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Effect of 17β-estradiol on chondrocyte membrane fluidity and phospholipid metabolism is membrane-specific, sex-specific, and cell maturation-dependent

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Abstract

In this study we examined the hypothesis that 17β-estradiol exerts both rapid and direct, nongenomic effects on cells in the endochondral pathway. To do this, we used a cell culture model in which chondrocytes at two distinct stages of cell maturation are isolated from the costochondral cartilage of male and female rats, and examined the short-term effect of 17α- and 17β-estradiol on [^{14}C]arachidonic acid turnover in the cell layer and phospholipase A_2 specific activity in plasma membranes and extracellular matrix vesicles isolated from similarly prepared cultures. In addition, the effect of 17α- and 17β-estradiol on plasma membrane and matrix vesicle membrane fluidity was assessed. The effect of hormone on arachidonic acid turnover was rapid, time- and concentration-dependent, stereo-specific, and cell maturation-specific. Only resting zone cells from female rats were affected, and only 17β-estradiol elicited a response. Similarly, only female rat resting zone chondrocytes exhibited a change in phospholipase A_2 activity after a 24 h exposure to hormone, causing an increase in enzyme activity in the matrix vesicles, but not plasma membranes. When isolated membranes were incubated directly with hormone, membrane fluidity was decreased in both plasma membranes and matrix vesicles isolated from female rat resting zone chondrocyte cultures. This nongenomic effect was dose-dependent and stereo-specific and differentially expressed in the two membrane fractions with respect to time course and magnitude of response. These results support the hypothesis that 17β-estradiol has a rapid action on chondrocyte membrane lipid metabolism and suggest that specific membrane components, characteristic of a particular sex and state of cell maturation, are involved in the nongenomic effects of this sex hormone on isolated matrix vesicles and plasma membranes.

Keywords: Chondrocyte; Estrogen; Fluidity; Arachidonic acid turnover; Membrane; Matrix vesicle; 17β-Estradiol

1. Introduction

It is widely accepted that sex hormones influence mammalian growth and development and that many of the effects of estrogens are indirect and mediated by other hormones and local factors secreted by cells in response to hormone stimulation [1–3]. Further, it is becoming increasingly clear from both in vivo and in vitro studies that estrogens exert direct effects on cells as well. In cartilage, it is known that 17β-estradiol inhibits chondrocyte proliferation [4–8] and stimulates RNA synthesis [8,9], sulfate incorporation [10,11], collagen production [8,10], and alkaline phosphatase [8,9] and creatine kinase [12] specific activities. In addition, many of these effects are gender-specific [7–11,13–16] and cell maturation-dependent [8,10].

It has been traditionally accepted that estrogens mediate their effects through classic steroid hormone receptor mechanisms. According to this view, following binding of 17β-estradiol to the receptor, the hormone-receptor complex undergoes translocation to the nucleus, which results

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in gene transcription and protein synthesis [17]. Specific receptors for estrogens have been found in articular cartilage [18,19], growth plate [16,20], and fracture callus [21].

Recent evidence also suggests that some of the effects of steroid hormones may be mediated by rapid, membrane-mediated mechanisms [22–25]. While some of these effects result in genomic regulation, others appear to be nongenomic, that is, those actions by a regulatory factor which occur via membrane receptors or as a result of the membrane's composition, eliciting effects that are independent of direct interaction with the genome. In general, studies of nongenomic effects use time course experiments to establish the nature of the response, based on the supposition that changes which occur within the first few minutes of exposure to a regulatory agent happen too quickly to involve genomic participation. Unless rigorously shown not to elicit new gene transcription, however, these rapid effects may be nonreceptor-mediated, but not necessarily nongenomic. To resolve this, another commonly used approach is to pharmacologically block gene transcription or protein translation.

Studies examining the response of rat costochondral chondrocytes to 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ have resulted in a model capable of assessing the nongenomic action of lipophilic hormones. In culture, these cells produce matrix vesicles, extracellular organelles associated with matrix calcification, which have been postulated to be regulated in the matrix via the nongenomic action of local mediators [22]. Supporting this hypothesis is the fact that addition of vitamin D metabolites to isolated matrix vesicles produces effects on alkaline phosphatase and phospholipase A_2 specific activities that are comparable to those observed in intact cultures [26,27]. Since isolated matrix vesicle preparations contain no DNA or RNA [28], there is no possibility for gene transcription or translation. The observation that 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ alter the phospholipid metabolism and fluidity of isolated membranes in a metabolite-specific, membrane-dependent manner [26,29] suggests that physical changes in the membrane, such as altered permeability and ion transport [22], might provide a mechanism explaining the nongenomic action of these hormones on chondrocyte cultures. Membrane receptors may account for specific membrane-mediated effects, as well.

Estradiol has been shown to alter the membrane fluidity of vaginal epithelial cells [30] indicating that this sex hormone may also exert some of its effects in cartilage via nongenomic regulatory pathways. This has been supported by the observation that estradiol exerts rapid effects in a variety of cell types [31,32]. Recent studies suggest that a membrane receptor for estradiol [33] may be responsible for mediating some of the rapid effects of this hormone.

To test the hypothesis that estradiol can act via nongenomic mechanisms, we used the same rat chondrocyte model used for our vitamin D studies in which cells at two distinct stages of cell maturation are isolated from two different zones of costochondral cartilage [22,27,28]. By use of this culture system, we can determine the sex specificity of the effect by examining chondrocytes from male and female rats. In addition, we can examine membrane specificity by comparing the effect of hormone treatment on isolated plasma membranes, matrix vesicles and intact cultures. In the present study, we determined whether 17β-estradiol has a rapid action on cell membrane lipid metabolism and if 17β-estradiol has a nongenomic effect on isolated plasma membranes and matrix vesicles by exposing them to hormone and assessing changes in membrane fluidity.

