

peroxidation, possibly promoted by decompartmentalised metal ions. This has implications for the prevention and treatment of such adverse reactions. Copper chelating agents, such as D-penicillamine, may be considered, but there is a theoretical risk that this agent, like phenothiazines and desferrioxamine, may alter the compartmentalisation of copper^{27,28} and thereby, at least in the short term, exacerbate the complications. The more logical approach of interrupting the process of lipid peroxidation by use of lipid-soluble antioxidants, such as vitamin E, may be more effective not only in treating the adverse effects of phenothiazine medication but possibly also in preventing them. One study appears to show that phenothiazine-induced tardive dyskinesia improves with vitamin E therapy.²⁹

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References continued at foot of next column

Preliminary Communication

ANTIFERTILITY ACTIONS OF THE PROGESTERONE ANTAGONIST RU 486 INCLUDE DIRECT INHIBITION OF PLACENTAL HORMONE SECRETION

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Summary The antifertility effects of the potent antiprogesterin RU 486 (mifepristone) during early pregnancy have been attributed to its blockade of progesterone receptors in the endometrium. Studies in cultured syncytiotrophoblasts have revealed an additional action of RU 486 at the placental level, where it impairs the production of human chorionic gonadotropin (hCG), human placental lactogen (hPL), and progesterone. RU 486 (10 nmol–10 μ mol/l) attenuated the production of all three placental hormones, in a dose-related manner, and its effects on hCG and hPL were reversed by addition of exogenous progesterone. The specific inhibitory effects of RU 486 on placental hormone secretion indicate that its antifertility actions are attributable to competitive inhibition of progesterone action in the trophoblast as well as in the endometrium.

INTRODUCTION

THE synthetic 19 norsteroid RU 486 or mifepristone (17 β -hydroxy-11 β -[4-dimethylaminophenyl]-17 α -[1-propynyl]estra-4, 9-dien-3-one) is a potent progesterone antagonist that inhibits the uterine actions of progesterone and has pronounced antifertility effects in rodents.¹ It acts by competitive antagonism of progesterone at the receptor level, with blockade of its biological effects in reproductive tissues including endometrium and cervix, and at the hypothalamic-pituitary level. In normally cycling monkeys and in women at the mid-luteal phase, administration of the antiprogesterin induces premature menstruation.²⁻⁵ In oophorectomised monkeys receiving replacement oestradiol and progesterone, RU 486 causes uterine bleeding in the presence of high plasma progesterone levels, consistent with a direct local action on the endometrium.⁴ When administered to women during early pregnancy,^{6,7} RU 486 causes vaginal bleeding that usually starts on the second day of treatment, followed by expulsion of the placenta on the fourth or fifth day or sometimes later.⁸ Couzin et al⁹ report that RU 486 causes complete abortion in 85 of 100 women when given within 10 days of the missed menstrual period. RU 486 also decreases or abolishes the daily increase in urinary hCG excretion that is characteristic of early pregnancy^{7,8} with a consistent and pronounced fall in plasma hCG by day 6.⁹ Such reversal of the normal rise in hCG secretion by RU 486 has been attributed to its inhibitory action on the decidualised endometrium and the

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detachment process that precedes expulsion of the placenta, but may also reflect an inhibitory effect of the drug at the placental level. We have investigated this aspect of the action of RU 486 in cultured placental cells.

