Potent inhibition of mammalian progesterone synthesis by 2 α -cyanoprogesterone

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2 α -Cyanoprogesterone potently inhibits the conversion of [3 H]pregnenolone into progesterone catalysed by bovine corpora lutea, bovine adrenal cortex and human term placenta microsomes (microsomal fractions), yielding IC₅₀ (concentration causing 50% inhibition) values of 66 nM, 120 nM and 700 nM respectively. By contrast, it is an exceedingly poor inhibitor of the isomerization of pregn-5-ene-3,20-dione, yielding IC₅₀ values between 50 and 70 μ M. On this basis, 2 α -cyanoprogesterone would appear to be an extraordinarily selective inhibitor of the 3 β -hydroxysteroid dehydrogenase. Dixon plots indicate that it is a very-tight-binding competitive inhibitor of the corpus-luteum enzyme, yielding a K_i of 15 nM. In the bovine adrenal cortex and human placenta the steroid is less potent and inhibits the dehydrogenase non-competitively with K_i values of 150 nM and 1.0 μ M respectively. Thus 2 α -cyanoprogesterone inhibits the corpus-luteum dehydrogenase with substantial selectivity. Because of its high affinity for the ovarian enzyme, the presence of low-micromolar concentrations of 2 α -cyanoprogesterone can promote a complete cessation of progesterone synthesis in corpora-lutea microsomes for several hours. Since this effect is observed in the presence of saturating concentrations of pregnenolone (50 μ M), it is predicted that this inhibitor may be even more potent *in vivo*. 2 α -Cyanoprogesterone displays very low affinity for the human progesterone receptor, yielding a K_d of 600 nM as against a K_d of 1.6 nM for progesterone. It is suggested that 2 α -cyanoprogesterone may be a selective inhibitor of ovarian progesterone synthesis and may act as an effective anti-gestational agent *in vivo*.

In the mammalian ovary, progesterone is synthesized from pregnenolone via two steps. The first involves the NAD⁺-dependent oxidation of pregnenolone to pregn-5-ene-3,20-dione and is catalysed by a 3 β -hydroxysteroid dehydrogenase. The second involves the isomerization of pregn-5-ene-3,20-dione to give progesterone, and is catalysed by the C₂₁- Δ^5 -3-oxosteroid isomerase. The develop-

ment of an agent that could selectively inhibit either of these ovarian enzymes would decrease progesterone synthesis and could have therapeutic potential as a post-coital contraceptive or abortifacient. The development of such an agent has been unpopular, since these enzymes are also present in the adrenal cortex, where progesterone is the precursor of the mineralo- and glucocorticoids. Thus inhibition of mammalian progesterone synthesis is believed to result in adverse effects on adrenal steroidogenesis. Such a belief rests on the assumption that both the corpora-lutea and adrenal enzymes are equally sensitive to inhibition.

Goldman (1968) was the first to demonstrate that the α -cyanoketone was a tight-binding inhibitor of the bovine adrenal 3 β -hydroxysteroid dehydrogenase (K_i = 0.7 μ M) and Δ^5 -3-oxosteroid iso-

Abbreviations used: 2 α -cyanoprogesterone, 2 α -cyanopregn-4-ene-3,20-dione; 2-hydroxymethylene progesterone, 2-hydroxymethylenepregn-4-ene-3,20-dione; 3 β -hydroxysteroid dehydrogenase, 3 β -hydroxysteroid:NAD⁺ oxidoreductase (EC 1.1.1.51); R5020, promegestone, 17,21-dimethyl-19-norpregna-4,9-diene-3,20-dione; α -cyanoketone, 2 α -cyano-4,4,17-trimethyl-androst-5-en-17 β -ol-3-one.