2. Materials and methods

2.1. Chondrocyte cultures

The culture system used in this study has been described in detail before [22,26,27]. Rib cages were removed from 125 g Sprague-Dawley female and male rats and placed in Dulbecco's modified Eagle's medium (DMEM). The resting zone and growth zone cartilage was separated, sliced, and incubated overnight in DMEM. Cells were released from the tissue by sequential incubations in trypsin and collagenase and collected by centrifugation. Cultures were seeded at a density of 10 000 cells/cm^2 for resting zone cells and 25 000 cells/cm^2 for growth zone cells and incubated in DMEM containing 10% fetal bovine serum (FBS) and 50 μg/ml vitamin C at 37°C in an atmosphere of 5% CO_2 and 100% humidity. Media were changed at 24 h and then at 72 h intervals. At confluence, cells were subcultured using the same plating densities and allowed to return to confluence. Fourth passage cells were used since prior studies have shown that differential response to vitamin D_3 metabolites is preserved at this passage [22,26–29,34].

2.2. Determination of arachidonic acid turnover

Hormone effects on chondrocyte phospholipid metabolism were assessed by measuring arachidonic acid turnover, as previously described [29,35]. Confluent, fourth passage growth zone and resting zone chondrocytes were pre-labeled by adding [1-^{14}C]arachidonic acid (0.05 μCi; 50 mCi/mmol, NEC-661; New England Nuclear/DuPont, Boston, MA) to the cultures for 60 min and the unbound label removed by washing. Cultures were then incubated for 60 min in 5 ml fresh DMEM containing 10% FBS. *p*-Chloromercuribenzoate (100 nmol/ml DMEM) (Sigma, St. Louis, MO) was added to half of the cultures to block reacylation and the incubation continued for an additional 60 min. At this time (time 0), 17β-estradiol was added to the medium and the incubation continued for up to 360 min. Since the concentration of 17β-estradiol in normal serum ranges from 10^{-9} to 10^{-8} M, we chose to test

17β-estradiol at both physiological and pharmacological concentrations ranging from 10^{-12} to 10^{-6}M. In parallel experiments, the inactive metabolite, 17α-estradiol, was used as a control. Both hormones were dissolved in absolute ethanol and diluted by at least 1000-fold. At 5, 15, 30, 60, 120, 180, 270 and 360 min, 50 μl aliquots of culture media were collected and the reaction stopped by addition of 5 ml chloroform:methanol (2:1, v/v). The 'percent release' of [^{14}C]arachidonic acid was calculated as the amount of [^{14}C]arachidonic acid released into the medium in the presence of *p*-chloromercuribenzoate at the specified time point divided by the total [^{14}C]arachidonic acid in the cell layer and medium at harvest at 360 min. This experimental design permits measurement of acylation and reacylation on the same population of cells. As a result, any incorporation of [^{14}C]arachidonate is the result of reacylation, since the only source of this fatty acid is the pre-labeled cell itself.

2.3. Determination of phospholipase A_2 activity

Confluent, fourth passage chondrocyte cultures were incubated for 24 h with medium containing vehicle or 10^{-11}–10^{-7} M 17β-estradiol. After treatment, conditioned media were discarded and the cells harvested by trypsin digestion. Matrix vesicles were isolated from the trypsin digest and plasma membranes from cell homogenates as described below.

Phospholipase A_2 (EC 3.1.1.4) activity in plasma membranes and matrix vesicles was measured as a function of release of [^{14}C]arachidonate from L-α-1-palmitoyl,2-arachidonyl phosphatidylethanolamine (PEC; cat. #NEC-783, 50 mCi/mmol; New England Nuclear/DuPont, Boston, MA), as previously described [34]. Phospholipase A_2 activity was calculated as the percent hydrolysis of [^{14}C]arachidonate from the total ^{14}C-PEC.

2.4. Preparation of membrane fractions

Matrix vesicles and plasma membranes were isolated from chondrocyte cultures as previously described [27]. Matrix vesicles were isolated by differential centrifugation of trypsin digests of the cell layer. Plasma membranes were isolated from cell homogenates by differential centrifugation, followed by sucrose density gradient centrifugation. The protein content of each fraction was determined [36]. Alkaline phosphatase specific activity [37] of each membrane preparation was assayed to ensure that matrix vesicles exhibited greater than a two-fold enrichment over their respective plasma membrane fraction [22,27].

2.5. Membrane fluidity

Membrane fluidity was assessed by using the lipophilic fluorophore, 1-[4-(trimethylamine)phenyl]-6-phenyl-hexa-1,3,5-triene (TMP-DPH) as previously described [29,38]. This probe has a high quantum yield, localizes with the phospholipid polar head groups, and is thought to be more selective than its parent compound DPH [39]. TMP-DPH labels solely those membranes that are in contact with the external medium [40]. It incorporates very rapidly into the cell membrane and remains localized in it for at least 30 min [41]. However, after this time, cell damage and internalization of the probe are possible. For these reasons, samples were exposed to low concentrations of the probe for time periods not exceeding 30 min.

In order to determine the direct effect of 17β-estradiol on membrane fluidity, matrix vesicles and plasma mem-

