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NOTE

BIOSYNTHETIC PREPARATION OF THE TRITIUM LABELLED 3α-HYDROXY-5α-PREGNAN-20-ONE, AN ALLOSTERIC MODULATOR OF GABAERGIC NEUROTRANSMISSION

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SUMMARY

A procedure for the biosynthetic preparation of 3α-hydroxy-5α-[1,2,6,7-^{3}H]-pregnan-20-one (^{3}H-HPO) with high specific activity convenient for binding studies was developed. ^{3}H-HPO was synthesized from [1,2,6,7-^{3}H]progesterone using a microsomal preparation from rat liver and NADPH. The progesterone metabolite was purified by silica gel 60 and Sephadex LH 20 chromatography. The identity of the product was confirmed by reverse isotopic dilution of chemically synthesized and highly purified 3α-hydroxy-5α-pregnan-20-one. The yield for radiolabelled HPO was 60% and its purity was > 98%.

Keywords: *3α-hydroxy-[1,2,6,7-^{3}H]-pregnan-20-one; biosynthetic preparation; progesterone; steroids*

INTRODUCTION

The $GABA_A$-receptor has besides the binding site for GABA at least three allosteric, pharmacologically identified binding sites. The effect of allosteric modulators of this receptor were commonly studied by determining alterations in the binding of ^{3}H-muscimol to $GABA_A$-receptor sites. Among the various modulators 3α-hydroxy-5α-pregnan-20-one (HPO), a metabolite of progesterone, has been suggested as an endogenous candidate mediating sex steroid effects on neurotransmission. To study the allosteric binding site for

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HPO in vitro, a highly purified tritium labelled ligand is necessary. This paper describes the biosynthetic preparation starting from commercially available tritium labelled progesterone. HPO (3) is formed from progesterone (1) via 5α-pregnanedione (2) by two metabolizing enzymes, 5α-reductase and 3α-hydroxysteroidoxidoreductase, in various mammalian tissues (Fig. 1).

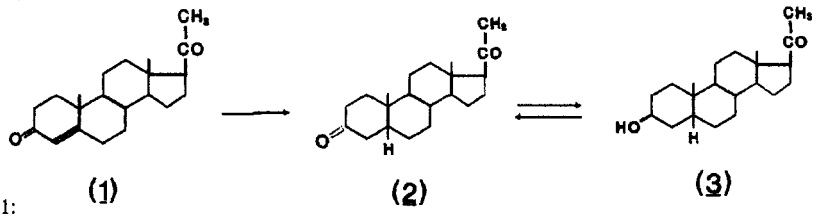

Fig. 1:

Biosynthetic formation of 3α-hydroxy-5α-pregnan-20-one (HPO) by 5α-reduction of progesterone and subsequent reversible 3α-reduction of 5α-dihydroprogesterone

MATERIAL AND METHODS

[1,2,6,7-^{3}H]-progesterone was purchased from Amersham-Buchler (Braunschweig, GER). The used buffer comprised 15 mM Tris-HCl, 8.7% glycerol and 10 mM monothioglycerol (= TGT-buffer, pH 7.4).

Biopreparation of 3α-hydroxy-5α-[1,2,6,7-^{3}H]pregnan-20-one (^{3}H-HPO). ^{3}H-HPO was synthesized from [1,2,6,7-^{3}H]progesterone with the aid of liver microsomes (preparation see next paragraph) in the presence of NADPH. 50 nmol NADPH and 2 nmol ^{3}H-progesterone, each dissolved in 1 ml TGT-buffer, were added to 5 ml of liver microsomes equivalent to 200 mg liver suspended in TGT-buffer. After incubation at 37°C for 60 min the reaction was stopped by adding 1 ml of ice-cold double distilled water. The steroids were then extracted with dichloromethane and subjected to chromatography on a silica gel 60 (Merck, Darmstadt, GER) column (230-400 mesh, length 20 cm, ID 1cm) with dichloromethane/acetone (10:1 v/v) as eluent system. Further purification of the ^{3}H-HPO was performed on a Sephadex-LH 20 (LKB-Pharmacia, Freiburg i. BRSG., GER) column (length 20 cm, ID 1 cm) with ethanol as eluent. Aliquots of the incubation assay as well as purified product were chromatographed on thin layer silica gel plates (Merck, Silica gel F_{254}) in the system dichloromethane/acetone (10:1 v/v).

Liver microsomes were prepared from rat (strain Han: SPRD, Zentralinstitut für Versuchstierzucht, Hannover, GER) livers that were perfused with physiological saline before homogenization of the tissue (TGT-buffer). A 25% (w/v) homogenate was made in TGT-buffer using a motor driven teflon glass homogenizer at 800 rpm. The homogenate was centrifuged at 10,000 g for 20 min (4°C) and the supernatant spun at 100,000xg for 60 min. The resulting pellet, equivalent to 200 mg liver, was washed, centrifuged as described above, resuspended in 5 ml TGT-buffer and frozen at -190°C till use for enzymatic formation of ^{3}H-HPO not later than 60 days.

A reference sample of inactive HPO was prepared essentially according to (2).

RESULTS AND DISCUSSION

1. Biopreparation of 3α-hydroxy-5α-[1,2,6,7-^{3}H]pregnan-20-one

It was the aim of the present study to obtain highly pure, tritium labelled 3α-hydroxy-5α-pregnan-20-one by stereoselective 3α- and 5α- hydrogenation of [1,2,6,7-^{3}H]progesterone. Monitoring the reaction course by TLC indicated that two parameters were critical: The ratio NADPH/substrate and incubation time. Under the conditions described here, the yield was 60% for 3α-hydroxy-5α-pregnan-20-one, which was the result of a compromise between the maximal extent of the hydrogenation of [1,2,6,7-^{3}H]progesterone and minimal transformation of [^{3}H]-HPO to more polar compounds.

A reference sample of inactive HPO was prepared essentially according to (2).

2. Identity of biosynthetically prepared tritium labelled 3α-hydroxy-5α-pregnan-20-one with the chemically synthesized compound.

A mixture of biosynthetically prepared 3α-hydroxy-5α-[1,2,6,7-^{3}H]pregnan-20-one with chemically synthesized HPO, crystallyzed from different solvents, exhibited constant specific activity (Tab. 1), indicating the identity of the radioactive compounds with the radioinert HPO.

Table 1. Reverse isotopic dilution of 3α-hydroxy-5α-pregnan-20-one

solvent	dpm/mg
cyclohexane	2866.7 ± 182.5
acetone	2916.6 ± 113.6
ethyl acetate	2829.1 ± 49.8

HPO was mixed with the putative ^{3}H-HPO in order to provide a specific activity of 100 Bq/mg.

The final radiopurity of ^{3}H-HPO was > 98%.

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