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Myometrial Estrogen and Progesterone Receptor Binding in Pregnancy: Inhibition by the Detergent Action of Phospholipids

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We characterized the phospholipid inhibition of estradiol and progesterone binding to guinea-pig and human myometrial receptors. Of twelve compounds studied, phosphatidylinositol (PI), lysophosphatidic acid and lysophosphatidylcholine (lyso-PC) were the most active inhibitors (50% inhibition at 10^{-5} M). Lyso-PC with fatty acid chain length C14:0 inhibited ligand binding both to estrogen receptor (ER) and progesterone receptor (PR), C16:0 only to PR and C18:0 neither to ER nor to PR. The lyso-derivates were more inhibitory than the parent compounds. The ionic detergent (sodium taurocholate) inhibited both ER and PR binding, but the non-ionic detergent (Triton X-100) only PR. Triton X-100 enhanced the PI-induced inhibition of ER binding by a factor of 10. PR was more sensitive to inhibition than ER in all cases. The type of inhibition was non-competitive. At term pregnancy, ligand binding to myometrial ER or PR was low or absent in humans, but moderate in the guinea-pig. Phospholipid extracts of human decidua and fetal membranes contained PI and phosphatidylserine rather than lyso-PC. The extract was a potent inhibitor of ligand binding to PR (50% inhibition at 10^{-6} M phospholipid phosphorus), but not to ER. The physicochemical environment, modulated by phospholipids acting as detergents, may regulate sex steroid function also *in vivo*. This might have special significance for pregnancy maintenance.

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INTRODUCTION

The steroid-mediated control of uterine smooth muscle activity by estradiol-17β (E2) and progesterone (P4) has been connected with the enzymatic activity of phospholipase A2 (PLA2), which hydrolyzes phospholipids to lysophospholipids and fatty acids. The arachidonic acid thus formed serves as a substrate for prostanoids, known regulators of uterine function [1]. The other products of this enzymatic reaction, lysophospholipids, had not earlier been thought to control parturition, although concomitant fetal pulmonary maturation depends on them [2].

Steroid receptor inactivation through PLA2-action reflects inactivation by the detergent lysophosphatides, not the enzyme itself [3]. Ligand binding is low at term pregnancy in porcine [4], monkey [5], and human [6] myometrial tissue. This low binding could relate to phospholipid metabolism, characterized during fetal development by increased formation of lysoderivates [2]. Among the different species, human and guinea-pig pregnancies have a close similarity in their myometrial P4-profile and in pregnancy maintenance, characterized by gradual withdrawal of P4-action, rather than decreasing the P4-concentration [7, 8].

The purpose of this study was to characterize how the physicochemical environment of plasma and other cellular membranes, modulated by the phospholipids acting as detergents, affects sex steroid ligand binding to the myometrial receptors, and to determine, whether pregnancy-associated tissues closely connected to the myometrium (fetal membranes, decidua) have the same properties. This may be significant for pregnancy maintenance.

It was therefore necessary to study the action of phospholipids on steroid (E2, promegestone R5020, P4) ligand binding to myometrial estrogen and progestin-progesterone receptors (ER, PR) in non-pregnant and pregnant human and guinea-pig. A comparison was made between lyso-compounds and their parent compounds and a search was made for the phospholipid metabolites most active in this respect; fatty acid chain length was studied as a qualitative and

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quantitative determinant of the possible inhibition difference between ER and PR. Moreover, the difference between ionic and non-ionic detergents when stabilizing either ER or PR was investigated, and, finally, the possible synergism between mere detergents and phosphatidylinositol (PI) when inhibiting steroid ligand binding was also investigated. The nature of this inhibition was determined. The phospholipid extract from human decidua and fetal membranes was examined for its ability to inhibit ligand binding during the first and third trimester of pregnancy, and the major compounds involved identified.