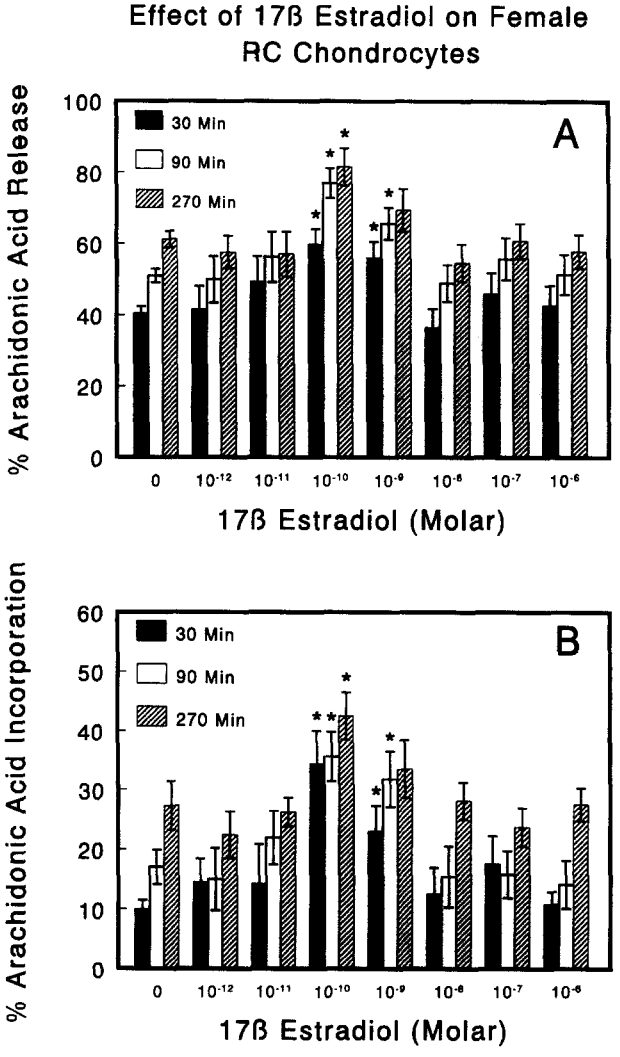

Fig. 1. Effect of 17β-estradiol on arachidonic acid turnover by resting zone chondrocytes derived from female rats. Confluent, fourth passage resting zone chondrocytes were pre-labeled with [^{14}C]arachidonic acid and then treated with varying concentrations of 17β-estradiol (10^{-12}–10^{-6} M) for 30, 90, or 270 min. Reacylation was blocked in one half of the cultures by inclusion of 100 nmol/ml *p*-chloromercuribenzoate. A: percent arachidonic acid release. B: percent arachidonic acid incorporation. All values are from one experiment and represent the mean ± SEM for six samples. Each experiment was repeated two or more times to verify the observations. * $P < 0.05$, treatment vs. untreated control.

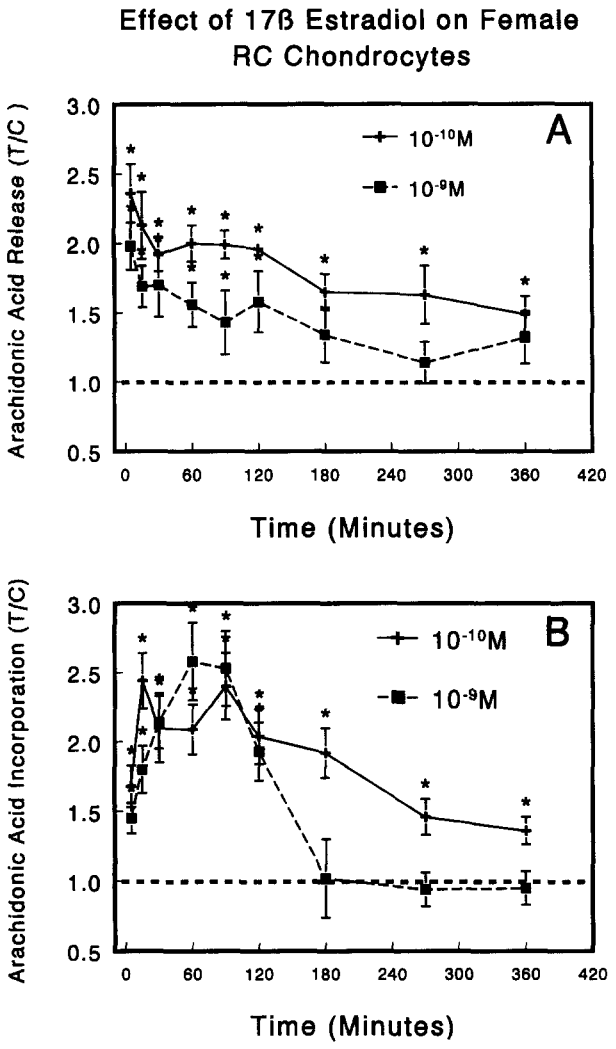

Fig. 2. Treatment:control ratios showing the effect of 17β-estradiol treatment on arachidonic acid turnover by resting zone chondrocytes derived from female rats. Confluent, fourth passage resting zone chondrocytes were pre-labeled with [^{14}C]arachidonic acid and then treated with varying concentrations of 17β-estradiol (10^{-11}–10^{-8} M) for up to 360 min. Only data for treatment with 10^{-10} and 10^{-9} M 17β-estradiol is shown for clarity. Reacylation was blocked in one half of the cultures by inclusion of 100 nmol/ml *p*-chloromercuribenzoate. A: percent arachidonic acid release. B: percent arachidonic acid incorporation. Values represent the mean ± SEM for treatment/control ratios of three separate experiments. * $P < 0.05$, treated vs. a treatment:control ratio of 1.

branes were isolated from cultures not previously exposed to exogenous hormone. Membrane fractions (15 μg matrix vesicle or plasma membrane protein/tube) were incubated in 3 ml HBSS containing 10% FBS plus vehicle alone or 10^{-9} or 10^{-10} M of 17α- or 17β-estradiol for the specified time. These concentrations of hormone were chosen based on the results of the arachidonic acid turnover studies. 30 min prior to terminating the assay, TMP-DPH was added so that the final concentration of the probe was 10^{-7} M.

Measurements were carried out on an SLM 5000 spectrofluorometer. Data acquisition was simplified by interfacing a personal computer to the signal processing unit of the spectrofluorometer. Changes in anisotropy were measured, following addition of TMP-DPH, using an excitation wavelength of 353 nm and an emission wavelength of 426 nm. The influence of experimental parameters on anisotropy was determined using the Perrin-Weber equation [42,43]. There is a direct correlation between the anisotropy and fluidity (viscosity) of the membrane such that a decrease in anisotropy is interpreted as an increase in membrane fluidity [44]. Each measurement was corrected for contributions due to scattering by measuring these components with blanks composed of solution constituents minus the fluorophore [45].