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merase ($K_i = 0.6 \mu\text{M}$). By using this compound *in vivo*, a marked inhibition of the adrenal-cortical, corpora-lutea and ovarian-follicular and stromal-cell enzyme was observed (Goldman *et al.*, 1965). Furthermore, after a single injection of the synthetic steroid the inhibition was found to persist *in vivo* for a period of 4 weeks. This persistent inhibition resulted in adrenal and corpora-lutea hyperplasia, the former being reversed by the co-administration of glucocorticoids (Potts *et al.*, 1963). These findings suggest that selective inhibition of the ovarian dehydrogenase/isomerase system may be difficult to achieve.

Although the α -cyanoketone can inhibit ovarian and adrenal steroidogenesis, the potential of this compound and its [2,3-*d*]isoxazole, Win 17,025 (17 β -hydroxy-4,4,17-trimethylandroster-5-en-[2,3-*d*]isoxazole) to act as anti-gestational agents *in vivo* has been assessed. Thus the [2,3-*d*]isoxazole can terminate pregnancy in both the pre- and post-implantation period in rats (Csapo & Resch, 1979a,b,c). Similar findings have been observed in rhesus monkeys, where no changes in plasma glucocorticoid levels were observed (Schane *et al.*, 1977). It is thus conceivable that low-dose isoxazole may act as an effective anti-gestational agent *in vivo*.

As a further development in this area we have synthesized the α -cyanoketone of progesterone, namely 2 α -cyanoprogesterone, and have shown it to be an active-site-directed affinity label for the crystalline Δ^5 -3-oxosteroid from *Pseudomonas testosteroni* (Penning, 1985). The present paper describes the potent inhibition of mammalian progesterone synthesis by 2 α -cyanoprogesterone and indicates that selective inhibition of ovarian progesterone synthesis is feasible.

Materials and methods

Chemicals

[7-³H]Pregnenolone (19.3 Ci/mmol), [17 α -methyl-³H]promegestone R5020, (80.0 Ci/mmol) and unlabelled R5020 were purchased from New England Nuclear (Boston, MA, U.S.A.). Pregnenolone, progesterone, 5 α -dihydrotestosterone and dexamethasone were products of Steraloids (Wilton, NH, U.S.A.). 2 α -Cyanoprogesterone, 2-hydroxymethylene progesterone, androst-5-ene-3,17-dione and pregn-5-ene-3,20-dione were synthesized as described previously (Penning, 1985). Bovine serum albumin was a product of Armour Pharmaceuticals (Kankakee, IL, U.S.A.).

Preparation of microsomal fractions from mammalian endocrine tissues

Adrenal cortex was removed from the capsule of fresh bovine adrenals. Corpora lutea were dis-

sected from fresh bovine ovaries. Placental tissue was separated from the membranous and cotyledonous material. The first two tissues were homogenized with 3 vol. of 50mM-Tris/HCl, pH 7.4, containing 250mM-sucrose, 5mM-MgCl₂ and 25mM-KCl. Placental tissue was homogenized in 2 vol. of 50mM-sodium phosphate buffer, pH 7.4, containing 250mM-sucrose. In each case homogenization was conducted in a Waring Blendor at 4°C. Differential centrifugation gave the microsomal pellets, which were washed once and then resuspended in their homogenization buffer to give final protein concentrations of approx. 10mg/ml. Aliquots (1ml) were then frozen and stored at -80°C for future use.