Table 1. Phospholipids and the other compounds used

Phosphatidylcholine (lecithin from fresh egg yolk, Sigma P-3556)
Lysophosphatidylcholine (lysolecithin from egg yolk, Sigma L-4129)
Lysophosphatidylcholine, myristoyl C14:0, Sigma L-6629
Lysophosphatidylcholine, palmitoyl C16:0, Sigma L-5254
Lysophosphatidylcholine, stearoyl C18:0, Sigma L-2131
Phosphatidylinositol (crude from soybean, Sigma P-6636)
Phosphatidylinositol (from soybean, sodium salt, Sigma P-0639)
Phosphatidyl-L-serine (from bovine brain, Sigma P-7769)
Phosphatidylethanolamine L-3 (Koch-Light Lab 1002h)
Phosphatidic acid, dipalmitoyl (Sigma P-4013)
Lysophosphatidic acid, oleoyl (sodium salt, Sigma L-7260)
1,2-Dipalmitoyl-sn-glycerol (Sigma D-9135)
1-Stearoyl-2-arachidonoyl-sn-glycerol (Sigma S-6389)
Cholic acid (Sigma C-1129)
Taurocholate (sodium salt, B.D.H.)
Triton X-100 (LKB 1243-226)

EXPERIMENTAL

Tissue sampling

Myometrial samples were collected (with the informed consent of patients and the approval of the Ethical Committee of the University of Turku) from three non-pregnant and six pregnant patients. The non-pregnant women at midcycle had a clinically normal myometrium when hysterectomy for menorrhagia was performed. The samples of term pregnant patients not in clinical labor, were collected during cesarean section with fetopelvic disproportion as an indication from the upper medial lip of the uterine incision. Additional samples were collected from four non-pregnant and three pregnant guinea-pigs at term, but not in labor. The myometrium was carefully freed from concomitant tissues (decidua, serosa), and washed free of blood with isotonic saline. Small sections of uterine muscle were frozen in liquid nitrogen, pulverized with a microdismembrator, and stored at −70°C.

Immediately before steroid binding assays, 1 g of the homogenate was suspended in 4 ml of 40 mmol/l Tris–HCl buffer (pH 7.4), containing EDTA (2 mmol/l), dithiothreitol (200 mg/l), NaN_3 (5 mmol/l) and glycerol (100 g/l). The suspension was centrifuged at 100,000 *g* for 1 h at 3°C and the supernatant was used as a receptor source.

Samples of decidua and fetal membranes were obtained from three first trimester patients, admitted for pregnancy termination by suction curetage. The three third trimester pregnant patients were at term labor, and similar samples were collected at c-section with arrested labor, fetal asphyxia or narrow pelvis as an indication, stored at −70°C, and used for phospholipid extraction.

Steroids and other chemicals

Estradiol (2,4,6,7-^{3}H(N)) and promegestone, [17α-methyl-^{3}H]R5020 were purchased from NEN-Products DuPont, and [1,2,6,7-^{3}H]progesterone from the Radiochemical Centre, Amersham. Table 1 shows the other chemicals and their suppliers.

Receptor binding

The receptor assay methods used are described in detail elsewhere [9]. Briefly, in available receptor concentration assays, dilution series of radiolabeled E2 (0.06–7.5 nmol/l), R5020 or P4 (0.18–15 nmol/l) were prepared in the buffer indicated above. Equal volumes of receptor cytosol and radioligand were mixed and incubated for 16 h at 0°C. Under these incubation conditions unoccupied ER [10] and total PR [11] binding sites were determined. Bound and unbound radioligands were separated with charcoal-dextran solution by centrifugation. The radioactivity of the bound ligand was measured in a liquid scintillation counter. Nonspecific binding was measured from tubes containing a 500-fold excess of nonradioactive steroid.

Table 2. Ligand binding capacities of different myometrial sources

Receptor source	ER	PR, R5020	PR, P4
Guinea-pig			
Non-pregnant ($n = 4$)	592 (444–720)	852 (670–1229)	ND
Pregnant ($n = 3$)	158 (140–187)	652 (486–819)	ND
Human			
Non-pregnant ($n = 3$)	83 (46–145)	1010 (534–1539)	2274 ($n = 1$)
Pregnant ($n = 6$)	<5	<50	ND

Numbers indicate average (range) unoccupied ligand binding capacity for E2 and total binding capacities for R5020 and P4, fmol/mg protein. ND, not determined.

The protein content was assayed by the method of Bradford [12]. The data was analyzed using the Scatchard plot, and the receptor content expressed as fmol/mg protein.