METHODS

Term placentae were obtained under aseptic conditions and brought to the laboratory within one hour after caesarean section of uncomplicated pregnancies. Several cotyledons were removed from the placenta and rinsed extensively in warm 0.9% sterile saline. The soft villous tissue was separated from the connective tissue and blood vessels and was processed essentially by the method of Hall et al.¹⁰ The minced villous tissue (30–40 g) was placed in a 250 ml sterile flask with 60 ml calcium/magnesium-free medium 199 (M199) containing 25 mmol/l HEPES, 1% bovine serum albumin, 0.125% trypsin (1:250, Sigma), 0.025% DNase I (400–600 Kunitz units/mg, ICN Biochemicals), 50 U/ml penicillin, and 50 µg/ml streptomycin, and was incubated at 37°C for 30 min in a shaking water bath. The supernatant suspension was collected in 50 ml conical polypropylene tubes containing 5 ml fetal bovine serum and centrifuged at 900 g for 10 min at room temperature. The cell pellet was resuspended in calcium/magnesium-free M199, and the remaining tissue in the flask was subjected to six further digestions of 20 min each. The cell suspensions from all digestions were pooled and resuspended in 5 ml Earle's balanced salt solution (EBSS) before purification on preformed density gradients of 5% to 70% Percoll in EBSS.¹¹ The gradients were centrifuged for 20 min at 1200 g at 4°C and the middle layer containing the cytotrophoblasts was washed twice with alpha minimal essential medium (MEM) and then resuspended in alpha MEM containing 4 mmol/l glutamine, 10% fetal bovine serum, 50 U/ml penicillin, and 50 µg/ml streptomycin. The cells were plated in 24-well tissue culture clusters at a concentration of 500 000 cells/ml and maintained in a 5% CO₂/air incubator at 37°C. The media were changed every day and analysed for chorionic gonadotropin (hCG), placental lactogen

(hPL), and progesterone by radioimmunoassay. All experiments were conducted in serum-free alpha MEM containing 0.01% bovine serum albumin.

RESULTS

The cytotrophoblasts cultured from human term placentae differentiated into syncytiotrophoblasts within 48 h and produced increasing amounts of hCG, hPL, and progesterone. The effects of RU 486 on placental hormone secretion were evaluated in cells cultured for a further 24 h with increasing concentrations of the antiprogestin from 10 nmol to 10 µmol per litre. As shown in fig 1, RU 486 caused dose-dependent inhibition of hCG, hPL, and progesterone production. Inhibition of hormone production was greatest in the case of hCG, which was significantly reduced by RU 486 in a concentration as low as 0.1 µmol/l. Secretion of hPL and progesterone was significantly suppressed by 10 and 1 µmol/l, respectively. Analysis of cells and media for the three hormones revealed that less than 2% of their total concentration was intracellular—ie, RU 486 primarily inhibits synthesis rather than release of placental hormones. Fig 2 shows the time-course of the inhibitory action of the antiprogestin on hormone secretion. In low concentrations the antiprogestin often caused a minor increase in hormone

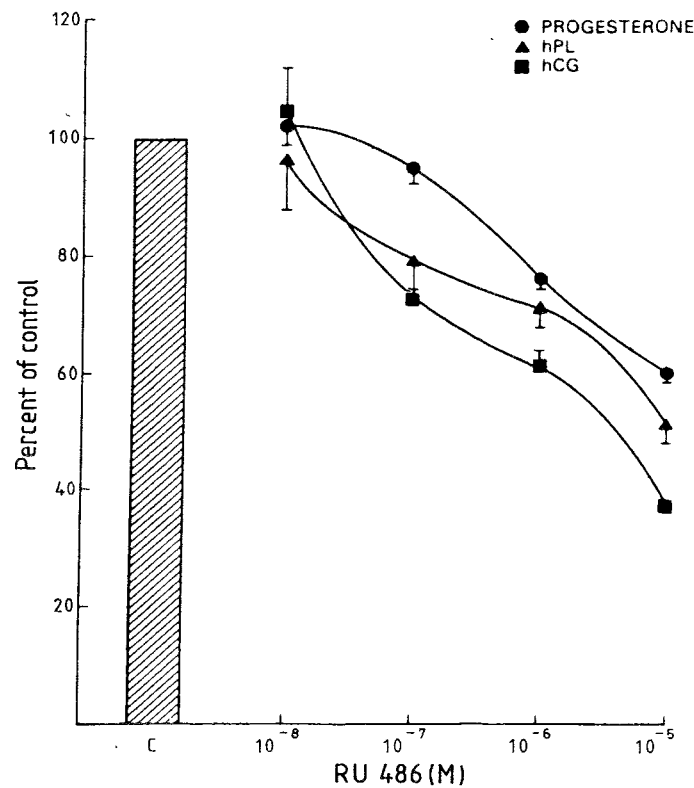


Fig. 1—Dose-dependent actions of RU 486 on 24 h hormone production by cultured human syncytiotrophoblast cells.

The data represent composite results from three experiments (mean and SE) each performed in quadruplicate. C denotes the mean control value expressed as 100%. RU 486 concentrations in mol/l.

