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INHIBITION OF FATTY ACID SYNTHESIS IN RABBIT MAMMARY ALVEOLAR EXPLANTS BY PROGESTERONE AND RELATED STEROIDS

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Summary—The possible inhibitory effect of steroids related to progesterone on prolactin-stimulated fatty acid synthesis in mammary alveolar explants from 11-day pseudopregnant rabbits, after culture, was investigated. Like progesterone, 17α-hydroxyprogesterone, 20α-dihydroprogesterone, medroxyprogesterone acetate, ethinodiol diacetate, ORG 2058 were inhibitory whereas pregnenolone and 5α-pregnan-3,20-dione were not.

INTRODUCTION

In most species plasma progesterone increases during early pregnancy and declines abruptly towards parturition or after expulsion of the placenta. These events are closely followed by induction of milk production in the mammary gland. It is recognised that progesterone is a suppressor of lactogenesis and the decrease of the hormone is associated with stimulation by the lactogenic hormones such as insulin, prolactin and corticosteroids acting on the gland to produce milk [1]. In our previous study we found that pharmacological concentrations of progesterone (0.5 μg/ml or greater) *in vitro* suppressed the stimulation of fatty acid synthesis and acetyl-CoA carboxylase [2, 3]. A significant negative correlation was also observed between glycerolipid synthesis or the activity of fatty acid synthetase in rabbit mammary explants after culture, and increasing concentrations of progesterone (0.05, 0.5, 5 μg/ml) when present along with insulin, corticosterone and prolactin [3]. The effect was selective in that it did not suppress the enzymes glucose 6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase [3], which are involved in lipid biosynthesis. In this study, we have tested progesterone-related steroids, some of which are used as contraceptives or in the measurement of progesterone receptors, others being metabolites of progesterone, with a view to compare their antilactogenic potency in terms of direct suppressive action on mammary epithelial cells in explant culture.

EXPERIMENTAL

Animals

Rabbits were kept under the natural cycle of light and darkness and fed *ad libitum*. Pseudopregnancy was induced in 9-month old virgin females by injection of 50–100 I.U. of human chorionic gonadotrophin into the marginal ear vein. The day of injection was taken as day 0 of pseudopregnancy, which was confirmed by counting new corpora lutea at the time of sacrifice.

Chemicals

ORG 2058 (16α-ethyl-21-hydroxy-19-nor-pregn-4-ene 3,20-dione) was a gift from Organon International, Netherlands. Ethynodial diacetate 3β, 17β-diacetoxy-17α-ethynyl-4-estrene was a gift from G. D. Searle and Co., Chicago, Illinois, U.S.A. 17α-Hydroxy progesterone was a gift from The Upjohn Co., Michigan, U.S.A. Medroxy progesterone acetate (6α-methyl-17-acetoxy progesterone), progesterone (4-pregnen-3,20-dione), 20α-dihydroprogesterone (20α-hydroxy-4-pregnen-3-one), pregnenolone (3β-hydroxy-5-pregnen-20-one), 5α-pregnan-3,20-dione, bovine insulin (24.9 I.U./mg) corticosterone and cortisol were obtained from Sigma Chemical Company, St Louis, Missouri, U.S.A., sodium [1-^{14}C]acetate, 1.8–2.09 GBq (49–57 mCi)/mmol, [4.5-^{3}H]L-leucine 37.0 GBq (1 Ci)/mmol were obtained from Amersham Australia Pty. Ltd, Sydney, N.S.W., Australia: human chorionic gonadotrophin from Schering, A.G., Berlin, Germany: Medium 199 from Commonwealth Serum Laboratories, Parkville, Victoria, Australia. Ovine prolactin (NIH-P-S-14: 25 I.U./mg) was a gift from Endocrine Study Section, National Institutes of Health, Bethesda, Maryland, U.S.A. Ovine prolactin-16(AFP-5915A), 30.5 I.U./mg was a gift from National Institute of Arthritis, Diabetes, Digestive and Kidney diseases (NIADDK), Bethesda, Maryland, U.S.A.

