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Inhibition of cholesterol side-chain cleavage by intermediates of an alternative steroid biosynthetic pathway

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Mitochondrial preparations from endocrine tissues were incubated with radioactive cholesterol and the effect of hydroxylated metabolites of 23,24-dinor-5-cholen-3β-ol (23,24-dinor-5-cholene-3β,20-diol and 23,24-dinor-5-cholene-3β,21-diol) on the production of pregnenolone was measured. These compounds are intermediates in an alternative, sesterterpene pathway for steroid hormone biosynthesis. It was found that these materials, like the analogous side-chain-hydroxylated derivatives of cholesterol (20α-hydroxycholesterol and 22*S*-hydroxycholesterol), inhibit cholesterol side-chain cleavage. The possibility that there could be a control mechanism whereby metabolites of 23,24-dinor-5-cholen-3β-ol inhibit steroidogenesis occurring by the cholesterol pathway is discussed.

Pregnenolone; Steroid hormone synthesis; Steroidogenesis inhibitor; 23,24-Dinor-5-cholen-3β-ol; (Ovary, Adrenal)

1. INTRODUCTION

It has been demonstrated, at least in vitro, that steroidogenesis can occur by a sesterterpene pathway in addition to the conventional pathway through cholesterol [1]. In this pathway, a sesterterpene hydrocarbon (five isoprene units) is formed and subsequently converted to steroid hormones via the intermediate 23,24-dinor-5-cholen-3β-ol (Guneribol) (scheme 1, I). Rat testes and adrenals can convert both 23,24-dinor-5-cholen-3β-ol and cholesterol to steroid hormones in vitro. The conversion of the former occurs in the microsomal fraction, that of cholesterol taking place in the mitochondrial fraction [2,3].

23,24-Dinor-5-cholene-3β,20-diol (II) and 23,24-dinor-5-cholene-3β,21-diol (III), metabolites of 23,24-dinor-5-cholen-3β-ol, are biosynthesised from radioactive acetate in vitro [1,4,5]. The conversion of 23,24-dinor-5-cholen-3β-ol to the diols (II, III) occurs in the mitochondrial fraction of rat adrenals [3], but they do not undergo side-chain cleavage in these organelles (cf. [6,7]). However, 23,24-dinor-5-cholene-3β,20-diol is converted to steroid hormones by bovine adrenal homogenates [1], presumably by the microsomes [2,3]. 23,24-Dinor-5-cholene-3β,20-diol is also converted to cortisol by the guinea-pig in vivo [8].

The initial step in the conversion of cholesterol to steroid hormones is side-chain cleavage in the mitochondria to form pregnenolone. Side-chain-hydroxylated derivatives of cholesterol, e.g. 20α-hydroxycholesterol (IV) [9–11] and 22*S*-hydrocholesterol (V) [12] inhibit this conversion. Evidence is now presented that 23,24-dinor-5-cholene-3β,20-diol and 23,24-dinor-5-cholene-3β,21-diol also inhibit the side-chain cleavage of cholesterol. The possible significance of this finding for the control of steroidogenesis is considered.

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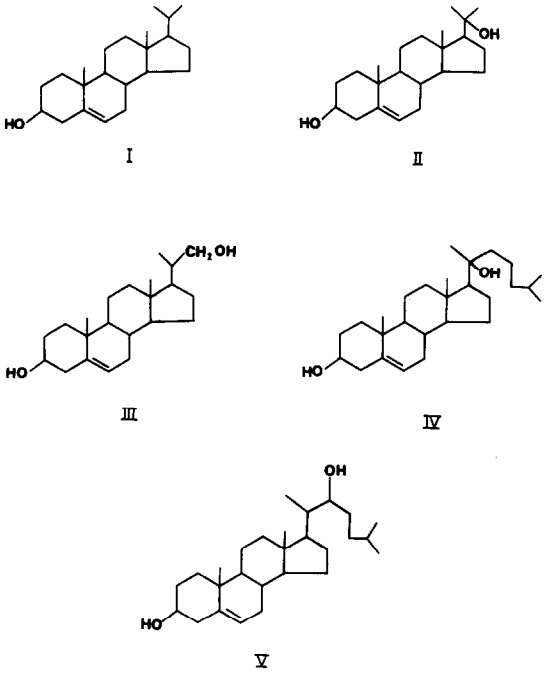

Scheme 1. Structural formulae of I–V.