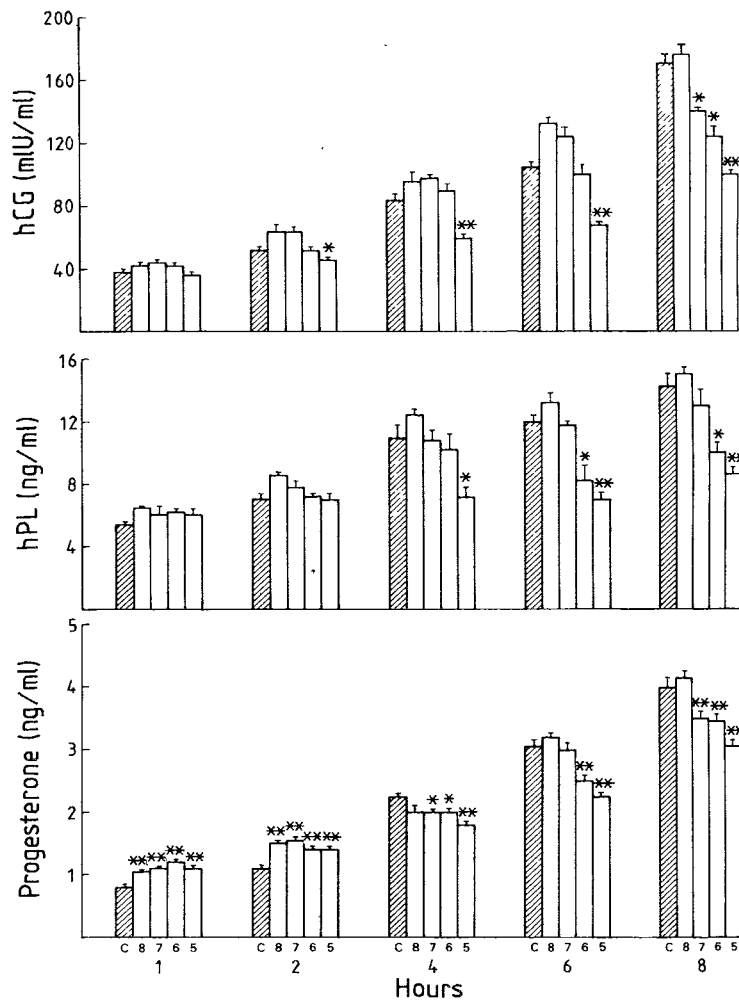


Fig. 2—Time course of inhibitory actions of RU 486 on hCG, hPL, and progesterone secretion by cultured human syncytiotrophoblasts.

Bars represent mean and SE of six replicates. Significance was calculated by Student's t-test; single asterisks represent $p < 0.05$ and two asterisks represent $p < 0.01$. Responses of control cells (hatched) and those to RU 486 concentrations from 10^{-8} to 10^{-5} mol/l (open bars indicated by negative log concentration) are shown for each of the three hormones during incubation for up to 8 h.

