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Effect of Meiotic Maturation on Yolk Platelet Lipids From *Bufo arenarum* Oocytes

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ABSTRACT Progesterone induces the resumption of meiosis in *Bufo arenarum* full-grown arrested oocytes through a nongenomic mechanism called meiotic maturation. Growing evidence indicates that lipids are involved in the maturation process. They are mainly located in yolk platelets, the principal organelles of amphibian oocytes. The aim of the present study was to analyze the effect of progesterone-induced maturation on lipids from *B. arenarum* yolk platelets. Ovarian oocytes, manually obtained, were incubated with progesterone to induce maturation. Yolk platelets were isolated by centrifugation at low velocity. Lipids were separated by thin-layer chromatography. For compositional analysis, they were derivatized by methanolysis, and were identified and quantified in a gas–liquid chromatograph. Phospholipid content decreased in progesterone-treated oocytes, mainly as a result of a decrease at the level of phosphatidylcholine (PC). The turnover of this lipid is considered crucial for the completion of meiosis. Sphingomyelin also underwent a decrease that could be related to the important role of ceramide as an inducer of germinal vesicle breakdown. Maturation effect on fatty acid composition registered significant changes in PC whose saturated fatty acids increased. A net increase in arachidonic acid was observed in phosphatidylserine after progesterone treatment. The contents of total triacylglycerols and diacylglycerols were not significantly modified by hormone effect while free fatty acids underwent a significant increase as a result of polyunsaturated fatty acids increase. Altogether, our results demonstrate that yolk platelet lipids are involved in the resumption of the meiotic cell cycle, thus suggesting that these organelles participate in a dynamic role during amphibian development. *J. Exp. Zool. 303A:813–822, 2005.*

Ovarian full-grown oocytes from *Bufo arenarum* are arrested at the first meiotic prophase and can be induced to develop into fertilizable eggs by the steroid hormone progesterone in a process called meiotic maturation. The maturing oocytes re-enter meiosis, undergo germinal vesicle breakdown (GVBD), and complete meiosis I with the extrusion of a polar body. The oocytes subsequently enter meiosis II and arrest at metaphase (Ferrell, '99; Nebreda and Ferby, 2000). Progesterone induces the resumption of meiosis through a nongenomic mechanism that involves the inhibition of adenylyl cyclase (Sadler and Maller, '81) and the reduction of intracellular cAMP (Schorderet-Slatkine et al., '78). In this respect, previous evidence indicates that the progesterone receptor in *Xenopus* oocytes is a membrane-bound receptor (Masui and Markert, '71; Godeau et al., '78). In contrast, two studies (Bayaa et al., 2000; Tian et al., 2000) report new evidence suggesting that the oocyte GVBD response could be mediated by the nuclear progesterone receptor. Interestingly, Edwards et al. (2002) proposed that progesterone receptor functions as an activator of Src signal transduction pathways in the cytoplasm and as a transcription factor in the nucleus.

An early response to progesterone is the synthesis of the onco-protein c-Mos, resulting in maturation-promoting factor (MPF) activation (Nebreda and Ferby, 2000). The biological activity of c-Mos is mediated by the activation of MAP kinase (MAPK) cascade through the phosphorylation of the MAPK-activating kinase, MEK (Nebreda and Hunt, '93). Another signaling pathway

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mediates the activation of MPF prior to GVBD. It involves the activation of the phosphatase Cdc25C by the *Xenopus* polo-like kinase xPlk1, which itself is activated by upstream kinase kinases such as the *Xenopus* polo-like kinase kinase, xPlkk1 (Qian et al., 2001; Kelm et al., 2002; Tunquist and Maller, 2003).

There is growing evidence that lipids are involved in the maturation process. The initial action of progesterone in *Rana* oocytes at its plasma membrane receptor triggers a series of enzyme activations that not only modify the membrane but also release a cascade of lipid messengers, such as ceramide (Morrill and Kostellow, '98) and multiple DAG species (Morrill and Kostellow, '99; Morrill et al., 2000). In addition, the first product of phosphatidylethanolamine (PE) *N*-methylation, phosphatidylmonomethylethanolamine, is a lipid mediator for progesterone action (Kostellow et al., 2001). In *Xenopus* oocytes, the steroidal hormone induces a phospholipid turnover (Stith et al., '92) and increases in DAG levels (Stith et al., '91) that could result in a pathway leading to the activation of PKC (Bement and Capco, '91; Johnson and Capco, '97). It has been demonstrated that progesterone-induced maturation leads to significant qualitative and quantitative modifications in *B. arenarum* oocyte lipids and their fatty acid composition (Alonso, 2000).

