The biosynthesis of 3β-hydroxy-5,7-androstadien-17-one by the horse fetal gonad

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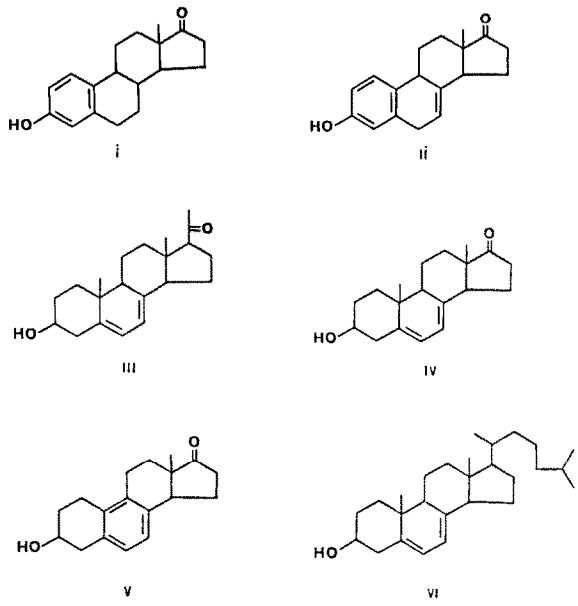
Horse fetal gonadal tissue was incubated with 3β-hydroxy-5,7-pregnadien-20-one and 5,7-cholestadien-3β-ol and it was shown that both substrates were converted to 3β-hydroxy-5,7-androstadien-17-one. These findings support the proposal that in this tissue there is a 5,7-diene pathway producing 3β-hydroxy-5,7-androstadien-17-one, the putative precursor of equilin in the placenta.

Equilin Horse Fetal gonad 3β-Hydroxy-5,7-androstadien-17-one 3β-Hydroxy-5,7-pregnadien-20-one 5,7-Cholestadien-3β-ol

1. INTRODUCTION

The blood and urine of pregnant mares contain high concentrations of estrogens, such as estrone (I), and also ring B unsaturated estrogens, such as equilin (II). The horse fetal gonad provides substrates for estrogen formation in the placenta [1,2]. It contains the steroidal 5,7-dienes, 3β-hydroxy-5,7-pregnadien-20-one (III) and 3β-hydroxy-5,7-androstadien-17-one (IV) as endogenous compounds [3] and can biosynthesise them from tritiated sodium acetate in vitro [4,5]. 3β-Hydroxy-5,7-pregnadien-20-one (III) has been proposed as the biosynthetic precursor of 3β-hydroxy-5,7-androstadien-17-one (IV), this compound being aromatised in the placenta to give equilin [4,5]. 3β-Hydroxy-5,7-androstadien-17-one could also be the precursor of 3β-hydroxy-5,7,9-estratrien-17-one (V) [6].

This report describes the in vitro conversion of 5,7-cholestadien-3β-ol (VI) to 3β-hydroxy-5,7-pregnadien-20-one and of the latter to 3β-hydroxy-5,7-androstadien-17-one by the horse fetal gonad. The structures of the above compounds are given in scheme 1.



Scheme 1. (I) Estrone, (II) 3-hydroxy-1,3,5(10),7-estratetraen-17-one, (III) 3β-hydroxy-5,7-pregnadien-20-one, (IV) 3β-hydroxy-5,7-androstadien-17-one, (V) 3β-hydroxy-5,7,9-estratrien-17-one, (VI) 5,7-cholestadien-3β-ol.

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2. EXPERIMENTAL

High performance liquid chromatography (HPLC) was carried out on a Waters radial compression C-18, 10 μM column. Thin-layer chromatography (TLC) was carried out on 0.30 mm layers of silicagel HF 254/366 (Merck). The solvent systems used were: petroleum ether (b.p. 60–80°C)-ethyl acetate ((A) 9:1, v/v); cyclohexane-acetone ((B) 3:2, v/v; (C) 2:1, v/v; (D) 4:1, v/v). Procedures for acetylation and hydrolysis of steroids were as described in [7]. 3β-Hydroxy-5,7-androstadien-17-one (IV) and 3β-hydroxy-5,7-pregnadien-20-one (III) were synthesised as in [8].