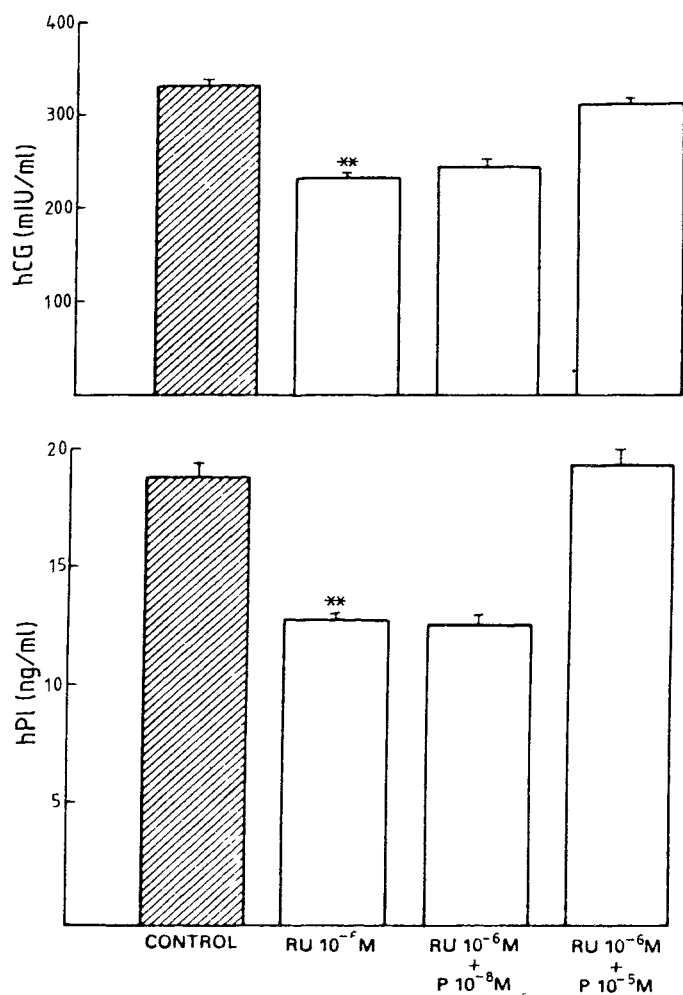


Fig. 3—Reversal by progesterone of the inhibitory effects of RU 486 on hCG and hPL secretion in cultured syncytiotrophoblasts.

RU 486 concentrations in mol/l.

production, but this effect was not statistically significant. No morphological evidence of cytotoxicity or changes in cell number were observed during incubation with RU 486.

In contrast to its prominent inhibitory actions on hCG and hPL secretion, all concentrations of RU 486 had a biphasic effect upon progesterone production, with a significant increase during the initial 1–2 h and a progressive decline at subsequent times up to 6 h. At the later periods, the antiprogesterone inhibited progesterone production in parallel with its suppressive effects upon hCG and hPL secretion. Addition of exogenous progesterone prevented the inhibitory effects of RU 486 on production of hCG and hPL (fig 3), whereas cortisol was only partly effective (not shown). A tenfold higher concentration of progesterone was required to overcome inhibition of protein hormone secretion by RU 486, consistent with the fivefold higher affinity of the antiprogesterone for binding to progesterone receptor sites.¹

DISCUSSION

Although the rates of hormone production by cultured syncytiotrophoblasts varied between cell preparations from individual placentae, RU 486 consistently inhibited placental hormone secretion in all cultures studied. Furthermore, the concentrations of the antiprogesterone that achieved these effects were in the range of the plasma levels that are required for its contragestive actions in women.⁸ Our findings also indicate that the syncytiotrophoblast contains receptors for progesterone which, like the

endometrial progesterone receptors, have higher affinity for RU 486 than for progesterone itself. The specificity of the inhibitory effects of RU 486 for the progesterone receptor was indicated by the ability of added exogenous progesterone to overcome its suppressive actions on hormone secretion. RU 486 is also a potent glucocorticoid antagonist,⁹ but cortisol was less effective than progesterone in overcoming its inhibitory actions on hormone production. These results also demonstrate the importance of progesterone in several of the secretory functions of the human placenta, since the antiprogesterone attenuates the production of both steroid and protein hormones by cultured placental cells.

These findings are highly relevant to the clinical use of RU 486 as an abortifacient, since the mechanism by which the compound interrupts pregnancy is not entirely clear.⁹ Although RU 486 primarily acts to block progesterone action at the receptor level, its contragestive properties have been attributed mainly to its antiprogesterone effect within the endometrium. Thus, administration of RU 486 caused decidual necrosis and endometrial sloughing during early pregnancy, but did not induce histological changes in the trophoblast.⁶ However, the present observations clearly demonstrate that RU 486 also compromises placental function by a direct action on the trophoblast, with reduced secretion of the major steroid and protein hormones produced during pregnancy. Such an action of RU 486 has also been observed in trophoblastic explants, where the drug inhibited production of hCG but not of hPL.¹² By exerting such inhibitory effects upon the trophoblast, RU 486 opposes maintenance of the conceptus by blockade of placental hormone production as well as through its established inhibitory action at the level of the endometrium. Such a combination of inhibitory actions upon endometrial and trophoblastic function may be responsible for the high efficacy of RU 486 in termination of early pregnancy.

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