Preparation and culture of mammary explants

Mammary alveolar explants from 11-day pseudopregnant rabbits were prepared under aseptic

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Table 1. Effect of progesterone and related compounds on fatty acid synthesis and protein synthesis in mammary alveolar explants from pseudopregnant rabbits, after culture with insulin (I, 2.5 ng/ml), corticosterone (C, 100 ng/ml) and prolactin-14 (P, 100 ng/ml)

Time in culture (h)	Hormones in culture	Progestin concentration μg/ml	[1-^{14}C] Acetate incorporation into fatty acid	[4,5-^{3}H]L-leucine incorporation into protein
			nmol/h/mg explant Mean ± SEM (6 observations on tissue from 2 rabbits)	
0	No hormones	—	0.08 ± 0.01	0.33 ± 0.03
47	IC	—	0.18 ± 0.04	0.60[a] ± 0.0
47	ICP (Control group)	—	1.46 ± 0.09	0.52 ± 0.06
47	ICP + Progesterone	0.5	0.73** ± 0.06	0.66 ± 0.11
47	ICP + 17α-hydroxyprogesterone	0.5	1.02* ± 0.16	0.58 ± 0.09
47	ICP + ORG 2058	0.5	0.69** ± 0.12	0.68 ± 0.5
47	ICP + Ethinodiol diacetate	0.5	0.95* ± 0.13	0.66 ± 0.10
47	ICP + Medroxyprogesterone acetate	0.5	0.91* ± 0.15	0.69 ± 0.14

[a]Mean of 5 observations on tissue from 2 rabbits.
** = $P < 0.01$, * = $P < 0.05$ (significance of progestin treatment against control).

conditions by the method described [4]. Explants were cultured in Medium 199, containing 15 mM $NaHCO_3$/20 mM HEPES buffer (pH 7.4), polymyxin B sulfate and neomycin sulfate antibiotics (1–2 U/ml of medium) in an atmosphere of air. Hormone solutions were prepared as described [2]. Steroids were dissolved in alcohol and added to the medium. The alcohol content in the medium was 0.12%.

Fatty acid synthesis

Rate of fatty acid synthesis was determined by measuring the incorporation of [1-^{14}C]acetate into fatty acids as described [5, 6]. Viability of mammary epithelial cells and alveolar integrity were confirmed by histological examination after staining with haematoxylin and eosin.

Protein synthesis

Rate of incorporation of [4,5-^{3}H]L-leucine into TCA-insoluble protein was carried out according to the method described [5, 6].

Statistics

Measurement of the significance of steroid treatments was determined by <0.05 least significant difference, obtained from analysis of variance.

RESULTS

Effect of progesterone and related compounds on fatty acid synthesis and protein synthesis

Table 1 shows that on day 11 of pseudopregnancy the rate of fatty acid synthesis remained at a low basal level and a slight increase observed when the explants were cultured in the presence of 2.5 ng/ml insulin and 100 ng/ml corticosterone for 47 h. When prolactin at 100 ng/ml was also present for 47 h in culture, fatty acid synthesis was stimulated markedly (8-fold). Fatty acid synthesis (as a measure of lactogenesis) in the presence of insulin, corticosteroid and prolactin served as the control in the present study. Addition of progesterone or any one of the progestins such as 17α-hydroxyprogesterone, ORG 2058, ethynodiol diacetate and medroxyprogesterone acetate, at 0.5 μg/ml to the other hormones significantly prevented prolactin-stimulation of fatty acid synthesis.

As shown in Table 1 the rate of protein synthesis (one of the indices of tissue viability) nearly doubled, when mammary alveolar explants were cultured in the presence of insulin and corticosterone. Addition of prolactin also did not affect protein synthesis significantly. Progesterone or other progestins at 0.5 μg/ml, along with insulin, corticosterone and prolactin in culture did not change the rate of protein synthesis significantly (Table 1).