Radiochemical assay for 3 β -hydroxysteroid dehydrogenase

The conversion of [³H]pregnenolone into progesterone was measured in 0.2ml systems containing 0.16ml of homogenization buffer, 10 μ l of NAD⁺ (80mg/ml), 2 μ l of pregnenolone (1.6mg/ml, dissolved in ethanol), 1 μ l of [³H]pregnenolone (40000 c.p.m.), 8 μ l of ethanol and 1–20 μ l of microsomal resuspension. Incubations were for 1–5 min at 37°C and the reactions were quenched by the addition of 0.25ml of ice-cold water followed by 1.0ml of ethyl acetate. The incubation mixture was extracted with 2 $\times$ 1ml aliquots of ethyl acetate, the extracts were dried, redissolved in 20 μ l of ethanol and 10 μ l aliquots were spotted on to 1.5cm lanes on 20cm $\times$ 20cm, 250 μ m-thick Merck silica-gel 60 plates (MCB Manufacturing Chemists, Cincinnati, OH, U.S.A.). Standards of progesterone and pregnenolone were applied to the outer lane on each edge of the plate. Plates were developed in chloroform/ethyl acetate (4:1, v/v), and standards (pregnenolone, R_F 0.41; progesterone, R_F 0.61) were detected with a methanolic conc. H₂SO₄ spray. Areas in each lane corresponding to these standards were scrapped from the plates and placed in 5ml of a toluene-based scintillation fluid containing 4.0g of PPO (2,5-diphenyloxazole) and 50mg of POPOP [1,4-bis-(5-phenyloxazol-2-yl)-benzene]/litre. Radioactive steroid was then quantified by liquid-scintillation counting. ³H radioactivity was determined as corrected c.p.m. and the number of nmol of progesterone formed were calculated from the specific radioactivity of the [³H]pregnenolone used in the assay.

Spectrophotometric assay for Δ^5 -3-oxosteroid isomerases

The standard spectrophotometric assay for mammalian Δ^5 -3-oxosteroid isomerases contained, in a final volume of 1.0ml, 100mM-potassium

phosphate buffer, pH 7.0, 1.0 μM neutralized bovine serum albumin and 4% (v/v) acetonitrile containing either 68 μM -androst-5-ene-3,17-dione or 13.6 μM -pregn-5-ene-3,20-dione. The reaction was initiated by the addition of resuspended microsomes, and the initial velocity of Δ^4 -3-oxosteroid formation ($\epsilon = 16300 \text{ M}^{-1} \cdot \text{cm}^{-1}$; λ_{max} , 248 nm) was measured at 25°C against a blank containing all the components except steroid and enzyme. IC_{50} values (concentration causing 50% inhibition) for cyanoprogesterone and hydroxymethylene progesterone were obtained by adding increasing concentrations of the steroidal inhibitors to the assay system. The initial velocities were corrected for non-enzymic isomerization rates, which were less than 10% of the enzyme-catalysed velocity.

Human progesterone receptor assays

Portions of human breast tumour known to contain high levels of progesterone receptor were minced, pooled and homogenized in 5 vol. (w/v) of 10 mM-Tris/HCl, pH 7.4, containing 1 mM-EDTA, 1 mM-dithiothreitol, 10 mM-sodium molybdate and 10% (v/v) glycerol. After centrifugation at 200 000g the supernatants were pooled and aliquots were stored at -80°C for future use.

Progesterin binding to the cytosolic receptor was assayed by using [^3H]R5020 as ligand and dextran-coated charcoal to remove unbound steroid (de Hertogh *et al.*, 1975). Briefly, portions of the cytosol containing 0.35 mg of protein/ml were incubated with various concentrations of [^3H]R5020 (i.e. 0.1 nM–3.0 nM) at 4°C for 22 h. To assess non-specific binding, duplicate tubes containing a 200-fold excess of unlabelled R5020 were included at the highest and lowest isotope concentrations. At the end of the incubation, dextran-coated charcoal was added to a final concentration of 0.25%, and after centrifugation at 1000g a portion of the supernatant was counted for radioactivity to determine the amount of protein-bound radioligand. By subtracting this value from the total radioactivity the amount of free [^3H]R5020 was determined. The pooled cytosol used in this study was found to contain $610 \pm 32 \text{ fmol}$ of progesterone receptor/mg of cytosol protein, with a K_d of 8.2×10^{-11} ($\pm 1.2 \times 10^{-11}$) M and was stable throughout the course of the work. In competition experiments a similar binding isotherm was generated in the presence of a fixed concentration of competitor. In all the experiments the stock solutions of steroids were made in 100% ethanol, and the final concentration of organic solvent was kept constant at 0.5%.