Subcellular studies carried out during early *B. arenarum* embryogenesis indicated that lipids are mainly located in yolk platelets (Alonso et al., '82; Alonso and Bonini de Romanelli, '86).

Amphibian yolk platelets are membrane-bound crystalline structures (Karasaki, '63) present in considerable quantities in the oocytes. They are generally considered as mere reservoirs storing materials for embryogenesis. An alternative but related function for sea urchin yolk as a site of catabolism is suggested by the activity of several acid hydrolases, including acid phosphatase (Schuel et al., '75). Such catabolic enzymes could be used to generate amino acids, fatty acids or carbohydrates from their stored macromolecular forms. In addition, organelles containing lysosomal enzyme activities were shown to be associated with a subpopulation of yolk platelets in *Xenopus* oocytes (Wall and Meleka, '85). It has been reported that yolk platelets are not simple lysosomes, but novel membrane-rich organelles that have the ability to regulate proton fluxes (Mallya et al., '92). Immunogold electron microscopy showed that the G-protein α_s subunit is present in the yolk platelet membranes from *Xenopus* oocytes suggesting that the target of progesterone could include yolk platelet membranes as well as the plasma membrane (Gallo et al., '95). G-proteins on yolk platelet membranes could also be related to the formation of the yolk platelets by endocytosis, endosome fusion, and polarized vesicular transport (Danilchik and Gerhart, '87) during oogenesis.

Furthermore, yolk platelets are capable of metabolizing triacylglycerols (TAG) rapidly and efficiently, since the diacylglycerols (DAG) and free fatty acids (FFA) do not accumulate or they function to supply TAG to other cellular sites for a latter metabolization (Alonso et al., '86). Besides, yolk platelets vesicular structure may indicate a role either in lipogenesis or in membrane biogenesis. In this sense, their ability to *de novo* biosynthesize glycerolipids has been demonstrated in unfertilized *B. arenarum* oocytes (Alonso, '89).

The aim of the present study was to analyze the effect of progesterone-induced maturation on polar and neutral lipids of yolk platelets from *B. arenarum* stage V oocytes.

MATERIALS AND METHODS

Ethics

Investigations were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* promulgated by the Institute for Laboratory Animal Research (ILAR) of the National Academy of Science.

Experimental system

Mature females of the toad *Bufo arenarum* Hensel, 1867 were collected in Bahía Blanca, Argentina, during the hibernation period (winter animals). The ovarian tissue was surgically removed and transferred to ND 96 solution (96 mM NaCl, 1 mM $MgCl_2$, 2 mM KCl, 5 mM HEPES; pH 7.4; Sigma, St. Louis, MO). Only those ovaries containing a large population of full-grown oocytes were used. Full-grown prophase-arrested oocytes, labelled as stage V according to Valdéz Toledo and Pisanó ('80), were isolated with watchmaker's forceps under a stereotaxic microscope.

Induction of oocyte maturation

Maturation was induced by incubation of defolliculated oocytes in ND 96 solution with progesterone 5 μg/ml final concentration for 7 hr, while control oocytes were incubated in ND 96 solution without progesterone for the same period of time.

The criterion used for successful induction of the meiosis reinitiation was the GVBD ascertained by dissection of heat-fixed oocytes. All experiments were carried out with oocytes from clutches in which GVBD underwent with a frequency of 100% after progesterone treatment. Spontaneous nuclear membrane breakdown was never observed in progesterone-untreated *B. arenarum* oocytes.

Subcellular fractionation

Oocyte homogenates were prepared in 50 mM Tris–HCl buffer, pH 7.4, containing 10^{-3} M EDTA and 0.3 M sucrose according to the procedure usually followed in this laboratory (Bonini de Romanelli et al., '81). The homogenate was centrifuged at 1,500*g* for 20 min. The pellet was rehomogenized in the same medium and centrifuged at 1,500*g* for 20 min. The washing was repeated, after which, the yolk platelet fraction was sedimented as previously indicated. Electron microscopy showed typical intact structures and no other membrane material (Alonso, '89). It is necessary to point out that oocyte plasma membrane is obtained when the homogenates are centrifuged at about 10,000*g*, a higher velocity than that required for yolk platelets (Blondeau and Baulieu, '84, '85). The pellet contained the plasma membrane as was demonstrated by enzyme markers (Alonso et al., '92).