Effect of progesterone and some of its metabolites on fatty acid synthesis

Table 2 shows that the rate of fatty acid synthesis in the mammary alveolar explants of 11-day pseudopregnant rabbits remained low when the explants were cultured in the presence of insulin 25 ng/ml and cortisol 25 ng/ml (cortisol forms the major portion of serum corticosteroids in rabbits and the concentration of unbound cortisol ranges from 5–21 ng/ml during late pregnancy and early lactation [7]) for 47 h. However in the presence of prolactin also, fatty acid synthesis was stimulated markedly. Prolactin-stimulation of fatty acid synthesis was suppressed significantly when progesterone or related steroids such as 17α-hydroxyprogesterone or 20α-dihydroprogesterone were included in the medium at high concentration (5 μg/ml). In contrast other progesterone related steroids such as pregnenolone or 5α-pregnan-3,20-dione were without any significant effect on fatty acid synthesis stimulated by prolactin, when added to the culture medium.

DISCUSSION

It is well established that prolactin induces fatty acid synthesis markedly (a measure of lactogenesis) in mammary alveolar explants from 11–12 day pseudopregnant rabbits, cultured for 2 days in the presence

Table 2. Inhibition of fatty acid synthesis by progesterone and some of its metabolites in mammary alveolar explants of pseudopregnant rabbits, after culture with insulin (I, 25 ng/ml), cortisol (C, 25 ng/ml) and prolactin-16 (P, 100 ng/ml)

Time in culture (h)	Hormones in culture	[1-^{14}C]Acetate incorporation into fatty acids Mean ± SEM (9 observations on tissue from 3 rabbits) nmol/h/mg explants
0	No hormones	0.12 ± 0.05
47	IC	0.15[a] ± 0.03
47	ICP (Control)	2.42 ± 0.47
47	ICP + Progesterone	0.30** ± 0.06
47	ICP + 20α-Dihydroprogesterone	0.92* ± 0.21
47	ICP + Pregnenolone	1.39 ± 0.28
47	ICP + 5α-Pregnone-3,20-dione	2.82 ± 0.43
47	ICP + 17α-Hydroxyprogesterone	1.18* ± 0.25

Progestin concentration = 5 μg/ml: [a]Mean of 6 observations for tissue from 2 rabbits.
** = $P < 0.01$, * = $P < 0.05$ (Significance of steroid treatment against control).

of insulin and corticosterone [5, 8]. However we showed that prolactin was unable to stimulate fatty acid synthesis unless insulin was also present in the culture medium and the effect was evident even at a low insulin concentration of 25 ng/ml [2]. Plasma insulin concentration in the pregnant rat has a range of 1.8 ng–5 ng/ml [9, 10] and in female rabbits it is about 0.8 ng/ml [11]. Table 1 shows that lowering of insulin concentration in the culture further to 2.5 ng/ml, close to the physiological range, is sufficient to elicit a marked prolactin response (8-fold). Prolactin-stimulation of fatty acid synthesis was suppressed significantly when progesterone at 0.5 μg/ml was added to the medium, which is in agreement with a significant negative correlation between increasing progesterone concentration (0.05, 0.5 and 5 μg/ml) and prolactin-stimulation of fatty acid synthesis shown earlier [2] in the presence of comparable insulin, corticosterone and prolactin concentrations.

ORG 2058 has been used as a substitute for progesterone in measuring the concentration of progesterone receptors in the uterus as well as mammary glands [12] and this synthetic progestin was also inhibitory to lactogenesis. Analogous to progesterone, ORG 2058 not only binds to the progesterone receptor in the mammary gland of pregnant rats [12] but it also exhibits high biological potency *in vitro* as shown by inhibition of fatty acid synthesis, which was stimulated by prolactin in this study. Synthetic progestins such as medroxyprogesterone acetate and ethynodiol diacetate are more potent than progesterone as components of oral contraceptives, since progesterone is relatively ineffective when given by mouth [13] and is metabolised fast. The greater progestational potency of synthetic progestins may also be due to relatively slower break down *in vivo*, since in humans the plasma concentration of medroxyprogesterone acetate remains elevated for 6 months after injection, under therapeutic conditions [14]. However they appear to be approximately equal to progesterone in terms of their antilactogenic activity, as exercised by direct action on mammary secretory cells. Also, the inhibitory effect of components of contraceptives on mammary fatty acid synthesis is compatible with the negative effect of a low dose combination oral contraceptive containing 0.093 mg ethynyl estradiol and 0.15 mg levonorgestrel on human lactation and infant growth [15].