After correcting bound radioactivity for non-

specific binding, double-reciprocal plots of bound R5020 versus the concentration of free R5020 were constructed. Data points were analysed by the Wilkinson (1961) hyperbolic method. In the absence of the competitor the B_{max} (total number of specific binding sites) and the K_d (the receptor–ligand dissociation constant) were computed from the reciprocals of the intercepts of the ordinate and abscissa respectively. In the presence of the competitive ligands, K_i values (dissociation constant of the receptor–competitive-ligand complex) were calculated from the relationship:

$$K_d(\text{app.}) = K_d(1 + [\text{I}]/K_i)$$

where $K_d(\text{app.})$ is computed from the intercept with the abscissa observed in the presence of the competitive ligand, K_d is the dissociation constant for R5020, and $[\text{I}]$ is the concentration of competitor.

Liquid-scintillation counting

[^3H]Radioactivity from the dehydrogenase assays was quantified on a Tracor Analytical model 43 scintillation counter whose machine efficiency for ^3H was 53%. ^3H radioactivity from the receptor-binding assays was quantified on a Tracor model 6892 scintillation counter with a ^3H efficiency of 32%.

Protein determinations

Protein concentrations were determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as standard.

Results

Progesterone synthesis in mammalian endocrine tissues

The conversion of [^3H]pregnenolone into progesterone was measured in microsomal resuspensions prepared from bovine corpora lutea, bovine adrenal cortex and human term placenta. In every instance the formation of progesterone was linear with respect to time (0–3 min) and protein concentration (result not shown). In general the reaction was complete within the first 5 min of the assay. Examination of the specific activities indicate that corpora-lutea microsomes are the most active, synthesizing 3 and 50 times more progesterone than either the adrenal or placental microsomes (Table 1). By using androst-5-ene-3,17-dione and pregn-5-ene-3,20-dione to monitor the C_{19} - and C_{21} - Δ^5 -3-oxosteroid isomerase(s) in these preparations it is clear that the 3β -hydroxysteroid dehydrogenase catalyses the rate-limiting step in the conversion of pregnenolone into progesterone.

Table 1. *Specific activities for the conversion of [^3H]pregnenolone into progesterone and the isomerization of Δ^5 -3-oxosteroids in mammalian endocrine tissues*

The specific activity for the conversion of [^3H]pregnenolone into progesterone catalysed by adrenal-cortex, corpora-lutea and placental microsomes was determined in incubations in which 1–20 μl of microsomal resuspensions were added to the standard radiochemical assay. The reaction was quenched at 1 min intervals, and the initial velocity of the reaction was calculated from plots of nmol of progesterone formed versus time. The specific activities for the isomerization of androst-5-ene-3,17-dione and pregn-5-ene-3,20-dione were determined by using the spectrophotometric assay described in the Materials and methods section.

Microsomal preparation	Specific activity ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)		
	[^3H]Pregnenolone conversion	C_{21} - Δ^5 -3-oxosteroid isomerase	C_{19} - Δ^5 -3-oxosteroid isomerase
Bovine adrenal cortex	19.9	504	221
Bovine corpora lutea	60.1	568	168
Human term placenta	1.3	6.8	18.9

Inhibition of mammalian progesterone synthesis by 2-substituted progesterones

2 α -Cyanoprogesterone and its synthetic precursor 2-hydroxymethylene progesterone were examined as inhibitors of mammalian progesterone synthesis by using [^3H]pregnenolone as substrate (Fig. 1). 2 α -Cyanoprogesterone was found to be an extraordinarily potent inhibitor of these reactions in bovine corpora lutea, bovine adrenal cortex and human placental microsomes, yielding IC_{50} values of 66 nM, 120 nM and 700 nM respectively. By contrast, the 2-hydroxymethylene progesterone was less potent, giving IC_{50} values that ranged from 1.4 to 35 μM .