In addition, thin-layer chromatograms of lipid extracts from yolk platelets in overloaded plates were found to be completely devoid of diphosphatidylglycerol or cardiolipin, a typical component of mitochondria.

Lipid analysis

Lipids were extracted from tissues as described by Folch et al. ('57). Phospholipids were separated by two-dimensional thin-layer chromatography (TLC) on silica gel H according to Rouser et al. ('70). Phospholipids were located by iodine vapors; the silica was scraped off and phosphorus was quantified after digestion with perchloric acid (Rouser et al., '70). Neutral lipids were separated by mono-dimensional TLC on silica gel G using hexane/diethylether/acetic acid (80:20:1, by vol.). Pure substances as standards were used.

For the determination of fatty acid composition, lipids were separated by TLC as described above, and spots were visualized under ultraviolet light after spraying with 2′-7′-dichlorofluorescein in methanol. Methanolysis was performed directly on the silica spots scraped off the plates according to the method of Morrison and Smith ('64) using boron trifluoride (14% w/v in methanol, Sigma, St. Louis, MO). Fatty acid methyl esters were purified by TLC using hexane/diethylether (95:5, by vol.) on silica gel 60 plates prewashed with methanol/diethylether (75:25, by vol.). Fatty acid analysis was carried out by temperature-programmed gas–liquid chromatography (GLC) using 21:0, added before methanolysis, as internal standard. Two glass columns (2 m × 0.2 cm i.d.) packed with 10% SP2330 on 80–120 Chromosorb WAW (Supelco, Bellefonte, PA) were connected to two flame ionization detectors operated in the dual-differential mode. The initial and final oven temperatures were 160°C and 220°C, respectively, and the rate of increase was 5°C/min. Injector and detector temperatures were 220°C and 230°C, respectively. The carrier gas was N_2 (30 ml/min). Chromatograms were quantified with a CDS-111 Varian integrator (Palo Alto, CA). Peaks were identified by comparison of retention times with those of standards. The unsaturation index (UI) was determined as the sum of percentages of individual unsaturated fatty acids times the number of double bonds.

During all the procedures (lipid extraction, solvent evaporation, TLC spotting, drying and spraying of the TLC plates, and derivatization), the lipids were kept in a N_2 atmosphere. All organic solvents were of analytical grade.

Other analytical methods

Proteins were determined by Lowry et al. ('51) after extraction with 1 N NaOH using crystalline bovine serum albumin as standard.

Statistical analysis

Statistical analysis was carried out using Student's *t*-test with the values representing the mean ± standard deviation of the total number of samples indicated in each legend.

RESULTS

Effect of progesterone-induced maturation on the content and fatty acid composition of phospholipids

The effect of progesterone treatment on the phospholipid content of yolk platelets was measured by phospholipidic phosphorus determination (Fig. 1). The total amount of phospholipids registered a significant decrease of about 23 nmol/mg protein when yolk platelets from control and