The inhibition of receptor binding by phospholipids was studied at 10^{-3} M through 10^{-7} M phospholipid concentrations. The molar concentration of the 50% inhibition (IC_{50}) of the particular compound studied were recorded. The type of inhibition of the ligand binding was calculated by means of a direct linear plot, according to Eisenthal and Cornish-Bowden [13]. For PR-studies, R5020 was selected as a specific ligand, and P4 as a physiologically acting compound. The ligand concentrations used were 2.5 nmol/l for E2 and 5 nmol/l for R5020 and P4.

Phospholipids from decidua and fetal membranes

Pieces of decidua and fetal membranes, about 1 g of each, were pulverized in a microdismembrator in liquid nitrogen. The homogenate was suspended in 0.04 mol/l Tris–HCl buffer, 2 mmol/l Na_2EDTA, pH 7.4, 1 g homogenate per 4 ml buffer. Phospholipids were extracted twice into 4 ml 1-butanol by shaking for 20 min at 400 shakes/min at room temperature. This procedure optimizes the lysolipid extraction [14]. The extracts were combined, the butanol evaporated and the residue dissolved in methanol at 1 g original homogenate per 0.5 ml solvent. The phosphorus concentration of this lipid solution was determined by the method of Duck-Chong [15]. Thin-layer chromatography of the phospholipids was performed as described previously [16]. PI and phosphatidylserine (PS) appeared at the same spot.

RESULTS

Ligand binding capacities of different myometrial sources

The unoccupied ligand binding capacities of the different myometrial tissue sources for E2 and the total binding capacities for R5020 and P4 are shown in Table 2. Only traces of ligands were bound in human myometrial tissue at term pregnancy: E2 <5 fmol/mg, R5020 <50 fmol/mg. When the endogenous steroids were extracted from cytosol by charcoal-dextran, no change in binding of E2 or P4 was observed. The myometrial tissues of pregnant humans were not used for any further studies.

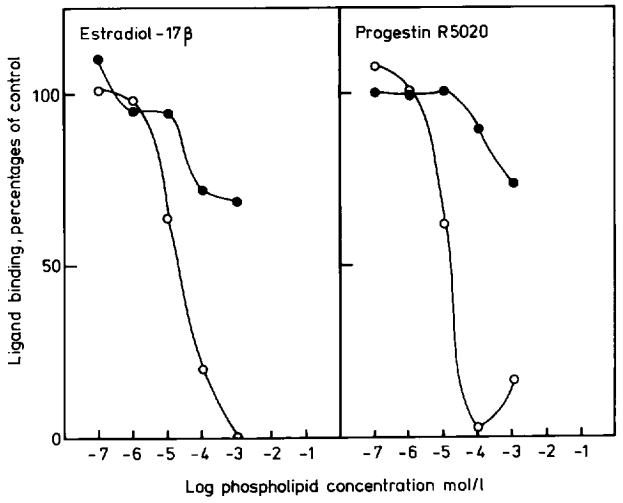

Fig. 1. The inhibitory effects of phosphatidylinositol (○) and egg yolk lysolecithin (●) on the estradiol-17β or progestin R5020 binding to myometrial receptors (human, non-pregnant).

Effect of egg yolk lecithin, lysolecithin and crude PI on steroid (E2, R5020) binding to its receptor

Addition of lecithin does not have any systemic effect on steroid binding, either in the guinea-pig or in humans. In the guinea-pig, lysolecithin inhibited R5020-binding at a lower concentration than E2-binding. In human myometrial homogenate, the effect of egg yolk lysolecithin was not significant; even at a relatively high concentration of 1×10^{-3} M a marginal 24–32% inhibition was observed. Crude PI, however, exhibited inhibition at a level of 10^{-5} M (Fig. 1). Human and guinea-pig myometrial homogenates gave approximately the same results, PR binding being slightly more sensitive than ER binding. Pregnancy itself did not affect the IC_{50} (Table 3).

