The effect of synthetic gestagens on progesterone formation *in vitro* in human placenta of early pregnancy

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Summary. Villous tissue from 26 placentae of 7–17 weeks was incubated with radio-active pregnenolone alone and with pregnenolone in the presence of progesterone and 9 synthetic gestagenic steroids and the progesterone formation was measured after 30 min. When progesterone was present in a concentration of 31 or 310 $\mu\text{mol/l}$ the conversion rate of labelled pregnenolone to progesterone was reduced to 88.6 and 82.2% of that of the respective control incubations. Dydrogesterone, allyloestrenol, lynoestrenol and norethynodrel under similar conditions did not inhibit the formation of progesterone. The inhibitory effects of megestrol acetate, medroxyprogesterone acetate and norgestrel were close to that of progesterone. Norethisterone and methylloestrenolone were the most effective inhibitors of progesterone formation: when incubated in an equimolar concentration (35 $\mu\text{mol/l}$) with pregnenolone (50 μg) the progesterone formation was reduced to 60.0–62.7% and 29.1–34.0% respectively of that of the respective control experiments.

Introduction

It has been shown that synthetic gestagenic steroids inhibit progesterone synthesis in the human cyclic corpus luteum *in vivo* (Johansson, 1971; Collins *et al.*, 1974; Lehmann, Just-Nastansky, Behrendt, Czygan & Bettendorf, 1974) and *in vitro* (Mukherjee, Wright & Davidson, 1972) and in the corpus luteum of pregnancy (7–14 weeks) *in vitro* (Saure, Karjalainen & Teräväinen, 1976). At this stage of pregnancy, however, the placenta is synthesizing increasing amounts of progesterone: the transference of progesterone production from the corpus luteum to the placenta is well advanced during the 7th week and the placental production of progesterone becomes progressively dominant from the 8th week onwards (Csapo, Pulkkinen, Ruttner, Sauvage & Wiest, 1972). It has been estimated that at 8–9 weeks of pregnancy about half of the circulating progesterone is produced by the corpus luteum and half by the placenta (Lebech, 1971).

We therefore investigated whether gestagens would exert effects on the placental Δ^5 - 3β -steroid dehydrogenase function similar to those obtained on luteal tissue in early pregnancy.

Materials and Methods

Steroids

The following synthetic gestagens were used: dydrogesterone (6-dehydro-retroprogesterone: Ferrosan, Malmö, Sweden); medroxyprogesterone (6 α -methyl-17 α -acetoxyprogesterone: Medipolar Oy, Oulu, Finland); megestrol (16 β -methyl-6-dehydro-17 α -acetoxyprogesterone: Orion Oy, Helsinki, Finland); allyloestrenol (17 α -allyl-4-oestren-17 β -ol: N. V. Organon, Oss, Holland); lynoestrenol (17 α -ethynyl-4-oestren-17 β -ol: N. V. Organon); methylloestrenolone (17 α -methyl-19-nortestosterone: N. V. Organon); norethisterone (17 α -ethynyl-19-nortestosterone: Leiras, Turku, Finland); norethynodrel (17 α -ethynyl-17 β -hydroxy-5(10)-oestren-3-one: Searle & Co., Chicago, U.S.A.); and norgestrel (13 β -ethyl-17 α -ethynyl-17 β -hydroxygon-4-en-3-one: Leiras). Pregnenolone and progesterone were obtained as commercial preparations from Sigma Chemical Co. (St Louis, U.S.A.). The purity of all these steroids was tested by thin-layer chromatography (t.l.c.) before use.

[4-¹⁴C]Pregnenolone (sp. act. 51 mCi/mmol) and [4-¹⁴C]progesterone (sp. act. 61 mCi/mmol) were obtained from the Radiochemical Centre (Amersham, England). Their radiochemical purity was reported as 98%: t.l.c. tests in our laboratory showed them to be 96% and 98% pure, respectively.

All steroids were dissolved in ethanol-propyleneglycol solution (1:1 v/v). Solvents were analytical grade and redistilled before use.

Tissue and incubations

Tissue from 26 placentae at 7–17 weeks of pregnancy, obtained at laparotomy in connection with legal abortions, was used. The period of gestation was calculated on the basis of the last menstrual period. A detailed description of the tissue preparation and the incubation procedure employed is given elsewhere (Saure, 1973). In brief, finely minced villous tissue was incubated as 500 mg portions in 5 ml 0.05 M-tris buffer medium (pH 7.4) at 37°C and under continuous oxygen flow for 30 min. Incubation was stopped by the addition of 2 ml boiling acetone.

Control incubations. In 22 experiments the substrate was [4-¹⁴C]pregnenolone alone, in a dose of 0.145 µCi (=339 × 10³ (ct/min)/0.9 µg) and at a concentration of 0.6 µmol/l. Ten incubations in which the labelled substrate was diluted with different amounts (10–500 µg, 6.2–310 µmol/l) of unlabelled pregnenolone were also carried out.