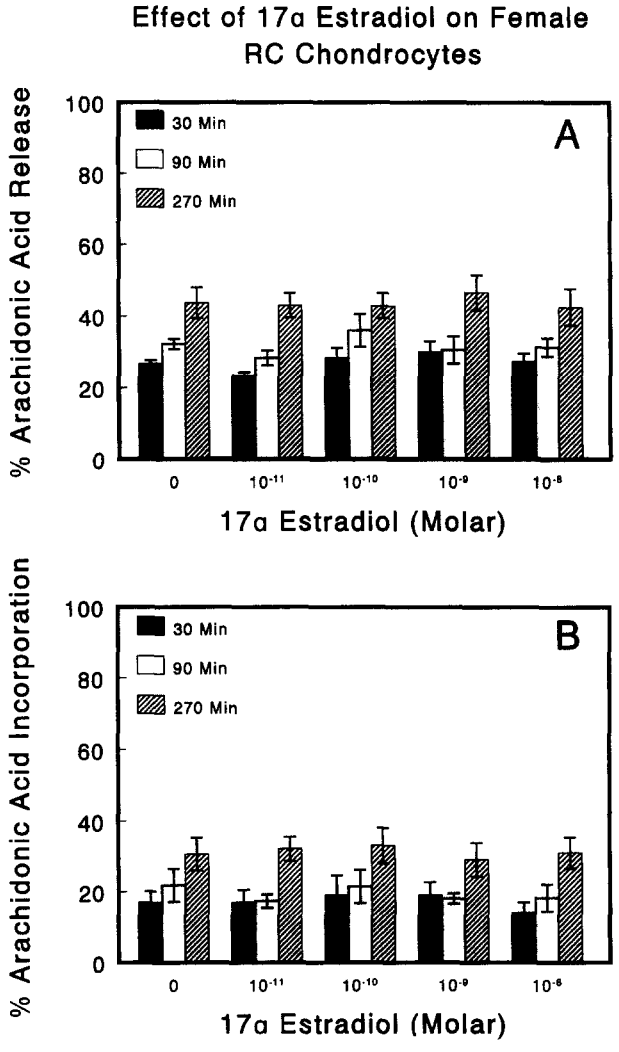

Fig. 3. Effect of 17α-estradiol on arachidonic acid turnover by resting zone chondrocytes from female rats. Confluent, fourth passage resting zone chondrocytes were pre-labeled with [^{14}C]arachidonic acid and then treated with varying concentrations of 17α-estradiol (10^{-11}–10^{-8} M) for 30, 90, or 270 min. Reacylation was blocked in one half of the cultures by inclusion of 100 nmol/ml *p*-chloromercuribenzoate acid incorporation. A: percent arachidonic acid release. B: percent arachidonic acid incorporation. All values are from one experiment and represent the mean ± SEM for six samples. Each experiment was repeated two additional times to verify the observations.

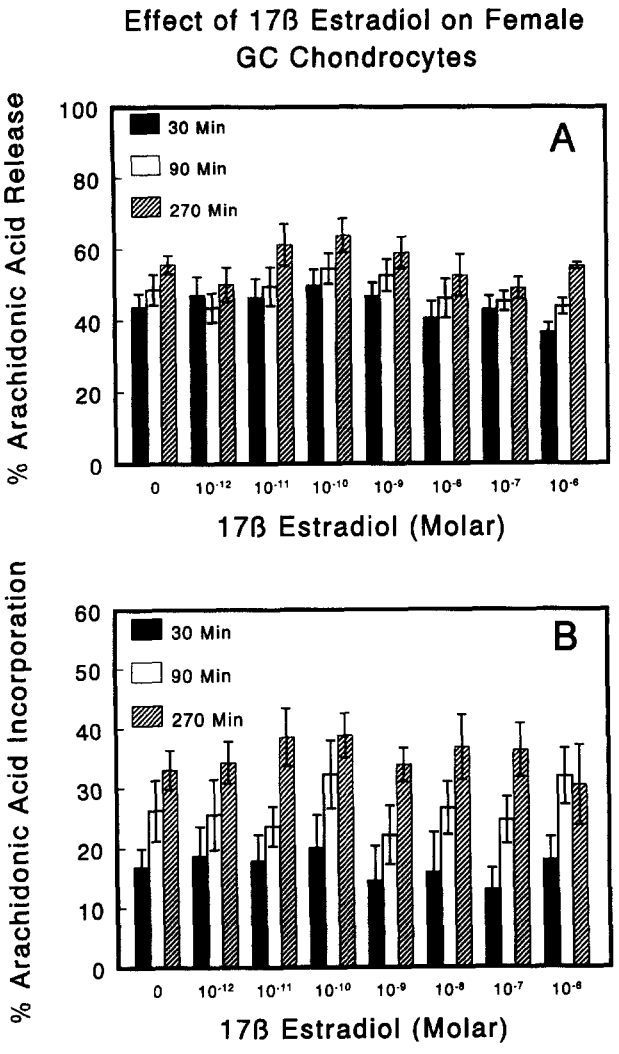

Fig. 4. Effect of 17β-estradiol on arachidonic acid turnover by growth zone chondrocytes derived from female rats. Confluent, fourth passage growth zone chondrocytes were pre-labeled with [^{14}C]arachidonic acid and treated as described in Fig. 1. A: percent arachidonic acid release. B: percent arachidonic acid incorporation. All values are from one experiment and represent the mean ± SEM for six samples. Each experiment was repeated two additional times to verify the observations.