Table 3. Effect of lecithin, lysolecithin and phosphatidylinositol on steroid (E2, R5020) ligand binding to their myometrial receptors in humans and in guinea-pig

Compound	Receptor source	ER	PR	
Lecithin	Human	Non-pregnant	None	None
	Guinea-pig	Non-pregnant	None	None
		Pregnant	None	None
Lysolecithin	Human	Non-pregnant	None	None
(from egg yolk)	Guinea-pig	Non-pregnant	1×10^{-3}	6×10^{-4}
		Pregnant	1×10^{-3}	6×10^{-4}
Phosphatidylinositol	Human	Non-pregnant	2×10^{-5}	3×10^{-5}
(crude, from egg yolk)	Guinea-pig	Non-pregnant	5×10^{-4}	8×10^{-5}
		Pregnant	5×10^{-4}	7×10^{-5}

Numbers indicate molar concentration at 50% inhibition.

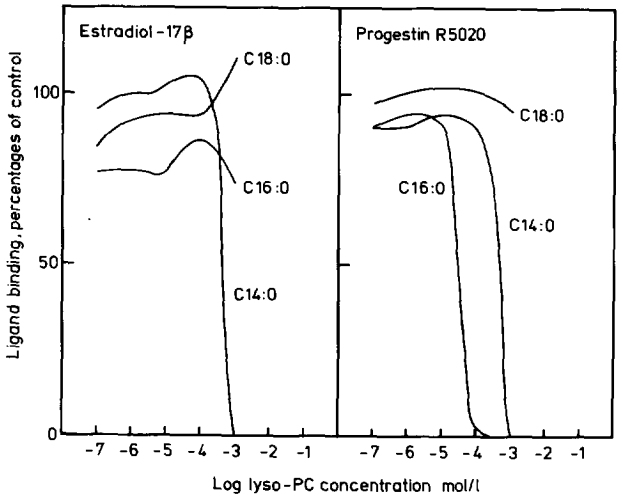

Fig. 2. The effect of the fatty acid chain length of lysophosphatidylcholine on estradiol-17β or progestin R5020 binding to myometrial receptors (human, non-pregnant).

Fatty acid chain length as a regulator of lysophosphatidylcholine-induced inhibition of ER or PR

The fatty acid substituents myristoyl C14:0, palmitoyl C16:0 and stearoyl C18:0 derivatives of lysophosphatidylcholine (lyso-PC) determined the lyso-PC-induced inhibition of ligand binding to ER and PR in their different ways (Fig. 2); C14:0 chain length lyso-PC inhibited ligand binding both to ER and PR, C16:0 only to PR and C18:0 neither to ER nor PR, irrespective of species, state of pregnancy, or whether the ligand for PR was R5020 or P4 (Table 4).

Compounds of phospholipid metabolism as inhibitors of ligand binding to ER or PR

Phosphatidylethanolamine (PE), PS, phosphatidic acid (PA), lyso-PA, dipalmitoyl-sn-glycerol and stearoylarachidonoyl-sn-glycerol (DAGs) were compared to PI with respect to their ability to inhibit ligand binding to ER or PR. Lyso-PA was found to inhibit the binding of E2 or R5020 to its receptor, in the same range of molar concentration as PI.

Table 5. Inhibition of oestrogen (E2) or progestin (R-5020, P4) binding to human myometrial receptors by compounds involved in phospholipid metabolism

Compound	ER	PR	PR
	E2	R-5020	P4
Phosphatidylinositol	1×10^{-4}	1×10^{-5}	9×10^{-6}
Phosphatidylethanolamine	1×10^{-3}	None	ND
Phosphatidyl-L-serine	1×10^{-3}	9×10^{-5}	6×10^{-5}
Phosphatidic acid	1×10^{-4}	1×10^{-4}	1×10^{-3}
Lysophosphatidic acid	7×10^{-5}	5×10^{-5}	ND
Dipalmitoyl-sn-glycerol	None	None	ND
Stearoylarachidonoyl-sn-glycerol	None	None	ND

Numbers indicate molar concentration at 50% inhibition. ND, not determined.

PA and PS inhibited ligand binding to ER or PR considerably less than PI. DAGs were ineffective. PE exerts a marginal inhibition of ER-binding only at a high concentration (Table 5).