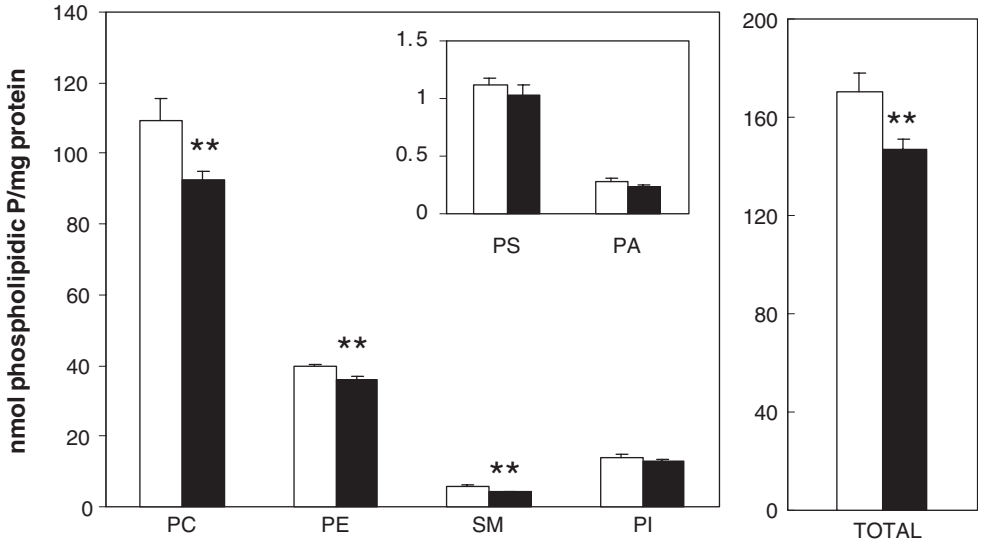

Fig. 1. Effect of progesterone-induced maturation on the content of phospholipids from yolk platelets of *Bufo arenarum* oocytes. Phospholipidic phosphorus was measured according to Rouser et al. ('70). Results are presented as nmol of phosphorus per mg protein and are mean values ± SD from four independent samples. (□) Control oocytes; (■) GVBD oocytes. Asterisks indicate significant ($^{**}P < 0.01$) differences with respect to control oocytes. GVBD: germinal vesicle breakdown; PC: phosphatidylcholine; PE: phosphatidylethanolamine; SM: sphingomyelin; PI: phosphatidylinositol; PS: phosphatidylserine; PA: phosphatidic acid.

progesterone-treated oocytes were compared. This change was mainly due to a decrease of 17 nmol/mg protein in phosphatidylcholine. This decrease represents 74% of the total phospholipid decrease. PE and sphingomyelin (SM) also showed significant decreases. Phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA) amounts remained constant. In addition, it is worthy of note that the total protein amount of yolk platelet fraction (0.18 mg/oocyte; data not shown) underwent no changes during meiotic maturation.

Compositional studies were carried out by GLC in yolk platelet fractions from control and progesterone-treated oocytes. Maturation effects on fatty acid compositions of PC, PE, and PI are shown in Table 1. In PC, an increase of about 3 mol% was found at the level of saturated fatty acids (SFA), mainly in palmitic acid (16:0), an abundant fatty acid. Significant changes were also observed at the level of 17:0, 17:1, and 20:5n3, which are minor acyl groups of PC. A few changes were observed in PE where stearic acid (18:0), one of the most abundant fatty acids, showed a net decrease of about 3 mol%. SFA, monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) underwent no changes during meiotic maturation. In PI, 18:0 underwent an important decrease of about 13 mol% while other fatty acids such as 16:0, 17:0, arachidonic acid (20:4n6), and 22:5n3 tended to increase. As a result, the fatty acid profile remained constant before and after progesterone stimulation.

The UI of PC, PE, and PI remained constant during meiosis reinitiation. The hormone effects on PS, PA, and SM in yolk platelets are shown in Table 2. The acyl group compositional distribution evidenced changes in PS in which a significant decrease was found in 18:0, the most abundant constituent. A net increase in 20:4n6 was also observed. However, total SFA, MUFA, and PUFA were not altered by hormone stimulation and the UI therefore remained unchanged. As to PA, a decrease was observed at MUFA level due to 16:1 and oleic acid (18:1), an important fatty acid in this phospholipid. A net decrease was measured in linoleic acid (18:2n6). PUFA, particularly the fatty acids with carbon chain lengths of 22, underwent a significant increase mainly in 22:5n3 and 22:6n3. The UI was higher in progesterone-treated oocytes.

SM differs from glycerophospholipids in that it contains almost exclusively saturated and monoenoic fatty acids. Almost all fatty acids underwent significant changes in their distribution during meiotic reinitiation. In control yolk platelets, 16:0 represented about 62 mol% of the total fatty acids and underwent a 50% decrease as a consequence of progesterone stimulation. Nervonic acid (24:1), a characteristic component of SM, was the most

TABLE 1. Effect of progesterone-induced maturation on fatty acid composition of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol in yolk platelets from Bufo arenarum oocytes