2.6. Statistical analysis

For analysis of [^{14}C]arachidonic acid turnover, data are expressed as the mean ± standard error of the mean (SEM) of six cultures. For isolated matrix vesicles and plasma membranes, each sample represents the combined membranes from three T75 flasks. Values are expressed as the mean ± SEM for six samples. The data presented are from representative experiments. However, all experiments were performed a minimum of two times using different batches of cells to ensure consistency of the observations. The data were analyzed by analysis of variance, and statistical significance was determined by comparing each data point to the control (containing the ethanol vehicle) using Bonferroni's *t*-test. Treatment/control ratios were compared using the Wilcoxon matched pair rank sum test. $P < 0.05$ was considered significant.

3. Results

3.1. Arachidonic acid turnover

The effect of 17β-estradiol on arachidonic acid turnover was dose- and time-dependent, sex-specific, and cell maturation-specific. Incubation of resting zone chondrocytes from female rats with 17β-estradiol for 30, 90, or 270 min produced a significant increase in acylation (release) and reacylation (incorporation) of arachidonic acid at 10^{-10}–10^{-9} M hormone (Fig. 1). The basal amount of acylation (Fig. 1A) and reacylation (Fig. 1B) increased with time, but the dose dependence of the 17β-estradiol effect was consistent at each of the time points examined. As indicated by the treatment/control ratios of three separate

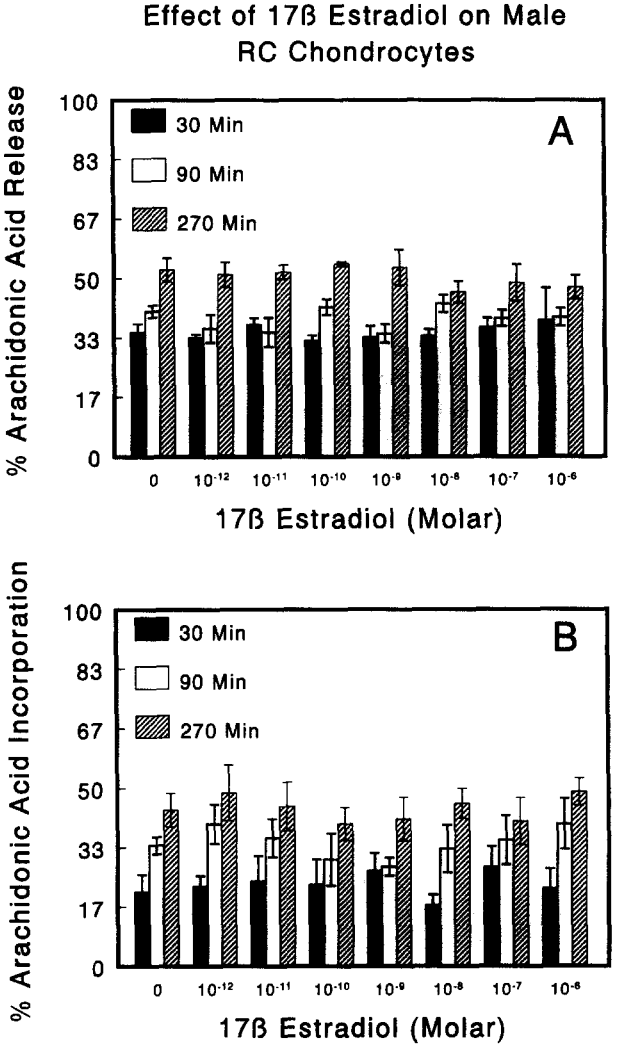

Fig. 5. Effect of 17β-estradiol on the incorporation and release of arachidonic acid by resting zone chondrocytes derived from male rats. Confluent, fourth passage resting zone chondrocytes were pre-labeled with [^{14}C]arachidonic acid and treated as described in Fig. 1. A: percent arachidonoic acid release. B: percent arachidonic acid incorporation. All values are from one experiment and represent the mean ± SEM for six samples. Each experiment was repeated a minimum of two times to verify the observations.

experiments (Fig. 2), the effect of hormone treatment was consistent. By correcting for the time-dependent changes in basal arachidonic acid turnover, the treatment/control ratios demonstrate that the effects of 17β-estradiol on release were greatest at 30 min and then gradually declined. The effect on incorporation was also rapid, remaining at peak levels over the first 2 h. In contrast, 17α-estradiol treatment of resting zone chondrocytes from female rats had no effect on either acylation (Fig. 3A) or reacylation (Fig. 3B) at any of the times or concentrations examined. Neither 17β-estradiol (Fig. 4) nor 17α-estradiol (data not shown) had any effect on either arachidonic acid release or incorporation in growth zone chondrocytes isolated from female (Fig. 4) or male (data not shown) rats. In addition, arachidonic acid turnover by male resting zone chondrocytes was unaffected by either 17β-estradiol (Fig. 5) or 17α-estradiol (data not shown) at any dose or time tested.

3.2. Phospholipase A_2 specific activity

When matrix vesicles and plasma membranes were isolated from female resting zone chondrocyte cultures treated with varying concentrations of 17β-estradiol for 24 h and assayed for phospholipase A_2 specific activity, it was observed that 10^{-10}–10^{-9} M 17β-estradiol produced a significant increase in matrix vesicle enzyme specific activity (Fig. 6). In contrast, 17β-estradiol had no effect on the plasma membrane enzyme activity. Similar treatment of growth zone chondrocytes from female rats had no effect on phospholipase A_2 specific activity in either the plasma membranes or matrix vesicles (Table 1), nor was there any effect of 17β-estradiol on phospholipase A_2 specific activity of either matrix vesicles or plasma membranes isolated from cultures of male rat resting zone or growth zone cells (data not shown).