Enhancement of inhibition by ionic and non-ionic detergents

The ionic detergent sodium taurocholate caused a 50% inhibition of E2-binding at 8×10^{-4} M, but inhibited P4-binding at a lower concentration (IC_{50} 5×10^{-5} M). Cholic acid is a weaker inhibitor: no inhibition for E2, but 2×10^{-3} M for P4. The non-ionic detergent Triton X-100 had no effect at all on E2-binding, but the IC_{50} for P4 occurred at 3×10^{-4} M. Triton X-100 (3×10^{-3} M) shifted the inhibition curve on E2-binding caused by PI to the left: the IC_{50} changed from 10^{-4} to 10^{-5} M.

Nature of phospholipid-induced inhibition of ligand binding to ER or PR

The type of inhibition was tested with PI, PA (Fig. 3) and lyso-PC (not shown). It was non-competitive. Phospholipid-induced inhibition was different when different steroids were bound: ligand binding to ER was less sensitive to phospholipids than ligand

Table 4. Fatty acid chain length as a variable in lysophosphatidylcholine-induced inhibition of steroid (E2, R5020, P4) ligand binding to human or guinea-pig myometrial receptors

Chain length	Receptor source		ER	PR, R-5020	PR, P4
C14:0	Human	Non-pregnant	7×10^{-4}	7×10^{-4}	5×10^{-5}
	Guinea-pig	Non-pregnant	7×10^{-4}	4×10^{-4}	ND
		Pregnant	6×10^{-4}	4×10^{-4}	ND
C16:0	Human	Non-pregnant	None	6×10^{-5}	6×10^{-5}
	Guinea-pig	Non-pregnant	None	5×10^{-4}	ND
		Pregnant	None	4×10^{-4}	ND
C18:0	Human	Non-pregnant	None	None	None
	Guinea-pig	Non-pregnant	None	None	ND
		Pregnant	None	None	ND

Numbers indicate molar concentration at 50% inhibition. ND, not determined.

