosine with tritium gas according to Birkofer and Hempel,[5] and synthesis of the peptides by the *p*-nitrophenyl ester method.[6,7]

Experimental. L-*Tyrosine(3-^{3}H).* 338 mg of L-3-iodotyrosine and 100 mg of palladium-active carbon (10 %) were suspended in 2.2 ml of 1.5 M methanolic potassium hydroxide, and treated with 29.1 ml of tritium gas of normal conditions (1.3 mole and about 70 Curie tritium) for 2 h. The yield of L-tyrosine(3-^{3}H) was 160 mg. 160 mg of non-radioactive L-tyrosine was added, and the whole amount was dissolved in 1 N hydrochloric acid, and tyrosine

* The following abbreviations are used: Bz, benzyl; Cbz, carbobenzoxy; Ph, phenyl; Tos, *p*-toluenesulphonyl. All amino acids (except glycine) have the L configuration.

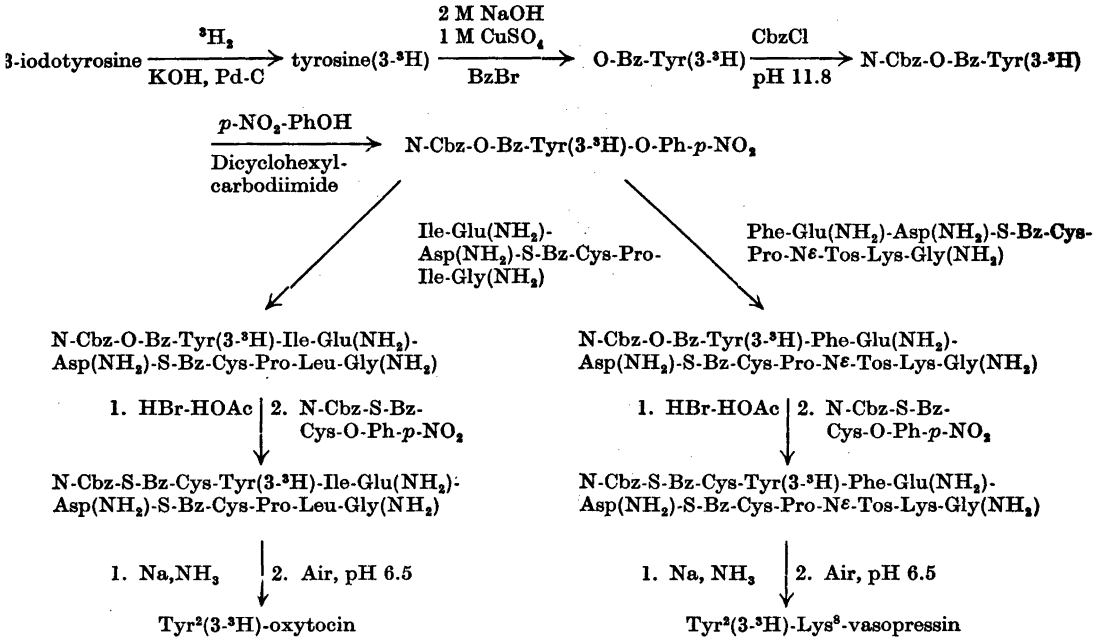

Fig. 1. Synthesis of Tyr^2(3-^{3}H)-oxytocin and Tyr^2(3-^{3}H)-lys^8-vasopressin.

reprecipitated at pH 6. Yield 300 mg. The tyrosine was radiochromatographically pure (descending paper chromatography in butanol-acetic acid-water, 4:1:5) and contained 7 Curie tritium/mmole.

N-Cbz-O-Bz-Tyr(3-^{3}H)-O-Ph-p-NO_2. The tyrosine was converted to its copper complex with sodium hydroxide and cupric sulphate, and benzylated under nitrogen with benzyl bromide. The O-benzyl-tyrosine was isolated (yield 218 mg) and suspended in water. The amino group was carbobenzoxylated with carbobenzoxy chloride at pH 11.8. The N-Cbz-O-Bz derivative was precipitated with acid (weight 316 mg). The substance was dissolved in ethyl acetate and esterified with *p*-nitrophenol and di-cyclohexyl-carbodiimide. Yield 360 mg of N-Cbz-O-Bz-Tyr(3-^{3}H)-O-Ph-*p*-NO_2.

Tyr^2(3-^{3}H)-oxytocin. From 200 mg of N-Cbz-Ile-Glu(NH_2)-Asp(NH_2)-S-Bz-Cys-Pro-Leu-Gly(NH_2), the carbobenzoxy group was split off in hydrogen bromide-acetic acid. To the free peptide dissolved in dimethylformamide and triethylamine, 130 mg of the labelled tyrosine derivative in dimethylformamide was added. The octapeptide obtained was treated with hydrogen bromide-acetic acid, and 243 mg of protected nonapeptide was obtained from the free peptide and N-Cbz-S-Bz-cysteine-*p*-nitrophenyl ester. After reduction in liquid ammonia with small pieces of metallic sodium and subsequent oxidation in dilute water solution, 80 000 international units of oxytocic activity estimated on the rat uterus were obtained. The specific radioactivity was 5.9 μC/IU or 2.9 C/mmole oxytocin.

Tyr^2(3-^{3}H)-lys^8-vasopressin. From 200 mg of N-Cbz-Phe-Glu(NH_2)-Asp(NH_2)-S-Bz-Cys-Pro-Nε-Tos-Lys-Gly(NH_2), the free heptapeptide was prepared in hydrogen bromide-acetic acid. The peptide was dissolved in dimethylformamide, and triethylamine was added together with 115 mg of the labelled and protected tyrosine ester in dimethylformamide. The protected octapeptide was formed during 20 h stay at room temperature. The free octapeptide was obtained after treatment with HBr-acetic acid, and the protected nonapeptide was prepared in dimethylformamide from the free octapeptide and N-Cbz-S-Bz-cysteine-*p*-nitrophenyl ester. Yield 209 mg of protected nonapeptide. The biologically active vasopressin was prepared in the same way as the labelled oxytocin. The pressor activity was totally 34 000 units, and the specific activity 6.5 μC/IU or 1.8 C/mmole vasopressin.

The labelled oxytocin and lysine-vasopressin were almost radiochemically pure, as could be judged after descending paper chromatography in butanol-acetic acid-water (4:1:5). About 95 % of the radioactivity was found on the same spot as the oxytocin monomer, and about 5 % in the place of the oxytocin dimer. After chromatography of lysine-vasopressin, the main amount of radioactivity (more than 95 %) was preceded and followed by small amounts of radioactivity.

A more detailed report will be given elsewhere.

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