Incubations with gestagens. In 44 experiments, [4-¹⁴C]pregnenolone (dose as in controls) was incubated with unlabelled progesterone or with a synthetic gestagen added in a concentration of 50 or 500 µg per incubation. Each synthetic gestagen was also incubated in the absence of pregnenolone (100 µg gestagen/incubation). In 11 additional experiments labelled pregnenolone (dose as in controls) was diluted with different amounts of unlabelled pregnenolone (10–500 µg) and incubated with equimolar concentrations of norethisterone or methyloestrenolone.

Isolation and determination of radioactive steroids

The methods used have been described previously (Suare, 1973; Saure *et al.*, 1976). The radioactivity of the incubates was extracted in ether and the neutral and phenolic steroid fractions were separated. After sampling for liquid scintillation counting, the radioactivity contained in the neutral fraction was submitted to t.l.c. (chloroform:acetone:ethanol, 84:15:1 by vol.) and collected in four fractions. The most apolar of these contained progesterone which was quantified by the measurement of radioactivity and u.v.-absorption. When the progesterone fraction from different chromatograms of control incubations was pooled and studied in two-dimensional t.l.c. (ethylacetate:cyclohexane:ethanol, 9:9:1 by vol.) with authentic unlabelled progesterone as carrier, 94–98% of the radioactivity was isolated in association with the carrier. Saure (1973) has shown that only progesterone in radiochemically homogeneous form can be eluted from the respective t.l.c. fractions of similar analyses, and more specific measures for the identification of the progesterone formed in the present incubations therefore seemed unnecessary.

The phenolic steroid fraction was not studied because earlier studies indicated that oestrogens were not formed in incubations of early placental tissue with pregnenolone (Saure, 1973). The recovery of [4-¹⁴C]progesterone was 89.6–90.3% after incubations with acetone-denatured tissue. The results presented are not corrected for the methodological losses.

Results

Control incubations

The mean (± S.D.) recovery of radioactivity in the neutral steroid fraction was 85.3 ± 5.0% of the dose, and 89.1 ± 4.2% (range: 75.8–93.6%) of this radioactivity was incorporated into the progesterone fraction. When pregnenolone was used at different concentrations the mean recovery of radioactivity in the neutral fraction was 90.9 ± 3.2% of the dose. The progesterone formed in these incubations was measured both in terms of radioactivity incorporation and in µg by u.v.-absorption

Table 1. The conversion of [4-¹⁴C]pregnenolone (339×10^3 ct/min = 0.9 µg per incubation) to progesterone by human placental tissue of 7–12 weeks in the presence of gestagens

Gestagen	Dose (µg)	No. of incubations	Progesterone formation (as % of control)
None	—		100
Progesterone	50	2	87.5, 89.6
	500	2	80.2, 84.1
Dydrogesterone	50	2	99.3, 101.8
	500	2	95.0, 101.5
Medroxyprogesterone acetate	50	2	93.4, 99.1
	500	2	72.1, 81.6
Megoestrol acetate	50	2	91.7, 88.9
	500	2	67.5, 70.3
Allyloestrenol	50	2	95.5, 102.1
	500	2	96.6, 100.9
Lynoestrenol	50	2	97.7, 97.0
	500	2	98.4, 99.8
Methyloestrenolone	50	2	60.9, 85.2
	500	3	28.0, 23.3, 24.5
Norethisterone	50	4	84.7, 82.7, 99.2, 99.4
	500	3	38.1, 19.9, 20.5
Norethynodrel	50	2	106.6, 100.7
	500	2	105.5, 97.0
Norgestrel	50	2	89.6, 97.6
	500	2	69.3, 73.9

(sensitivity = 2 µg, precision 0.5 µg). The results obtained by these two procedures correlated well, indicating that radioactive and unlabelled pregnenolone were equally metabolized under the present experimental conditions. When 10, 25, 50, 250 or 500 µg pregnenolone were added, the progesterone formed was 8 µg (n , no. of incubations, = 1), 18.5 and 20 µg (n = 2), 32.5 µg (27.5–38; n = 5), 67 µg (n = 1) and 64.5 µg (n = 1), respectively.

Incubations with gestagens

The mean ($\pm$ S.D.) total recovery of radioactivity in the neutral fraction was $81.7 \pm 4.6\%$ of the dose. The capacity of the placental tissue to convert radioactive pregnenolone to progesterone in the presence of added progesterone or other gestagens is shown in Table 1.