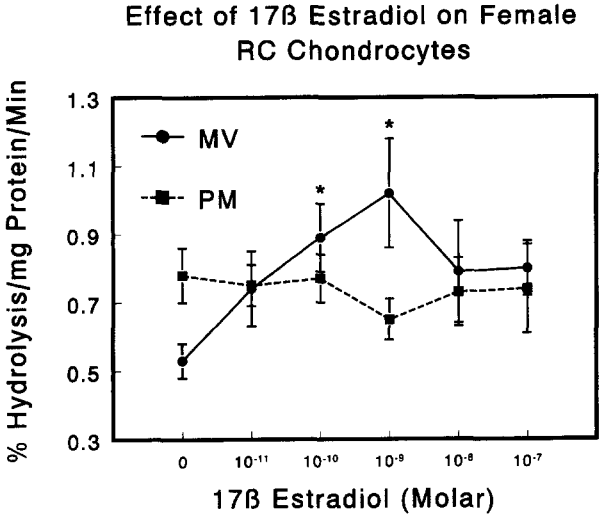

Fig. 6. Effect of 17β-estradiol on phospholipase A_2 specific activity of matrix vesicles and plasma membranes isolated from confluent cultures of resting zone chondrocytes derived from female rats treated with 17β-estradiol for 24 h. All values are from one experiment and represent the mean ± SEM for six samples. Each experiment was repeated two additional times to verify the observation. * $P < 0.05$, treated vs. untreated control.

Table 1
Phospholipase A_2 specific activity in matrix vesicles (MV) and plasma membranes (PM) isolated from female rat growth zone chondrocyte cultures treated with 17β-estradiol

17β-Estradiol	% Hydrolysis/mg protein/min	
(M)	MV	PM
–	0.47 ± 0.22	0.47 ± 0.10
10^{-11}	0.45 ± 0.11	0.66 ± 0.10
10^{-10}	0.36 ± 0.05	0.56 ± 0.12
10^{-9}	0.59 ± 0.11	0.53 ± 0.12
10^{-8}	0.47 ± 0.12	0.71 ± 0.15
10^{-7}	0.45 ± 0.13	0.40 ± 0.12

Data are mean ± SEM; $n = 6$ samples, where each sample is the combined membranes of three cultures.

3.3. Membrane fluidity

When plasma membranes and matrix vesicles were isolated from female rat resting zone chondrocyte cultures and then incubated directly with 17β-estradiol for 30 min, there was an increase in anisotropy at 10^{-10}–10^{-9} M hormone (Fig. 7A). Although incubation with 10^{-8} M 17β-estradiol also increased anisotropy relative to unexposed membranes, the effect was not significant. The dose-dependent effect of 17β-estradiol on membrane fluidity was consistently observed in all experiments ($n = 5$), although the relative intensity of the response varied (Fig. 7B). Incubation of plasma membranes and matrix vesicles isolated from cultures of male rat resting zone chondrocytes with 17β-estradiol for 30 min had no effect on membrane anisotropy. There was also no effect of 17β-estradiol on the anisotropy of matrix vesicles or plasma membranes isolated from cultures of either female or male rat growth zone chondrocytes (data not shown). In addition, the effect of 17β-estradiol on membrane fluidity was stereo-specific, as 17α-estradiol was without effect (Table 2).

The effect of 17β-estradiol on the membrane fluidity of matrix vesicles (Fig. 8A) and plasma membranes (Fig. 8B) isolated from female rat resting zone chondrocyte cultures was also time-dependent and membrane-specific. During the first 20 min of incubation, the anisotropy of untreated matrix vesicles decreased, while that of those incubated with hormone increased (Fig. 8A). From 30 min through 180 min, the anisotropy of the treated matrix vesicles was consistently greater than that seen in the untreated membranes, irrespective of changes in the basal levels. By 3 h

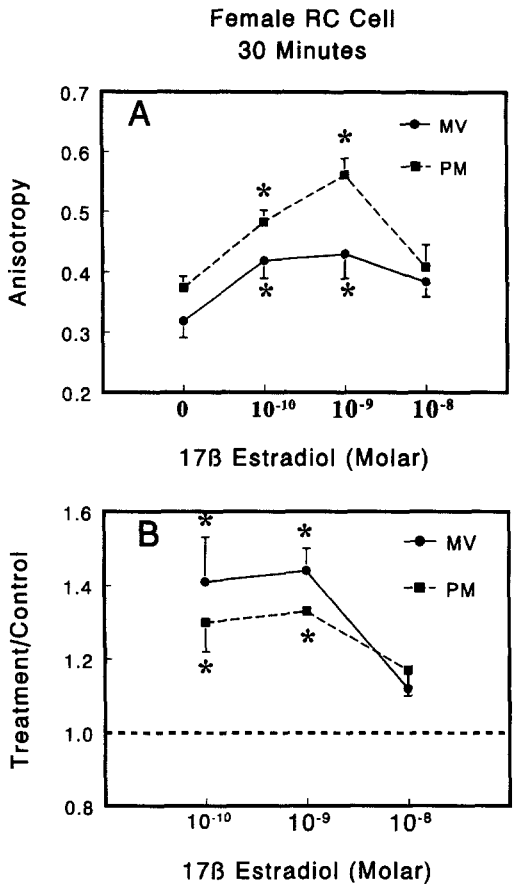

Fig. 7. Effect of 17β-estradiol on the membrane fluidity of matrix vesicles and plasma membranes isolated from cultures of resting zone chondrocytes derived from female rats. Matrix vesicles (MV) and plasma membranes (PM) were isolated and treated for 30 min with varying concentrations of 17β-estradiol (10^{-10}–10^{-8} M), TMP-DPH added, and the change in anisotropy determined by spectrofluorometry. A: results from one representative experiment. B: average treatment:control ratios for five independent experiments. All values in A represent the mean ± SEM for six membrane samples. * $P < 0.05$, treated vs. untreated control.