	PC		PE		PI	
Fatty acids	Control	GVBD	Control	GVBD	Control	GVBD
14:0	0.66 ± 0.12	0.80 ± 0.15	0.15 ± 0.04	nd	nd	nd
15:0	0.58 ± 0.05	0.66 ± 0.09	0.18 ± 0.03	nd	nd	0.15 ± 0.02
16:0	21.05 ± 1.43	24.08 ± 1.69*	11.20 ± 0.46	11.75 ± 1.37	11.70 ± 4.07	14.04 ± 3.66
16:1	5.25 ± 0.52	5.32 ± 0.35	2.04 ± 0.16	2.76 ± 0.39*	nd	nd
17:0	1.35 ± 0.07	1.06 ± 0.07**	1.21 ± 0.34	1.17 ± 0.14	nd	1.41 ± 0.41
17:1	0.99 ± 0.07	0.75 ± 0.07**	0.75 ± 0.19	0.77 ± 0.12	nd	0.51 ± 0.10
18:0	3.58 ± 0.69	3.75 ± 0.61	15.58 ± 1.76	12.65 ± 1.52*	33.72 ± 5.29	20.82 ± 0.93**
18:1	22.36 ± 1.96	22.78 ± 2.02	24.64 ± 2.63	23.23 ± 1.78	6.84 ± 0.35	5.91 ± 2.73
18:2n6	19.38 ± 1.44	20.06 ± 2.12	11.64 ± 1.37	13.50 ± 1.28	3.82 ± 1.04	3.14 ± 1.06
18:3n3	4.05 ± 0.89	3.79 ± 0.61	2.28 ± 0.61	2.54 ± 0.90	nd	0.54 ± 0.24
20:3n6	0.88 ± 0.22	0.65 ± 0.18	0.47 ± 0.04	0.53 ± 0.03	nd	nd
20:4n6	8.25 ± 1.97	7.36 ± 0.70	14.67 ± 1.67	16.49 ± 2.81	42.06 ± 4.57	50.88 ± 9.05
20:5n3	4.33 ± 0.84	3.02 ± 0.65*	5.22 ± 0.90	5.13 ± 0.98	1.86 ± 0.19	nd
22:5n6	nd	0.54 ± 0.09	nd	0.45 ± 0.14	nd	nd
22:5n3	3.46 ± 0.74	2.84 ± 0.71	3.79 ± 0.91	3.59 ± 0.47	nd	2.59 ± 0.35
22:6n3	3.82 ± 1.17	2.56 ± 0.84	6.17 ± 1.47	5.47 ± 0.97	nd	nd
SFA	27.23 ± 2.01	30.34 ± 1.50*	28.32 ± 2.12	25.56 ± 2.19	45.42 ± 5.13	36.43 ± 4.87
MUFA	28.60 ± 2.42	28.85 ± 1.98	27.43 ± 2.88	26.76 ± 1.72	6.84 ± 0.35	6.42 ± 2.82
PUFA	44.17 ± 4.07	40.81 ± 2.52	44.25 ± 3.66	47.68 ± 3.78	47.74 ± 5.36	57.15 ± 7.61
UI	187.76 ± 18.82	168.85 ± 7.91	210.62 ± 17.25	218.28 ± 19.62	200.18 ± 19.18	240.57 ± 29.22

Phospholipids were isolated by bidimensional TLC and analyzed by GLC. Results are presented as mol% and are mean values ± SD from four independent samples. Asterisks indicate significant (*$P < 0.05$ or **$P < 0.01$) differences with respect to control oocytes; nd: not detected; GVBD: germinal vesicle breakdown; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UI: unsaturation index as described in Materials and Methods.

abundant very long-chain unsaturated fatty acid before and after maturation. Fatty acids with carbon chain lengths of 20 and more carbon atoms showed net increases. The net decrease observed in SFA and the increase in MUFA, which was produced by 18:1, led to a significant increase in the UI.

Altogether, our results indicate that, after progesterone treatment, SM showed the lowest UI among all the phospholipids analyzed while PI was the most unsaturated one (Tables 1 and 2).

Similar results were obtained when phospholipid contents were measured either by phospholipidic phosphorus determination or by GLC (data not shown).

Effect of progesterone-induced maturation on the content and fatty acid composition of neutral lipids

In order to determine whether or not neutral lipids level and their composition were modified during maturation, they were analyzed by GLC in yolk platelets from control and progesterone-treated oocytes (Table 3). The total amount of TAG evidenced a tendency to increase after meiosis reinitiation. The compositional study showed a net increase in the content of 18:0 and a decrease in the UI, both of which have also been observed in whole oocytes of *B. arenarum* (Alonso, 2000). The content of total DAG was not significantly modified by the hormone effect as it was similarly found in whole oocytes (Alonso, 2000). A significant decrease at SFA level was registered, mainly in 16:0, the most abundant fatty acid. MUFA showed a net increase that was produced mainly by 18:1. In addition, 20:4n6 underwent a significant decrease. However, total PUFA amount remained constant.

The treatment with progesterone showed a significant increase of about 3.7 nmol/mg protein in the total amount of yolk platelet FFA. This increase was mainly due to PUFA increase.

The UI increased in both DAG and FFA during meiosis maturation.