2 α -Cyanoprogesterone is a selective inhibitor of the 3 β -hydroxysteroid dehydrogenase

In theory, 2 α -cyanoprogesterone could inhibit mammalian progesterone synthesis at one of two steps. First, it could prevent the oxidation of pregnenolone catalysed by the 3 β -hydroxysteroid dehydrogenase; alternatively it could inhibit the isomerization of the resulting pregn-5-ene-3,20-dione catalysed by the Δ^5 -3-oxosteroid isomerase. To identify the target enzyme we have examined whether 2 α -cyanoprogesterone can prevent the isomerization of androst-5-ene-3,17-dione and pregn-5-ene-3,20-dione. With either substrate the IC_{50} values generated were 400–1000 times higher than those observed for the conversion of pregnenolone into progesterone (Table 2). Such data confirms that 2 α -cyanoprogesterone acts as an extraordinarily potent and selective inhibitor of the 3 β -hydroxysteroid dehydrogenase.

By contrast, 2-hydroxymethyleneprogesterone inhibits the C_{21} - Δ^5 -3-oxosteroid isomerase at IC_{50} values comparable with those observed for its inhibition of the conversion of pregnenolone into progesterone (Table 2). The possibility thus exists

that 2-hydroxymethylene progesterone may exert all of its effect on the steroid isomerase.

2 α -Cyanoprogesterone as a tight-binding competitive inhibitor of corpora-lutea 3 β -hydroxysteroid dehydrogenase

Since 2 α -cyanoprogesterone acts as an irreversible active-site-directed inhibitor of the bacterial oxosteroid isomerase (Penning, 1985), the possibility existed that the tight binding to the mammalian 3 β -hydroxysteroid dehydrogenase was a reflection of covalent modification. To test this hypothesis, microsomes were preincubated with an excess of 2 α -cyanoprogesterone, and samples were removed over a 2 h time period and diluted into the normal assay mixture. In such experiments no time-dependent inactivation of enzyme activity was observed. These data suggest that cyanoprogesterone acts as a reversible inhibitor of the dehydrogenase.

Dixon plots reveal that, in corpora-lutea microsomes, 2 α -cyanoprogesterone acts as a potent competitive inhibitor of the 3 β -hydroxysteroid dehydrogenase, yielding a K_i value of 15 nM (Fig. 2). This value was obtained in two independent experiments and is substantially lower than the IC_{50} value (66 nM) for the reaction. By contrast, 2 α -cyanoprogesterone appears to be a non-competitive inhibitor of the dehydrogenase in adrenal-cortex and human term placenta microsomes, giving K_i values of 150 nM and 1.0 μM respectively. As these K_i values are essentially identical with the IC_{50} values for these reactions, this is further evidence for the non-competitive nature of the inhibition. It is noteworthy that 2 α -cyanoprogesterone displays a 10-fold higher affinity for the corpus-luteum enzyme than it does for the isofunctional adrenal-cortex enzyme. Such findings suggest that 2 α -cyanoprogesterone displays reason-

Fig. 1. Inhibition of mammalian progesterone synthesis by 2-substituted progesterones

Inhibition of progesterone synthesis in corpora lutea (●), adrenal-cortex (○), and human term placental (■) microsomes, by 2-cyanoprogesterone (a) and 2-hydroxymethylene progesterone (b) is shown. Plots show percentage inhibition of progesterone formed as a function of inhibitor concentration. Depending on the endocrine tissue, the amount of microsomal protein added to the assay and the time of the incubation varied. Thus, for the adrenal cortex, 16 μg of microsomal protein were incubated for 2 min; for the corpora lutea, 8 μg of microsomal protein were incubated for 5 min; and for human placenta, 24 μg of microsomal protein were incubated for 5 min. In all assays the inhibitor was dissolved in ethanol and the final concentration of organic solvent was maintained at 0.5% (v/v).

able selectivity for the ovarian 3β -hydroxysteroid dehydrogenase.