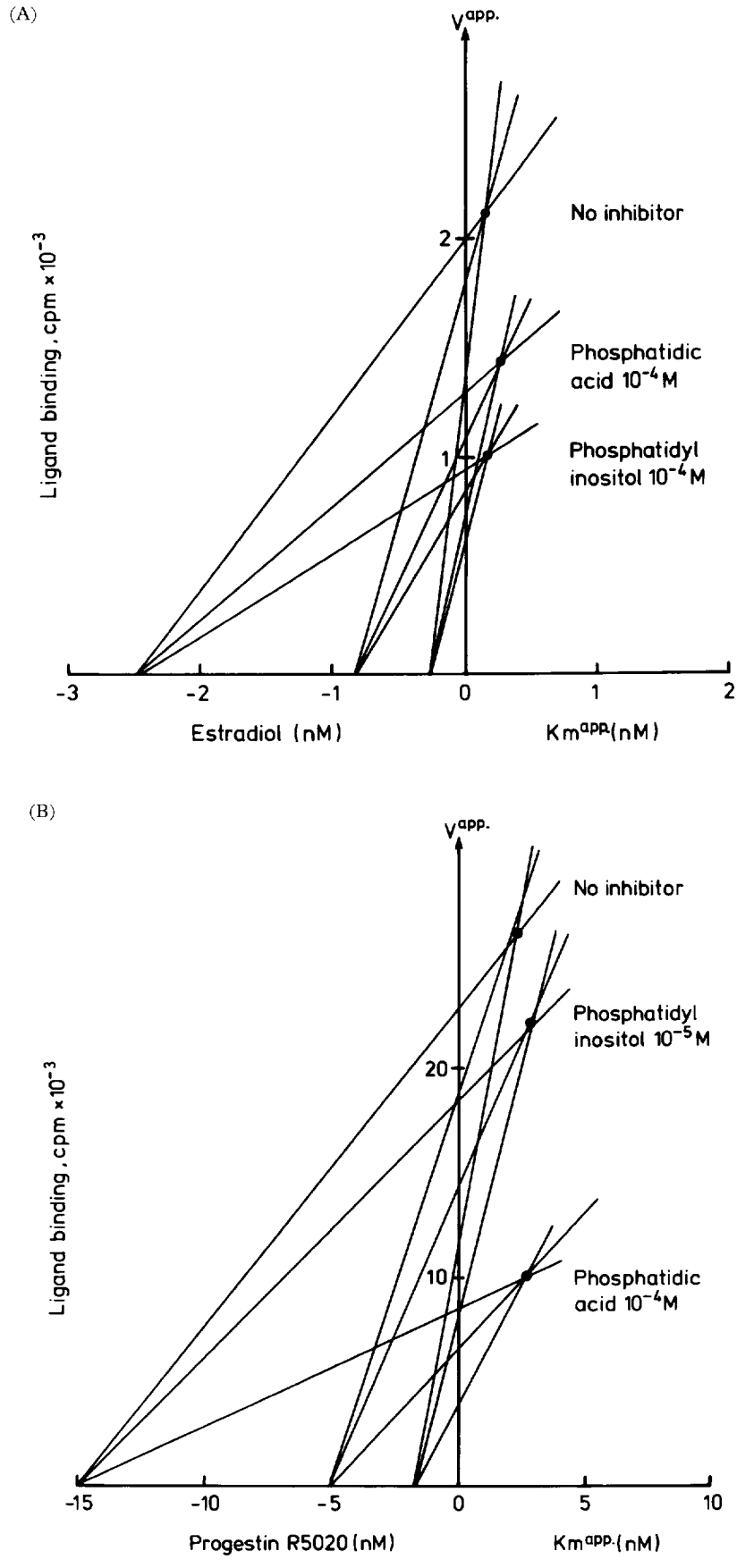

Fig. 3. The direct linear plot according to Eisenthal and Cornish-Bowden [13] indicates a non-competitive inhibition by phosphatidylinositol and phosphatidic acid, when either estradiol-17β (A) or progestin R5020 (B) binds to human myometrial receptors.

Table 6. Phospholipids of decidual and fetal membranes as inhibitors of steroid (E2, P4) ligand binding to human myometrial receptor

	First trimester				Third trimester			
	Decidua		Fetal membranes		Decidua		Fetal membranes	
Patients	ER	PR	ER	PR	ER	PR	ER	PR
1	None	1×10^{-5}	None	5×10^{-6}	None	4×10^{-5}	None	1×10^{-5}
2	None	8×10^{-5}	6×10^{-5}	6×10^{-6}	None	6×10^{-5}	6×10^{-4}	3×10^{-5}
3	None	1×10^{-4}	3×10^{-4}	8×10^{-6}	None	8×10^{-5}	3×10^{-4}	8×10^{-6}

Numbers indicate molar concentration of phospholipid phosphorus/l at 50% inhibition.

binding to PR; R5020 and PR had nearly the same sensitivity (Tables 3–5).

Phospholipids of decidua and fetal membranes

The mean phospholipid phosphorus concentration of the three decidual tissues studied during the first trimester was 120 (range 99–147) μg/g, and during the third trimester 63 (range 42–84) μg/g. Corresponding values for fetal membranes were 119 (range 82–156) μg/g and 82 (range 69–142) μg/g, respectively. Thin-layer chromatographic evaluation of the lipid extracts showed the principal phospholipids to be PE, PI/PS, PC and sphingomyelin. Two unindentified minor fractions were also found in the sulphatide-area. Only a trace amount of lyso-PC was detected. Samples obtained from the first and third trimester pregnancies showed similar fractions of each phospholipid.

The capacity of phospholipid extract of decidual and fetal membranes to inhibit steroid (E2, P4) binding to the human myometrial receptor is illustrated in Table 6. Decidual tissue extract did not inhibit ligand binding to ER, but that of fetal membranes did slightly. Ligand binding to PR was more sensitive than to ER: IC_{50} occurred in the order of 10^{-5} M (decidual phospholipid phosphorus/l) or in the order of 10^{-6} M (fetal membranes), during both first and third trimesters.

DISCUSSION

Low progestin ligand binding [6] in the human myometrium at term was confirmed. At term, myometrial P4-level is about 50 ng/g tissue both in the guinea-pig and in humans [7, 17]. The present pilot studies with egg yolk lecithin, lysolecithin and crude phosphatidylinositol also confirmed the findings of Westphal, that lyso-PC has an effect on steroid receptors [18]. In addition to that, phosphatidylinositol, even as a crude preparation, exerted inhibition at the 10^{-5} M level, irrespective of the species (human, guinea-pig) or whether the myometrium originated from a pregnant or non-pregnant subject.