2. MATERIALS AND METHODS

[7(n)-^{3}H]Cholesterol (spec. act. 185 GBq/mmol) was purchased from Amersham International (Amersham, Bucks). Syntheses of 23,24-dinor-5-cholen-3β-ol, 23,24-dinor-5-cholene-3β,20-diol and 23,24-dinor-5-cholene-3β,21-diol, general procedures and sources of materials have been described [2,4]. The solvent systems used for thin-layer chromatography were toluene-ethyl acetate [A, 3:2 (v/v); B, 1:4 (v/v)] and cyclohexane-acetone [C, 7:1 (v/v)].

Tissue (0.3–2 g) was homogenised by hand, using a glass homogeniser, in sucrose solution (0.25 mol/l, 2 ml/g tissue). The homogenate was centrifuged at 800 × g for 10 min and the supernatant again centrifuged at 10500 × g for 25 min. The residue obtained was washed by dispersion in sucrose solution (0.25 mol/l, 5 ml) and centrifugation, first for 10 min at 800 × g (when the residue was discarded) and then for 15 min at 10500 × g. This washing procedure was repeated and the 10500 × g residue, referred to as the mitochondrial fraction, was suspended in 5 ml sucrose (0.25 mol/l) containing $CaCl_2$ (0.01 mol/l) and 2α-cyano-4,4,17α-trimethyl-17-hydroxy-androst-5-en-3-one (cyanoketone, 50 μmol/l). The homogenisation and centrifugation steps were carried out at 4°C. Portions of the mitochondrial suspension (1 ml) were incubated at 37°C for 0.5 h in phosphate buffer (0.1 mol/l, pH 7.4, 1 ml) containing [7(n)-^{3}H]cholesterol [370 kBq, 0.77 μg in propylene glycol (50 μg)] and NADPH (1.3 mmol/l). With some incubations 23,24-dinor-5-cholen-3β-ol, 23,24-dinor-5-cholene-3β,20-diol or 23,24-dinor-5-cholene-3β,21-diol (60 μmol/l) were added to assess their effect on the cholesterol side-chain-cleavage activity.

Steroids were extracted from the incubations [2] and carrier steroid, pregnenolone (0.25 μmol), was added to the extracts which were then chromatographed in system A. The area corresponding to pregnenolone was eluted, acetylated and rechromatographed sequentially in systems B and C. Further pregnenolone acetate (150 μmol) was added to a portion of the eluted pregnenolone acetate area from the final chromatograms and crystallised to constant specific activity, which was considered to be achieved when the values for three consecutive crops agreed to within ± 5%. The specific activities are reported as means ± SD.

A mitochondrial preparation from the adrenal of a patient with an aldosterone secreting tumour and homogenates of human ovarian tissue were prepared and incubated with 23,24-dinor-5-[7a-^{3}H]cholen-3β-ol as described [2,3].

3. RESULTS

The conversion of cholesterol to pregnenolone was inhibited by 23,24-dinor-5-cholene-3β,20-diol and 23,24-dinor-5-cholene-3β,21-diol (table 1). The 3β,21-diol was a more effective inhibitor than the 3β,20-diol. The parent compound 23,24-dinor-5-cholen-3β-ol (I) also inhibited cholesterol side-chain cleavage (table 1, expt 1) but to a lesser extent than either of the diols (II,III).

23,24-Dinor-5-cholene-3β,20-diol was isolated from the human ovarian incubations and crystallised to constant specific activity (314 ± 7.2 dpm/μmol). The crystals obtained were combined, acetylated and again crystallised to constant specific activity (305 ± 9.7 dpm/μmol). From a second experiment the specific activities were found to be 376 ± 13.7 and 401 ± 4.0 dpm/μmol for 23,24-dinor-5-cholene-3β,20-diol and its acetate, respectively. 23,24-Dinor-5-cholene-3β,21-diol was also isolated from these incubations. From the first experiment the specific activities amounted to 105 ± 2.7 and 128 ± 1.3 dpm/μmol for the free steroid and its diacetate, respectively. From the second experiment the corresponding values were 116 ± 3.4 and 120 ± 5.0 dpm/μmol for the free steroid and its diacetate.