In the presence of progesterone the rate of formation of radioactive progesterone was slightly reduced. There was little effect on the incorporation of radioactivity into progesterone when dydrogesterone, allyloestrenol, lynoestrenol or norethynodrel were added. When medroxyprogesterone acetate, megoestrol acetate, norgestrel or norethisterone were present at the lower concentration (50 µg), the formation of radioactive progesterone was reduced slightly but no more so than in the presence of 50 µg progesterone. The higher concentration of 500 µg caused a reduction in progesterone formation. The most inhibitory of the gestagens tested were norethisterone and methyloestrenolone.

The results of incubations with equimolar concentrations of norethisterone and methyloestrenolone with pregnenolone are shown in Table 2.

Discussion

A major steroidogenic function of the placenta is the conversion of Δ^5 - to Δ^4 -steroids (Diczfalusy, 1969). Labelled pregnenolone was almost quantitatively converted to progesterone in the control incubations of the present study, as found earlier with first trimester placentae (Saure, 1973). Placental

Table 2. The conversion of pregnenolone incubated in different concentrations with human placental tissue of 11–12 weeks to progesterone in the presence of equimolar amounts of methyltestosterone and norethisterone

Pregnenolone (µg) + gestagen (µg)	No. of incubations	Progesterone formation			
		Methyltestosterone		Norethisterone	
		µg	% of control	µg	% of control
10 + 10	1	7.5	93.8	—	—
25 + 25	4	10, 12	54.1, 60.0	16, 18.5	86.5, 92.5
50 + 50	4	8, 11.5	29.1, 34.0	16.5, 21	60.0, 62.7
500 + 500	2	18	27.9	27	41.9

progesterone is mainly derived from maternal plasma cholesterol which is converted to pregnenolone in the placenta (Diczfalusy, 1969; Burstein & Gut, 1971). At term the placenta contains a relatively large pool of cholesterol (Villem, VanLeusden & Zelewski, 1966), but only a small portion of the pool contributes to progesterone synthesis *in vitro*. According to Maeyama, Tuchida & Matuoka (1968), the net synthesis of progesterone from endogenous precursors in placental minces of 35–40 weeks was 0.6–1.9 µg/g during incubation for 2 h. The endogenous progesterone content of this tissue before incubation was 1.1–2.0 µg/g. In the present incubations, therefore, the synthesis of progesterone from endogenous precursors probably had no influence on the incorporation of radioactivity in progesterone.

The present results confirm and extend our preliminary observations (Karjalainen, Saure & Teräväinen, 1972) on the effect of gestagens on the placental Δ^5 -3 β -dehydrogenase activity in early pregnancy. It seems possible that dydrogesterone, allyloestrenol and norethynodrel might even stimulate the formation of progesterone in placental tissue. Allyloestrenol has been shown to increase the urinary pregnanediol excretion during pregnancy (Szontágh & Sas, 1964) and to stimulate the conversion of radioactive pregnenolone to progesterone in term placental homogenates (Barlas *et al.*, 1974).

Norgestrel and the 17 α -acetoxyprogesterone derivatives used had an inhibitory effect comparable to that of progesterone. Norgestrel given in doses of 10 mg/day in early pregnancy decreased plasma progesterone level in only one of three subjects treated (Nygren & Johansson, 1975). Similar reports concerning 17 α -acetoxyprogesterone derivatives are not available.

Norethisterone and methyltestosterone, both Δ^4 -3-keto-19-nortestosterone derivatives, were the most inhibitory in the present incubations. The substitution of the 13-methyl group of norethisterone for an ethyl group as in norgestrel seemed to reduce the inhibitory activity. When a 17 α -ethynyl group was substituted for 17 α -methyl, as in methyltestosterone, the inhibitory effect was clearly enhanced (see Table 2). Methyltestosterone was more inhibitory than norethisterone in all concentrations tested, even at a concentration of 15.3 µmol/l. Methyltestosterone was also the most effective of these nine gestagens studied in reducing the *in-vitro* formation of progesterone in luteal tissue of early pregnancy (Saure *et al.*, 1976).

It is known that HCG counteracts the inhibitory effect of some gestagens, including norethisterone, on progesterone synthesis in the corpus luteum of the menstrual cycle *in vivo* (Johansson, 1971). HCG is evidently present and possibly also synthesized by placental preparations during incubation. The addition of HCG, however, has no effect on the formation of progesterone from cholesterol and pregnenolone in placental tissue *in vitro* (Saure, 1973), even after neutralization of the endogenous HCG with a specific antiserum (Macome, Bischoff, Uma Bai & Diczfalusy, 1972).

In vivo, however, the large amount of HCG present during early pregnancy may counteract the effect of gestagens, especially in the corpus luteum, thus explaining the finding that treatment of women, 35–56 days pregnant, with norethisterone resulted in only a transient decrease of the plasma progesterone (Nygren & Johansson, 1975).

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Received 21 January 1977