The non-competitive nature of inhibition is in accordance with earlier findings that PLA2-induced receptor stabilization is a detergent action of lysophospholipids produced in an enzymatic process, but not the action of the enzyme itself [3]. It also accords with the physical chemistry: lysolipid accumulation around proteins results in an extraction of integral proteins into soluble micelles [19]. Inhibition of dexamethasone binding to glucocorticoid receptor by polyunsaturated fatty acids is described to be of the mixed non-competitive type [20], but inhibition of E2-binding to ER by arachidonic acid only non-competitive [21].

The detergent biophysical action of lyso-PC and related compounds as pulmonary surfactant depends, among other things, on the fatty acid substituent and its properties [2]. We used chain length as a parameter, even though other factors, like double bonds, also have an effect [2, 19]. The equilibrium of binding (Table 4) and the size of the micelles formed [19] are determined by the length of the aliphatic chain of the lysophosphatides. E2 and P4, or P4 only can be selected for down regulation, by blocking the receptor binding with a phospholipid of different fatty acid chain length. P4-binding to the glucocorticoid receptor is inhibited by physiological concentrations of nonesterified fatty acids as a function of dose, degree of unsaturation and chain length of the fatty acid [20].

Arachidonic acid is one of the modulators of ER and PR in the uterus [21]. As found in this study with lyso-PC, PI and related compounds, there is a lower sensitivity for ER to the arachidonate effect than for PR, regulated also by the fatty acid chain length. The fatty acid chain length was a parameter not only in the binding of progestin R5020 but also of progesterone, the pregnancy-maintaining hormone [22].

When the performance of ionic and non-ionic detergents was compared, it appeared that a non-ionic detergent can act on the P4-receptor, but not on the E2-receptor. By contrast, an ionic detergent (sodium salt of taurocholic acid) can block both E2 and P4 action by inhibiting ligand binding. If a non-ionic detergent is present at the same time as PI when the ligand is bound to ER, it enhances the reaction by a factor of 10. This accords with the modulation of lecithin-dependent parturition of Ca^{2+} by combination of cholate + lysolecithin: avian lysolecithin is barely capable of transporting Ca^{2+} but the combination of

cholate + lysolecithin increases its capability by a factor of 10 [23]. When the catalytic and membrane-perturbing properties of lyso-PC are studied, the difference between ionic and non-ionic detergents is found to lie in their critical micelle concentration, which determines the energy difference at the aqueous-hydrophobic interface [19]. The chemical step is not rate-limiting during the hydrolysis by PLA2 of mixed micelles of phospholipid and detergent [24].

Among the seven compounds related to phospholipid metabolism, PE and DAGs did not stabilize PR. PS and PA were equally active, but PI was most effective (Table 5). The lyso-derivative of PA, like the lyso-PC from lecithin, is more effective than the parent compound. This could be another type of PLA2-action during pregnancy, in addition to arachidonic acid and prostanoid formations [1, 21]. Phospholipid extracts from decidua and fetal membranes resembled a non-ionic detergent and C16:0 fatty acid chain: they did not affect ER but PR binding.

The concentrations of phospholipids that were found to inhibit steroid ligand binding to myometrial receptors in this study exist in many tissues [16], including decidua and fetal membranes (Table 6). Free fatty acids are at higher concentration close to the target cell than in the general circulation, and mediate conformational changes in the structure of corticosteroid binding globulin. This is associated with marked changes in the functional properties (like P4-binding) of this protein both *in vivo* and *in vitro*, confirming the biological significance of the physicochemical environment for steroid function [25].

It can be concluded, that certain phospholipids may inhibit the binding of sex steroids to their receptors, through their detergent properties, thus changing the functional environment of the receptors. Fetal membranes and decidual tissue contain sufficient phospholipids to provoke the changes described above in the activity of the steroid receptors. It will be important in future to determine whether there are pregnancy-induced changes in the composition of phospholipids that may modify the functioning of E2 and P4. This could regulate pregnancy maintenance [22], as indicated by the labor-inducing properties of these compounds [26]. Induction of labor after treatment with phospholipids, through phospholipid action on the myometrial P4-block, provides a model with which to study the regulation of steroid hormone effects [27].

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