23,24-Dinor-5-cholene-3β,20-diol was isolated from the human adrenal mitochondrial incubation and crystallised to constant specific activity (703 ± 32.5 dpm/μmol); the crystals were combined, acetylated and again crystallised to constant specific activity (778 ± 12.4 dpm/μmol).

Table 1

The effect of various steroids on pregnenolone production by mitochondrial preparations incubated with cholesterol

Expt no.	Additions	Pregnenolone mean specific activities of crops (dpm/μmol) (SD)	Total radioactivity in pregnenolone (dpm)	Percentage inhibition
(1) Bovine corpus luteum	none	437 (4.6)	7.8×10^4	–
	23,24-dinor-5-cholen-3β-ol	319 (12.8)	5.0×10^4	35.9
	23,24-dinor-5-cholene-3β,20-diol	162 (4.2)	3.0×10^4	61.5
	23,24-dinor-5-cholene-3β,21-diol	99.4 (4.7)	1.9×10^4	75.6
(2) Bovine corpus luteum	none	36.5 (1.2)	6.3×10^3	–
	23,24-dinor-5-cholene-3β,20-diol	27.2 (1.0)	4.6×10^3	27.0
	23,24-dinor-5-cholene-3β,21-diol	22.7 (1.0)	3.6×10^3	42.9
(3) Bovine adrenal	none	2670 (111)	4.3×10^5	–
	23,24-dinor-5-cholene-3β,20-diol	2077 (20.8)	3.0×10^5	30.2
	23,24-dinor-5-cholene-3β,21-diol	684 (6.0)	1.3×10^5	69.8
(4) Rat adrenal	none	83.7 (0.6)	1.3×10^4	–
	23,24-dinor-5-cholene-3β,20-diol	46.3 (1.5)	8.1×10^3	37.7
	23,24-dinor-5-cholene-3β,21-diol	39.5 (1.2)	6.2×10^3	52.3

Mitochondrial preparations were incubated in phosphate buffer (0.1 mol/l, pH 7.4) containing [7(n)-^{3}H]cholesterol (370 kBq, 0.8 μg), cyanoketone (25 μmol/l) and NADPH (1.3 mmol/l). With some indications further steroids (60 μmol/l) were added to assess their effect on pregnenolone production

$$\text{Percentage inhibition} = \frac{\text{control dpm} - \text{sample dpm}}{\text{control dpm}} \times 100$$

4. DISCUSSION

The conversion of 23,24-dinor-5-cholen-3β-ol to steroid hormones (e.g. testosterone or corticosterone) takes place in the microsomal fraction from rat testis and adrenals, respectively [2,3]. However, when 23,24-dinor-5-cholen-3β-ol is incubated with preparations of rat [3] or human adrenal mitochondria (above) metabolism appears to stop after 20- or 21-hydroxylation (cf. [6,7]). In this fraction these compounds may have another function, namely inhibition of cholesterol side-chain cleavage and thereby regulation of the production of active hormones by the C_{27} pathway.

These inhibitors are analogous to the side-chain-hydroxylated cholesterols (20-hydroxy- and 22-hydroxycholesterols) which have been recognised as inhibitors of cholesterol side-chain cleavage [9–12]. Sheets and Vickery [7] have also found that 23,24-dinor-5-cholene-3β,21-diol, named 23,24-dinor-5-cholene-3β,22-diol to draw the structural analogy with 22-hydroxycholesterol, is not converted to pregnenolone and is an inhibitor (40%) of pregnenolone production from cholesterol by a reconstituted cytochrome P-450$_{scc}$ enzyme system.

Hydroxylated derivatives of 23,24-dinor-5-cholen-3β-ol have been observed in several instances ([1,3–5] and here) and it is now shown that they can inhibit the production of pregnenolone from cholesterol, and thus inhibit steroid hormone production from cholesterol. This raises the possibility of a control mechanism whereby, in cells where the sesterterpene pathway is functioning to produce one steroid hormone, it may suppress steroidogenesis by the cholesterol pathway.

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