In all the cases analyzed in the present study, very long-chain unsaturated fatty acids were minor constituents. Their identification on the

TABLE 2. Effect of progesterone-induced maturation on fatty acid composition of phosphatidylserine, phosphatidic acid, and sphingomyelin in yolk platelets from Bufo arenarum oocytes

	PS		PA		SM		
Fatty acids	Control	GVBD	Control	GVBD	Fatty acids	Control	GVBD
14:1	nd	0.30 ± 0.08	0.77 ± 0.14	1.92 ± 0.60*	14:0	0.45 ± 0.22	nd
15:0	nd	0.29 ± 0.07	nd	1.24 ± 0.37	14:1	nd	0.31 ± 0.13
16:0	8.55 ± 0.99	11.45 ± 3.44	20.97 ± 0.76	24.52 ± 4.67	15:0	0.70 ± 0.17	0.47 ± 0.14
16:1	nd	3.08 ± 0.82	4.37 ± 0.10	nd	16:0	62.45 ± 1.44	32.44 ± 2.83**
17:0	1.73 ± 0.22	0.76 ± 0.19**	nd	nd	17:0	4.22 ± 0.83	2.11 ± 0.71**
17:1	nd	nd	nd	0.42 ± 0.10	18:0	6.65 ± 1.43	11.63 ± 2.04**
18:0	32.28 ± 0.51	23.87 ± 4.25*	20.50 ± 1.16	18.08 ± 4.64	18:1	5.22 ± 0.81	12.69 ± 1.21**
18:1	8.67 ± 0.07	10.22 ± 4.10	25.17 ± 1.32	15.11 ± 0.87**	18:2n6	3.69 ± 1.83	6.83 ± 2.10
18:2n6	9.40 ± 1.30	7.35 ± 1.21	11.79 ± 1.46	7.69 ± 1.26*	20:0	1.44 ± 0.33	2.56 ± 0.78*
18:3n3	1.00 ± 0.21	1.30 ± 0.21	1.28 ± 0.20	0.94 ± 0.13	20:1	nd	0.60 ± 0.03
20:3n6	0.81 ± 0.00	nd	nd	nd	22:0	5.53 ± 0.49	5.82 ± 4.78
20:4n6	19.69 ± 1.24	26.46 ± 2.26**	11.48 ± 2.00	8.39 ± 1.05	24:0	1.91 ± 0.49	3.44 ± 0.44**
20:5n3	8.37 ± 2.84	nd	nd	nd	24:1	3.36 ± 0.73	6.41 ± 0.78**
22:4n6	1.81 ± 0.41	nd	nd	5.60 ± 1.97	25:0	2.24 ± 0.37	7.05 ± 0.55**
22:5n3	2.11 ± 0.14	3.75 ± 0.95*	1.25 ± 0.18	4.89 ± 0.99**	26:0	2.12 ± 0.38	7.64 ± 1.58**
22:6n3	5.58 ± 0.62	11.17 ± 4.77	2.40 ± 0.48	11.19 ± 4.53*			
SFA	42.56 ± 1.36	36.38 ± 4.51	41.48 ± 1.85	43.84 ± 6.42	SFA	87.73 ± 1.25	73.16 ± 3.04**
MUFA	8.67 ± 0.07	13.60 ± 3.69	30.32 ± 1.31	17.46 ± 1.11**	MUFA	8.58 ± 0.76	20.02 ± 1.08**
PUFA	48.77 ± 1.41	50.02 ± 4.34	28.21 ± 2.94	38.70 ± 5.51*	PUFA	3.69 ± 1.83	6.83 ± 2.10
UI	215.28 ± 12.43	237.42 ± 25.23	131.45 ± 8.05	200.14 ± 37.69*	UI	17.13 ± 2.95	33.20 ± 4.98**

Phospholipids were isolated by bidimensional TLC and analyzed by GLC. Data are mean values ± SD (mol%) of four independent samples. Asterisks indicate significant (*$P < 0.05$ or **$P < 0.01$) differences with respect to control oocytes; nd: not detected; GVBD: germinal vesicle breakdown; PS: phosphatidylserine; PA: phosphatidic acid; SM: sphingomyelin; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UI: unsaturation index as described in Materials and Methods.

basis of chromatographic behavior with respect to known standards is tentative. The role of these fatty acids in higher animals and plants has not been fully elucidated to date.

DISCUSSION

Lipids are involved in the production of intracellular signals and in the regulation of processes as diverse as cell proliferation, chemotaxis, differentiation, stress responses, senescence, and apoptosis (Pyne and Pyne, 2000; Brownlee, 2001; Repp et al., 2001; Deacon et al., 2002). There is general consensus in that progesterone begins to act at amphibian oocyte plasma membrane receptor within seconds to induce phospholipid turnover as well as to release a cascade of lipid messengers (Chien et al., '91; Kostellow et al., '93; Morrill et al., '94).