The decrease in plasma progesterone concentration towards the end of pregnancy in most mammals occurs due to changes in the rate of metabolic processes, such as decreased synthesis and secretion of progesterone mainly from the ovary and placenta, increased degradation into inactive compounds by liver which are excreted as glucuronides and redirection of the metabolism towards formation of estrogens. In the sheep towards the end of pregnancy, induction of the 17α-hydroxylase enzyme occurs in the placenta, favouring the formation of 17α-hydroxyprogesterone an intermediate in the synthesis of 17β-estradiol [1]. It has been found that in humans, during late pregnancy, plasma progesterone concentration increases maximally to about 200 ng/ml and 17α-hydroxyprogesterone to about 60 ng/ml [16]. In the rat, the decline in plasma progesterone 1–2 days before parturition is attributed to the induction of luteal 20α-steroid dehydrogenase which redirects progesterone towards the formation of 20α-dihydroprogesterone. Injection of 20α-dihydroprogesterone into pregnant, ovariectomised rats did not prevent the induction of lactose in the mammary glands, whereas progesterone was significantly suppressive [17]. Similarly in pseudopregnant-rabbit mammary explants, these steroids (unlike progesterone) did not inhibit the induction of lactose synthetase and galactosyl transferase which occur in the presence of prolactin, insulin, and corticosterone [18]. These observations are contrary to their antilactogenic activity observed in the present study (Tables 1 and 2). Intrauterine injection of 20α-dihydroprogesterone produced 40% of progestational activity compared to progesterone

[19], which is consistent with the inhibitory effect on fatty acid synthesis (Table 2). 17α-Hydroxyprogesterone is thought to have weak progestational activity [13] but 17α-hydroxyprogesterone caproate has found clinical use currently in the prevention of miscarriage [20] and for contraception [21] and it possibly acts through the progesterone receptor. The minimum structural components necessary for progestational activity (antiovulatory) which are the 17α-hydroxy group and the double bond in ring A [22] may have been sufficient to cause inhibition of fatty acid synthesis comparable to progesterone. Moreover as these steroids are naturally occurring and prove to be weakly antilactogenic, the cumulative effect of progesterone and progestins either natural or introduced may be more important than the effect of progesterone alone. The mechanism by which progesterone or other progestins inhibit fatty acid synthesis cannot be decided from these experiments. They may act either through the progesterone receptor directly or compete with glucocorticoid receptor binding of glucocorticoids. Evidence pointing to the latter is that progesterone inhibition is more marked at low glucocorticoid concentrations in the culture medium [2].

Though the above steroids inhibited fatty acid synthesis, protein synthesis was unaffected. Prolactin has been shown to stimulate protein synthesis significantly [5] in mammary alveolar explants from 11–12 day pseudopregnant rabbits after culture for 48 h in contrast to the result obtained in this study (Table 1). The reason may be because of the relatively low concentration of lactogenic hormones, insulin, corticosterone and prolactin used in this study.

Mammary glands of goat [23, 24, 25] and cow [26] have been found to be an important site of degradation of progesterone. 5α-Pregnan-3,20-dione, a catabolite of progesterone, is present in significant quantities in the milk of cow [27, 28] and goat [25].

Of all the steroids tested pregnenolone and 5α-pregnan-3,20-dione did not inhibit fatty acid synthesis, despite the high concentration (5 μg/ml) used. These studies indicate that the antilactogenic effect of progesterone is specific and selective since only closely related steroids and steroids of known progestational activity were found to suppress prolactin-stimulated fatty acid synthesis in rabbit mammary secretory cells.

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