3β-Hydroxy-5,7-[4-^{14}C]pregnadien-20-one (spec. act. 2 GBq/mmol) was synthesised from [4-^{14}C]-pregnenolone acetate [8]. The product was purified by TLC in system A followed by HPLC, the mobile phase being 15% water in methanol, flow rate 2 ml/min. Retention times were 10.5 and 13.5 min for 3β-hydroxy-5,7-pregnadien-20-one and pregnenolone acetates, respectively. The 3β-hydroxy-5,7-[4-^{14}C]pregnadien-20-one acetate was hydrolysed and the free steroid purified by TLC in system C.

5,7-[1α,2α(*n*)-^{3}H]Cholestadien-3β-ol (VI) (spec. act. 35.7 GBq/mmol) was synthesised from [1α,2α(*n*)-^{3}H]cholesterol acetate [8]. The product was purified by TLC in benzene followed by HPLC, the mobile phase being methanol, flow rate 2 ml/min. Retention times were 18.5 and 22.25 min for 5,7-cholestadien-3β-ol and cholesterol acetates, respectively. The 5,7-[1α,2α(*n*)-^{3}H]-cholestadien-3β-ol acetate was hydrolysed and the free sterol purified by TLC in system C.

Fetal horse gonadal tissue (3.5 g from an 8–9 month fetus) was homogenised in 10 ml of phosphate buffer (0.1 mol/l, pH 7.4) using a glass homogeniser with a motor driven teflon pestle, 250 rpm, for 1 min. The buffer also contained nicotinamide (3 mmol/l), magnesium chloride (5 mmol/l) and reduced glutathione (7.5 mmol/l).

A portion of the homogenate (5 ml) was incubated at 37°C for 1 h in air with 5,7-[1α,2α-(*n*)-^{3}H]cholestadien-3β-ol (74 kBq, 0.8 μg) in propylene glycol (50 μl), 3β-hydroxy-5,7-androstadien-17-one (15 μmol/l), 3β-hydroxy-5,7-pregnadien-20-one (15 μmol/l), NADPH (0.7 mmol/l), glucose 6-phosphate (6 mmol/l) and glucose-6-phosphate dehydrogenase (0.2 unit/ml).

A further 5 ml of the homogenate was incubated at 37°C for 1 h in air with 3β-hydroxy-5,7-[4-^{14}C]-pregnadien-20-one (18.5 kBq, 2.9 μg) in propylene glycol (50 μl), 3β-hydroxy-5,7-androstadien-17-one (15 μmol/l), NADPH (0.7 mmol/l), glucose 6-phosphate (6 mmol/l) and glucose-6-phosphate dehydrogenase (0.2 unit/ml).

The incubations were extracted as previously described [4,5] and the extracts separated into sterol and steroid fractions by TLC in system B. The sterol fraction was acetylated and rechromatographed in system D giving cholesterol acetate which was purified further by HPLC using methanol, flow rate 2 ml/min, as the mobile phase. The steroid fraction was acetylated, carrier steroids 3β-hydroxy-5,7-pregnadien-20-one, 3β-hydroxy-5,7-androstadien-17-one, pregnenolone and dehydroepiandrosterone (DHA) acetates (0.15 μmol of each) were added, and the mixture chromatographed in system D giving a fraction containing these four steroids. This mixture was resolved into its components by HPLC using 10% water in methanol, flow rate 1 ml/min, as the mobile phase.

Carrier steroid (150 μmol) was added to a portion of the extracts from the final chromatograms and crystallised to constant specific activity [4,5].

3. RESULTS

Table 1 shows that, when 5,7-cholestadien-3β-ol was incubated with homogenates from two different horse fetal gonads, cholesterol, pregnenolone, DHA, 3β-hydroxy-5,7-pregnadien-20-one and 3β-hydroxy-5,7-androstadien-17-one were formed.

Similarly, incubations with 3β-hydroxy-5,7-[4-^{14}C]pregnadien-20-one as substrate gave 3β-hydroxy-5,7-androstadien-17-one. This was characterised as its acetate (spec. act. 1070 ± 10 dpm/μmol), 5,7-androstadiene-3β,17β-diol 3-acetate (spec. act. 1080 ± 40 dpm/μmol) and 5,7-androstadien-3β,17β-diol diacetate (spec. act. 1040 ± 20 dpm/μmol). With tissue from a second horse, the specific activities for the same three compounds were 648 ± 33; 661 ± 33 and 669 ± 19 dpm/μmol, respectively.

The DHA and pregnelone fractions isolated from the incubations with 3β-hydroxy-5,7-[4-^{14}C]-pregnadien-20-one were not radioactive.