Data from our study showed a significant decrease in the total amount of phospholipids in yolk platelets mainly as a result of PC decrease. Similar results were found when the effect of progesterone-induced maturation on the content of polar lipids was analyzed in whole *B. arenarum* oocytes (Alonso, 2000). In addition, it has been reported that in *Rana pipiens* oocytes, progesterone triggers an early and transient increase in DAG derived from PC in the plasma membranes mainly through the stimulation of the PC-PLD pathway and the phosphatidate phosphohydrolase activity (Kostellow et al., '96). This DAG transient appears and is terminated within 60–90 sec after progesterone stimulation. PC turnover itself, or one of its downstream products, PA or DAG, is likely essential for the completion of meiosis (Howe and McMaster, 2001). The phospholipase D relocalization and catalytic activity are both required for a crucial function at spindle poles (Rose et al., '95) and in regulated membrane formation during meiosis (Rudge et al., '98). Generation of PA or DAG could alter the characteristics of membranes to promote membrane curvature similar to what is postulated to underlie the generation of Golgi vesicles (Ktistakis et al., '96; Kearns et al., '97).

In our study, the level of DAG did not increase after progesterone treatment (Table 3). Never-

TABLE 3. Effect of progesterone-induced maturation on the content and fatty acid composition of neutral lipids in yolk platelets from Bufo arenarum oocytes

	TAG		DAG		FFA	
Fatty acids	Control	GVBD	Control	GVBD	Control	GVBD
14:0	2.48 ± 0.68	3.18 ± 0.45	nd	nd	nd	nd
14:1	nd	1.37 ± 0.26	nd	nd	nd	nd
15:0	0.93 ± 0.18	1.13 ± 0.21	nd	nd	nd	nd
15:1	0.25 ± 0.01	0.27 ± 0.09	nd	nd	nd	nd
16:0	22.57 ± 3.05	26.93 ± 3.28	2.23 ± 0.43	0.61 ± 0.08**	0.85 ± 0.11	1.13 ± 0.37
16:1	11.40 ± 1.74	12.90 ± 2.76	0.31 ± 0.06	0.21 ± 0.05	0.49 ± 0.21	0.49 ± 0.05
17:0	1.32 ± 0.15	1.51 ± 0.38	0.05 ± 0.02	0.08 ± 0.04	0.08 ± 0.02	nd
17:1	1.46 ± 0.14	1.47 ± 0.38	nd	nd	0.03 ± 0.01	nd
18:0	2.49 ± 0.42	3.59 ± 0.64*	0.26 ± 0.05	0.27 ± 0.06	0.54 ± 0.08	0.54 ± 0.17
18:1	20.43 ± 2.47	22.74 ± 4.14	0.29 ± 0.08	0.65 ± 0.09**	1.38 ± 0.44	2.02 ± 0.45
18:2n6	21.00 ± 1.04	23.22 ± 4.14	0.48 ± 0.08	0.49 ± 0.08	1.91 ± 0.14	2.95 ± 0.45*
18:3n6	nd	0.80 ± 0.16	nd	nd	nd	nd
18:3n3	6.63 ± 0.60	6.65 ± 1.34	0.21 ± 0.04	0.12 ± 0.04*	0.72 ± 0.77	0.95 ± 0.14
20:4n6	2.65 ± 0.08	3.11 ± 0.37	0.56 ± 0.12	0.32 ± 0.05*	0.42 ± 0.02	0.46 ± 0.01
20:4n3	0.65 ± 0.07	0.45 ± 0.16	nd	nd	nd	nd
20:5n3	1.85 ± 0.22	1.59 ± 0.25	nd	0.24 ± 0.09	0.24 ± 0.02	1.85 ± 0.22**
22:4n6	0.40 ± 0.11	0.37 ± 0.04	0.14 ± 0.03	0.05 ± 0.02*	nd	nd
22:5n6	nd	0.10 ± 0.01	nd	nd	nd	nd
22:5n3	2.06 ± 0.34	1.64 ± 0.20	0.11 ± 0.02	0.05 ± 0.01**	nd	nd
22:6n3	1.41 ± 0.47	0.96 ± 0.16	nd	0.06 ± 0.01	nd	nd
Total	33.33 ± 3.41	37.99 ± 5.84	2.31 ± 0.46	1.58 ± 0.20	6.66 ± 0.69	10.38 ± 1.77*
SFA	29.81 ± 4.25	36.35 ± 4.85	2.54 ± 0.50	0.96 ± 0.12**	1.46 ± 0.21	1.66 ± 0.53
MUFA	33.54 ± 4.21	38.76 ± 7.10	0.60 ± 0.14	0.86 ± 0.06*	1.90 ± 0.50	2.51 ± 0.45
PUFA	36.65 ± 2.56	38.87 ± 6.21	1.49 ± 0.28	1.34 ± 0.27	3.29 ± 0.24	6.20 ± 0.78**
UI	138.51 ± 7.01	127.76 ± 3.57*	119.25 ± 0.69	173.16 ± 10.16**	162.10 ± 4.49	216.41 ± 10.54**