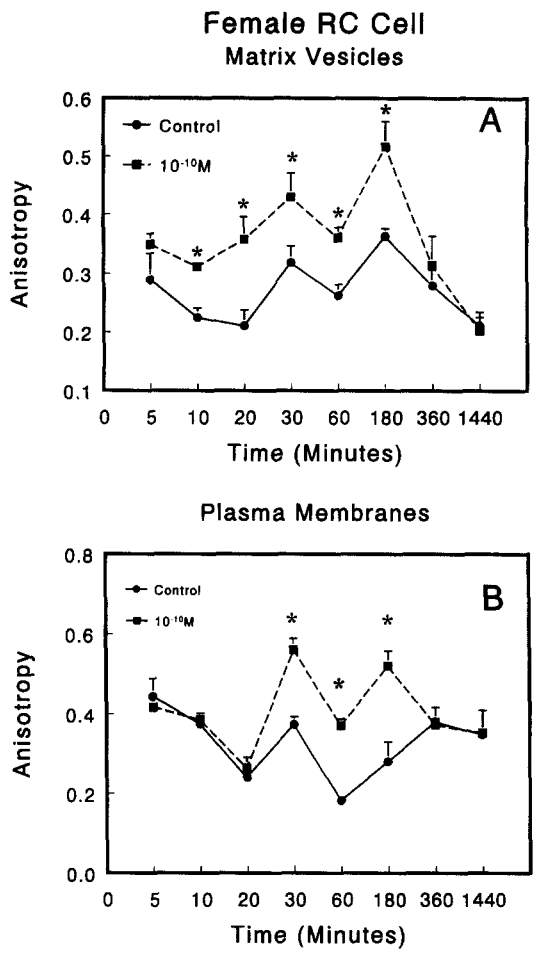

Fig. 8. Effect of 17β-estradiol on the membrane fluidity of matrix vesicles and plasma membranes isolated from cultures of female rat resting zone chondrocytes over time. Matrix vesicles (A) and plasma membranes (B) were isolated and treated for 5–1440 min with 17β-estradiol (10^{-10} M), TMP-DPH added, and the change in anisotropy determined by spectrofluorometry. Control cultures were treated with vehicle alone and the assay performed. All values are from one experiment and represent the mean ± SEM for six samples. Each experiment was repeated two additional times to verify the observations. * $P < 0.05$, treated vs. untreated control.

of incubation, the anisotropy of treated and untreated matrix vesicles was the same. Plasma membrane anisotropy decreased during the first 20 min of incubation in both treated and untreated samples (Fig. 8B). Changes in plasma membrane anisotropy, due to the presence of 17β-estradiol, were not detected until the membranes had been exposed to hormone for 30 min. When these studies were repeated three more times, similar results were obtained. Further, the oscillation in anisotropy values over time, in both treated and untreated membranes, was also consistently observed, suggesting that time-dependent fluctuations were an inherent characteristic of the membranes and independent of hormone treatment.

In contrast to the responsiveness of female rat resting zone chondrocyte membranes, no change in anisotropy was seen in matrix vesicles (Fig. 9) or plasma membranes (data not shown) isolated from male rat resting zone chondrocyte cultures at any time examined, whether or not 17β-estradiol was present. Similarly, there was no effect of hormone on the anisotropy of plasma membranes or matrix vesicles from cultures of either female or male rat growth

Table 2
Direct effect of 17α-estradiol on anisotropy of matrix vesicles and plasma membranes isolated from female rat resting zone chondrocyte cultures

Anisotropy			
17α-Estradiol	Matrix vesicles	Plasma membranes	*n*
Vehicle only	0.32 ± 0.02	0.38 ± 0.02	6
10^{-10} M	0.33 ± 0.01	0.37 ± 0.01	6
10^{-9} M	0.33 ± 0.01	0.39 ± 0.01	5
10^{-8} M	0.31 ± 0.02	0.38 ± 0.01	6

Data are the mean ± SEM. Each *n* represents the combined membranes from three cultures.

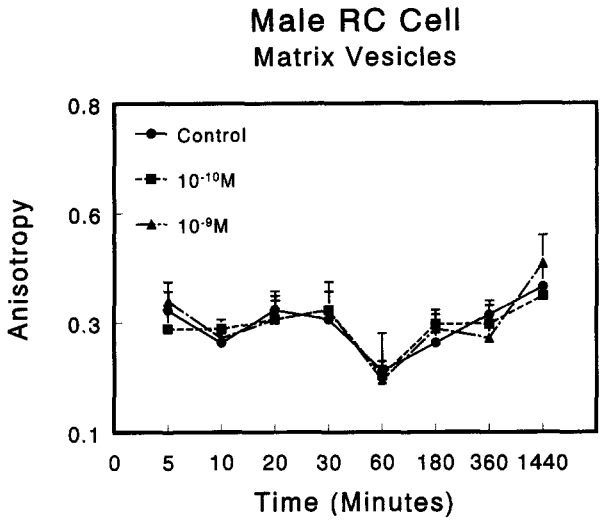

Fig. 9. Effect of 17β-estradiol on the membrane fluidity of matrix vesicles isolated from cultures of male rat resting zone chondrocytes over time. Matrix vesicles were isolated and treated for 5–1440 min with 17β-estradiol (10^{-10} or 10^{-9} M), TMP-DPH added, and the change in anisotropy determined by spectrofluorometry. Control cultures were treated with vehicle alone and the assay performed. All values are from one experiment and represent the mean ± SEM for six samples. Each experiment was repeated two additional times to verify the observations. * $P < 0.05$, treated vs. untreated control.

zone chondrocytes at any time or dose examined (data not shown).

4. Discussion

As shown by others [31,32], 17β-estradiol exerts rapid effects on its target cells. The regulation of arachidonic acid turnover in chondrocytes by estradiol is rapid, stereospecific, and dose-dependent. Further, the results of this study show that these effects are also sex- and cell maturation-specific and only seen at physiologically relevant concentrations. The fact that these rapid effects were limited to resting zone chondrocytes derived from female rats suggests that the composition of the cell membranes or their lipid metabolism is unique. It is possible that the classical intracellular estrogen receptor may play a role in this; however, our previous studies have demonstrated that receptors are present in both resting zone and growth zone chondrocytes from both female and male rats [16]. While the number of receptors was greater in the chondrocytes derived from female rats, there was no difference as a function of cell maturation. Thus, it is unlikely that intracellular estrogen receptor-mediated events could account for all of the observed differences. Recently, Pappas et al. [33] demonstrated the presence of a membrane receptor for estrogen in GH_3/B6 rat pituitary cells. If such a receptor can be shown to exist in female rat resting zone chondrocytes, it would be an attractive candidate for mediating the rapid effects on arachidonic acid turnover observed in this study.