2 α -Cyanoprogesterone prevents mammalian progesterone synthesis for several hours

Since 2 α -cyanoprogesterone is a reversible inhibitor of the 3β -hydroxysteroid dehydrogenase, it is predicted that it will decrease the rate of progesterone formation and that it will take longer for the reaction to reach completion. It is also predicted that, in the presence or absence of inhibitor, the amount of progesterone eventually

Table 2. Comparison of IC_{50} values for 2 α -cyanoprogesterone and 2-hydroxymethylene progesterone

The IC_{50} values shown for the inhibition of 3β -hydroxysteroid dehydrogenase were taken from Fig. 1. IC_{50} values for the inhibition of the C_{19} - and C_{21} - Δ^5 -3-oxosteroid isomerases were generated by conducting spectrophotometric assays with the appropriate Δ^5 -3-oxosteroid in the presence of various concentrations of inhibitor. In these spectrophotometric assays the steroids were dissolved in acetonitrile and the final concentration of organic solvent in the assay was maintained at 4% (v/v). Abbreviation used: N.D., not determined because of the low isomerization rates.

Microsomal preparation	Inhibitor	IC_{50} value (μM)		Ratio of IC_{50} values (C_{21} - Δ^5 -3-oxosteroid isomerase/ 3β -hydroxysteroid dehydrogenase)	
		3β -Hydroxysteroid dehydrogenase	C_{21} - Δ^5 -3-oxosteroid isomerase	C_{19} - Δ^5 -3-oxosteroid isomerase	
Bovine adrenal cortex	Cyanoprogesterone	0.120	50.0	80.0	416
	Hydroxymethylene progesterone	4.0	4.5	24.0	1.1
Bovine copora lutea	Cyanoprogesterone	0.066	70.0	60.0	1060
	Hydroxymethylene progesterone	1.4	11.0	13.0	7.8
Human placenta	Cyanoprogesterone	0.70	N.D.	70.0	N.D.
	Hydroxymethylene progesterone	35.0	N.D.	21.0	N.D.

Fig. 2. Dixon plots for the inhibition of progesterone synthesis by 2α -cyanoprogesterone in corpora-lutea, adrenal-cortex and human placenta microsomes

In each experiment the initial velocity for the conversion of either $10\mu\text{M}$ ($\circ$), or $50\mu\text{M}$ ($\bullet$)-pregnenolone to progesterone was measured as described in the legend to Table 1. These velocities were then re-determined in the presence of the four to six concentrations of 2α -cyanoprogesterone indicated. In every instance velocities were measured in duplicate. In these assays the amount of radioactivity was kept constant at 40000 c.p.m. and the number of nmol of progesterone formed were calculated from the final specific radioactivity of $[^3\text{H}]$ pregnenolone used in the assay.

formed should be the same. Because 2α -cyanoprogesterone inhibits the dehydrogenase in the nanomolar range, we wished to determine whether micromolar concentrations of this inhibitor could prevent the reaction from ever reaching completion. In such experiments progesterone synthesis was monitored in corpus-luteum, adrenal-cortex and placenta microsomes over 2h in the presence of concentrations of 2α -cyanoprogesterone that

Fig. 3. Cessation of mammalian progesterone synthesis by 2α -cyanoprogesterone

The conversion of $[^3\text{H}]$ pregnenolone into progesterone catalysed by adrenal-cortex (a), corpora-lutea, (b) and human term placenta microsomes (c) was measured in the presence of the concentration of 2α -cyanoprogesterone indicated (0.5 – $20\mu\text{M}$). Incubations contained $8\mu\text{g}$ of corpora-lutea microsomes, $16\mu\text{g}$ of adrenal-cortex microsomes and $24\mu\text{g}$ of placental microsomes plus the components of the regular radiochemical assay. At the time points indicated the incubation was quenched and the total number of nmol of progesterone formed were determined.

exceed the IC_{50} values generated in Fig. 1 by as much as 80-fold. The results indicate that cyanoprogesterone was most effective in the corpora-lutea microsomes; thus $0.5\mu\text{M}$ caused a significant decrease in progesterone synthesis, whereas $5.0\mu\text{M}$ promoted a complete cessation in the synthesis of this hormone over 2h (Fig. 3). Since progesterone synthesis will proceed to completion in the presence of lower concentrations of inhibitor (results not shown) the prolonged reductions observed in hormone synthesis are not due to a depletion of the cofactor, NAD^+ . Since these effects are observed in the presence of saturating amounts of pregnenolone (i.e. $50\mu\text{M}$), it is predicted that considerably less 2α -cyanoprogesterone would be required to inhibit ovarian progesterone