Neutral lipids were isolated by monodimensional TLC and their fatty acid composition was quantified by GLC. Results are presented as nmol per mg protein and are mean values ± SD from four independent samples. Asterisks indicate significant (*$P < 0.05$ or **$P < 0.01$) differences with respect to control oocytes; nd: not detected. The total amount of triacylglycerols and diacylglycerols was divided by three and two, respectively. GVBD: germinal vesicle breakdown; TAG: triacylglycerols; DAG: diacylglycerols; FFA: free fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UI: unsaturation index as described in Materials and Methods.

theless, the possibility that DAG could be generated and rapidly used to form other lipids such as TAG cannot be discarded. In fact, TAG showed a tendency to increase during maturation. In agreement with this, a stimulation of triacylgycerol biosynthesis, which is consistent with the putative employment of these lipids in supplying the energy required for the fertilization process, has been reported in the whole system (Alonso et al., '86; Alonso, 2000).

It is interesting to note that in the present study SM decreased after meiotic reinitiation. The treatment of *Xenopus* oocytes with progesterone also resulted in a decrease in the mass of SM through activation of an Mg^{2+}-dependent neutral sphingomyelinase, and a time- and concentration-dependent increase in the mass of ceramide, an important intracellular second messenger (Strum et al., '95). Similarly, a ceramide increase was found in *Rana* oocytes during maturation (Morrill and Kostellow, '98). Although ceramide is an effective inducer of nuclear breakdown in gonadotropin-primed oocytes from either *Xenopus* or *Rana*, its effect may depend on the priming action of subthreshold levels of progesterone at the plasma membrane (Morrill and Kostellow, '98).

Diacylglycerol fatty acid profile showed significant changes in the acyl group distribution (Table 3). PUFA such as 20:4n6, 22:4n6, and 22:5n3 denoted decreases. As to FFA, a net increase was observed as a result of 20:5n3 and 18:2n6 (the precursor to 20:4n6) increases after progesterone treatment. A significant increase in 20:4n6 was determined in PS (Table 2).

Usually, the free cellular level of 20:4n6 is very low because the liberated fatty acid is rapidly metabolized to prostaglandins, to thromboxanes, to leukotrienes and various hydroxyeicosatetrae-

noic acids, and to epoxyeicosatrienoic acids. Alternatively, 20:4n6 can be re-esterified into phospholipids by acyltransferases. In addition, 20:4n6 may play a second messenger role in the signal transduction mechanism while it is in the free liberated fatty acid state (Naor, '91). In starfish, arachidonic acid induces oocyte maturation at very low concentrations as well as its lipoxygenase products (Meijer et al., '84). Similarly, some prostaglandins stimulated oocyte maturation in a dose- and time-dependent manner in the marine teleost *Dicentrarchus labrax* (Sorbera et al., 2001).

To our knowledge, there are no data on unusual fatty acids as 14:0, 15:0, 15:1, 17:0, and 17:1 in different biological systems. The presence of these fatty acids may involve an oxidation mechanism in yolk platelets different from β-oxidation. Furthermore, some of these unusual fatty acids may be probably synthesized from propyonylCoA.

This different β-oxidation mechanism may occur in yolk platelets and/or it may suggest a possible structural and functional relationship between these organelles and mitochondria in agreement with previous studies (Ward, '62; Massover, '71; Vallejo et al., '79).

In other biological systems, it has been reported that proteolysis of yolk proteins occurs during seabream *Sparus aurata* oocyte meiotic reinitiation (Carnevali et al., '99) and that in *Verasper moseri*, two forms of vitellogenin, which are components of yolk platelets, play different roles during oocyte maturation and early development (Matsubara et al., '99). In this respect, our results provide the first evidence that lipids from amphibian yolk platelets are also involved in the physiological process of oocyte maturation. Altogether, the data suggest a more dynamic role of yolk platelets in development.

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