Table 1

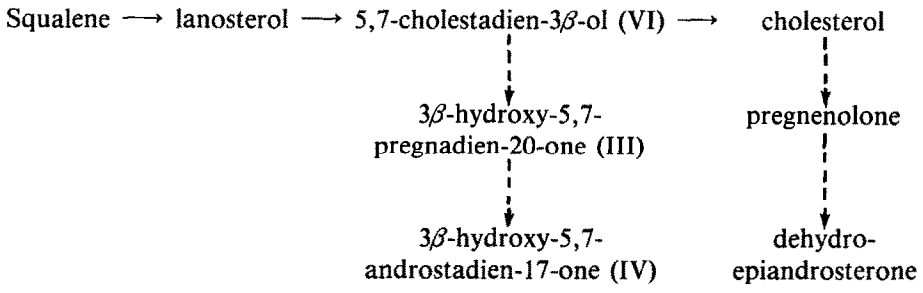
Identification of the steroids produced on incubating 5,7-cholestadien-3β-ol with homogenates of fetal horse gonadal tissue

Steroid	Derivative formed	Specific activity[a] dpm/μmol (SD)	
		Incubation (1)	Incubation (2)
Cholesterol		4530 (190)	3950 (100)
	cholesterol acetate	4560 (90)	3920 (50)
Pregnenolone		111 (2)	30.1 (0.7)
	5-pregnene-3β,20β-diol diacetate	109 (4)	30.7 (1.2)
Dehydroepiandrosterone		–	–
	androstenedione	19.4 (1)	69 (2.2)
3β-Hydroxy-5,7-pregnadien-20-one		–	–
	3β-hydroxy-5,7-pregnadien-20-one acetate	183 (1)	91.7 (1.8)
	5,7-pregnadiene-3β,20β-diol 3-acetate	184 (3)	89.3 (2.5)
	5,7-pregnadiene-3β,20β-diol diacetate	177 (3)	84.1 (3.5)
3β-Hydroxy-5,7-androstadien-17-one		–	–
	3β-hydroxy-5,7-androstadien-17-one acetate	129 (6)	311 (7)
	5,7-androstadiene-3β,17β-diol 3-acetate	132 (8)	303 (6)
	5,7-androstadiene-3β,17β-diol diacetate	137 (1)	320 (9)

[a] The specific activities were judged to be constant when three crops agreed to within ±5%. For brevity only the means and SD of the specific activities of each derivative are given

4. DISCUSSION

The conversion of 5,7-[1α,2α(*n*)-^{3}H]cholestadien-3β-ol and 3β-hydroxy-5,7-[4-^{14}C]pregnadien-20-one to 3β-hydroxy-5,7-androstadien-17-one supports the proposal that there is a 5,7-diene pathway operating in fetal horse gonadal tissue [4]. This, with the previous report that the tissue can biosynthesise these three compounds from radioactive sodium acetate [4,5], suggests that 3β-hydroxy-5,7-androstadien-17-one, the proposed precursor of equilin, is biosynthesised by the triterpene pathway.



This tissue was also able to transform 5,7-cholestadien-3β-ol to cholesterol, pregnenolone and DHA, implying that the classical pathway for steroid hormone biosynthesis, in which 5,7-cholestadien-3β-ol is reduced to cholesterol, was operational in addition to the 5,7-diene pathway. Also, since neither pregnenolone nor DHA was a product of the 3β-hydroxy-5,7-pregnadien-20-one incubations, it appears that they are produced from 5,7-cholestadien-3β-ol via cholesterol.

In contrast to these in vitro findings, it has been reported that [^{14}C]squalene was not converted to [^{14}C]equilin, on infusion in vivo into the equine feto-placental unit [9], implying that 5,7-cholestadien-3β-ol, and possibly 3β-hydroxy-5,7-pregnadien-20-one and 3β-hydroxy-5,7-androstadien-17-one, are not intermediates in equilin biosynthesis.

It might be argued that the use of homogenates brings into play enzymes to which the precursors are not exposed in vivo. Alternatively, infused precursors may not fully enter biosynthetic sequences in vivo because of differences in permeability and pool sizes [9]. These contrasting findings could be resolved by an in vivo experiment to test whether 5,7-cholestadien-3β-ol, injected into the umbilical circulation of the pregnant mare, is converted to the ring B unsaturated estrogens.

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