Changes in arachidonic acid turnover could have a number of consequences for the cell. By retailoring the fatty acid residues of the membrane phospholipids, the cell can markedly alter the physical characteristics of the membrane, resulting in altered fluidity [46–48] and function, including ion flux and enzyme activity [22,29,49–51]. Released arachidonic acid may contribute to the production of prostaglandins and other autocoid mediators known to have a regulatory influence on these cells. Other by-products of the acylation reaction can also contribute to signal transduction, for example, via protein kinase C activation [52,53]. In fact, 17β-estradiol has been shown to increase protein kinase C in GH_3 pituitary cells [54] and to enhance ion transport in granulosa cells [31].

In addition to the rapid effect on arachidonic acid turnover, 17β-estradiol also exerts a long-term effect on membrane phospholipid metabolism. After a 24 h exposure to hormone, matrix vesicles produced by resting zone chondrocytes derived from female rats exhibited a dose-dependent increase in phospholipase A_2 specific activity. These experiments demonstrate the sex specificity (only chondrocytes from female rats were affected) and cell-maturation dependence (only resting zone chondrocytes showed a change in enzyme specific activity) of the action of 17β-estradiol on membrane phospholipid metabolism. Moreover, they indicate the membrane selectivity of the effect, since only matrix vesicles were affected.

Our studies confirm those of Reddy et al. [30] demonstrating that estradiol alters membrane fluidity in vaginal epithelial cells. The action of effector molecules such as proteins, steroids, or other hydrophobic compounds [48,55], either via effects on membrane phospholipid metabolism like those described in the present study, or simply by the physical presence of the effector molecule in the membrane, may be responsible for changes in membrane fluidity. Plasma membranes and matrix vesicles isolated from cultures of resting zone and growth zone chondrocytes differ in their phospholipid composition [27], phospholipid metabolism [29,35], and relative distribution of enzyme activities [34,56]. Moreover, membranes isolated from cultures of male rat chondrocytes differ from membranes isolated from female rat chondrocytes [8,57]. This suggests that plasma membranes and matrix vesicles are structurally different and provides a partial rationale for the differences in response to both vitamin D metabolites and 17β-estradiol.

Changes in membrane fluidity, as a function of cell maturation, have been reported by others as well. Fluorescence steady-state anisotropy measurements comparing the fluidity of plasma membranes of chondrocytes from resting and ossifying zones of epiphyseal cartilage indicate that considerable modification of the chondrocyte plasma membrane occurs as the cells hypertrophy, resulting in higher membrane fluidity [51]. It is clear that membrane fluidity is also sensitive to the physiological state of the cell, since it has been noted that membrane fluidity is

altered in several different clinical diseases such as diabetes [58] and plasmalogen deficiency [39]. It is also obvious that changes in membrane fluidity are important because the plasma membrane is the locus of early cell response to external stimuli [23].

The present study supports our prior observations showing that membrane function can be altered in a differential manner by the vitamin D metabolites, 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$. Using matrix vesicles and plasma membranes from costochondral chondrocytes, the effects of these hormones on membrane fluidity were shown to be metabolite-specific, membrane-specific, and cell maturation-specific [27,28,34]. Similarly, the vitamin D metabolites had a direct effect on phospholipase A_2 activity. 1,25-$(OH)_2D_3$ stimulates this enzyme in plasma membranes and matrix vesicles isolated from growth zone chondrocytes, whereas 24,25-$(OH)_2D_3$ inhibits this enzyme in membranes isolated from cultures of resting zone chondrocytes. Further, both metabolites regulate arachidonic acid turnover, and the effects are cell maturation-dependent and metabolite-specific.

The effects of 17β-estradiol, however, are distinct from those of either 1,25-$(OH)_2D_3$ or 24,25-$(OH)_2D_3$ when one examines changes in fluidity as a function of time, changes in phospholipase A_2 activity, or changes in arachidonic acid turnover. This suggests that direct membrane effects may be a general property of steroids and related secosteroid hormones and indicates a function for these hormones that is independent of the classic hormone receptor mediated pathway. Nonetheless, the membrane response to lipophilic mediators is specific to the hormone. This is supported by the stereospecificity of the 17β-estradiol effect on the fluidity of matrix vesicles from female rat resting zone chondrocyte cultures. In addition, the effects occur at hormone concentrations lower than would be expected if all of the observations were simply the result of hormone intercalation in the membrane. Further, since the basal chemical composition of the plasma membranes and matrix vesicles used to assess the effect of 17β-estradiol was comparable to that of membranes used to assess the vitamin D effects, it is likely that specific membrane receptors or structural units for each hormone exist [22–25].

In summary, we have shown that 17β-estradiol can exert a rapid effect on chondrocyte phospholipid metabolism that is cell maturation-dependent and sex-specific. In addition, the hormone exerts a long-term effect on phospholipid metabolism that is specifically targeted to extracellular matrix vesicles, resulting in increased phospholipase A_2 activity. 17β-estradiol also exerts a direct, sex-specific effect on matrix vesicles and plasma membranes, evidenced by changes in membrane fluidity. The time course of this effect suggests that the alteration in fluidity may be secondary to the rapid effect on fatty acid acylation. Stereo-selectivity and sex and cell maturation dependence of the estradiol response, particularly the nongenomic regulation of membrane fluidity in isolated matrix vesicles and plasma membranes, suggests a specific interaction of 17β-estradiol with the membrane, potentially mediated via a membrane receptor.

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