Fig. 4. Affinity of 2 α -cyanoprogesterone for the human progesterone receptor

A binding isotherm for [^3H]R5020 was generated as described in the Materials and methods section (control curve). The ability of 2 nM unlabelled R5020, 4 nM-progesterone and 4000 nM-cyanoprogesterone to displace [^3H]R5020 was assessed, and in each instance the amount of free versus bound [^3H]R5020 was plotted by using a Lineweaver-Burk transformation. K_d and K_i values were calculated as described in the Materials and methods section.

synthesis *in vivo*. By contrast, the amount of 2 α -cyanoprogesterone required to completely arrest progesterone synthesis in the adrenal cortex and human placenta would appear to exceed 20 μM .

Progesterone-receptor assays

From the data, 2 α -cyanoprogesterone has the potential to act as an anti-gestational agent *in vivo*. Since this compound is an analogue of progesterone, it may also act as a progestin by binding to the cytoplasmic receptor. In competitive-binding assays, it was found that 4 nM-progesterone could effectively displace [^3H]R5020 from the human progestin receptor; however, similar displacement of the radioligand with cyanoprogesterone could only be achieved with concentrations that approached 4000 nM (Fig. 4). From these assays the K_i of progesterone was found to be 1.6 nM and that for 2 α -cyanoprogesterone was found to be 600 nM. Such findings indicate that 2 α -cyanoprogesterone displays very little affinity for the progestin receptor. The K_i value for 2 α -cyanoprogesterone can be placed into further perspective when it is compared with similar values generated for 5 α -dihydrotestosterone (K_i 788 nM) and dexamethasone (K_i 840 nM). These findings suggest that cyanoprogesterone is no more a progestin than an active androgen or glucocorticoid.

Discussion

2 α -Cyanoprogesterone is a potent reversible inhibitor of the rate-limiting step in the conversion

of pregnenolone into progesterone catalysed by mammalian endocrine tissues. Thus it inhibits the 3 β -hydroxysteroid dehydrogenase in the nanomolar range, and it can cause complete cessation of progesterone synthesis for several hours in the low-micromolar range. Its inhibitory effect is particularly powerful in the corpus luteum, where it acts as a competitive inhibitor, yielding an exceedingly low K_i of 15 nM. This K_i value is ten times lower than that observed for the 3 β -hydroxysteroid dehydrogenase of adrenal cortex and suggests that this compound may act as a selective inhibitor of ovarian progesterone synthesis. Since 2 α -cyanoprogesterone has a very low affinity for the cytoplasmic progesterone receptor, this compound has the potential to act as an anti-gestational agent *in vivo*.

Although 2 α -cyanoprogesterone resembles the cyanoketone studied by Goldman *et al.* (1965) and Goldman (1968) and the [2,3- d]isoxazole examined by Csapo & Resch (1979a,b,c), it should be emphasized that this compound differs from these other two inhibitors in some important respects. First, its K_i for the 3 β -hydroxysteroid dehydrogenase(s) indicates that its affinity exceeds that observed for the α -cyanoketone and [2,3- d]isoxazole by at least two orders of magnitude. Second, it displays extraordinarily high selectivity for the inhibition of the dehydrogenase; thus its potency for this enzyme is 400–1000-fold higher than that observed for the Δ^5 -3-oxosteroid isomerase (see Table 2). It should be recalled that the α -cyanoketone studied by Goldman (1968) displayed almost equal affinity for both the dehydrogenase and the isomerase of bovine adrenal cortex.

Further experiments are needed to determine whether 2 α -cyanoprogesterone will traverse the cell membrane and inhibit progesterone synthesis in isolated corpora-lutea cells. If such experiments are successful, one will be able to progress to studies *in vivo* and evaluate the effectiveness of this compound as an anti-gestational agent.

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