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METABOLISM OF LIPOIDAL DERIVATIVES OF ESTRADIOL-17β IN HUMAN MAMMARY CANCER TISSUE AND CELL LINES

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Summary—Estradiol-17β (E_2) is converted exclusively to intracellular metabolically formed lipoidal estrogens [long chain fatty acid 17β-esters (E_2-L)], by human mammary cancer tissue and cell lines. In order to further evaluate the biological role of lipoidal estrogens, rates of saturation of the estrogen receptor (ER) along with formation of [^{3}H]E_2-L have been measured in human mammary cancer cells exposed to 5 nM [^{3}H]E_2. Extensive specific binding of E_2 to ER in MCF-7 cells (~37%) and ZR-75-1 cells (~62%) occurred before appreciable synthesis of E_2-L was evident and the maximum level of E_2-L attained was only 3–9% of the E_2 specifically bound to ER. In these ER positive cell lines, and in the ER negative cell line MDA-MB-231, an initial rise in the rate of E_2-L formation was followed by a decrease at ~6 min and re-establishment of a new rate, indicating turnover of the E_2-L fraction by esterification–de-esterification reactions. This data does not support the concept that E_2-L acts in the transport of E_2 to nuclear receptors, but rather that liberation of E_2 from E_2-L could serve to maintain occupancy of ER necessary for initiation of DNA synthesis. The esterase, as studied in pooled human mammary cancer tissue, was found to hydrolyse E_2-17β-long chain fatty acid esters at different rates—the enzyme being less active towards E_2-17β-stearate compared to E_2-17β-oleate, -linoleate and -linolenate. Esterase activity was significantly higher in MDA-MB-231 cells compared to MCF-7 cells. Treatment of MCF-7 cells with E_2 did not alter the specific activity of the esterase towards E_2-17β-oleate as substrate. Similarly, addition of dibutyryl c-AMP to ZR-75-1 cell cultures was without effect on E_2-L, both during the time when E_2-L was accumulating, or during a subsequent phase when E_2-L was decreasing following transfer to medium lacking E_2. Calcitonin, which increases endogenous c-AMP in MCF-7 cells, had no effect on E_2-L in this latter phase using this cell line. Thus, no evidence could be provided that the esterase was under E_2 control, or control by polypeptide hormones which utilize c-AMP as a second messenger.

INTRODUCTION

In 1981 Schatz and Hochberg [1] discovered that estradiol-17β (E_2) could be converted to a mixture E_2-17β-long chain fatty acid esters (E_2-L) upon incubation with various rat tissues *in vitro* —this esterification occurring to a greater degree with E_2-target tissues [1]. When such incubations were carried out with bovine endometrium, some nine individual E_2-L were separated and characterized [2]. Because of their lipophilic properties (termed lipoidal derivatives by Hochberg's group), these esters are retained within the cell. When estrogen receptor (ER) negative human breast cancer cells were exposed to E_2 for 2 h, E_2-L represented the major intracellular form of the estrogen, whereas in ER positive cells, E_2-L was present at lower concentrations. In these cells, E_2 specifically bound to ER was the dominant form [3]. Accumulation of E_2-L in such cells represented the net result of esterification and de-esterification reactions. After allowing E_2-L to accumulate by exposure to hormone for 16 h, subsequent withdrawal of hormone led to hydrolysis of E_2-L and regeneration of E_2 [3]. Furthermore, turnover of the E_2-L fraction occurred during the time of exposure to hormone, as evident from a change in composition of the subcomponents of the total E_2-L fraction measured by reverse phase HPLC. This was observed both with E_2 [4] and the adrenal-derived estrogen 5-androstene-3β, 17β-diol which was also converted to lipoidal derivatives by human mammary cancer cells [5–7].

By employing bovine placenta cotyledons as a convenient tissue to study the biochemistry of E_2-L metabolism, it was established that the acylation system was present in microsomes,

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and to a lesser extent in mitochondria [4, 8]. An esterase, which hydrolysed E_2-17β-fatty acid esters and long-chain fatty acid esters of testosterone and dehydroepiandrosterone, was present in the cytosol. This enzyme was partially purified and was very similar, if not identical to bovine hormone sensitive lipase [9]. The latter occurs in adipose tissue, adrenals and ovaries where it is under the control of catecholamines, glucagon and ACTH (adipose tissue), ACTH (adrenal) and luteinizing hormone (ovary); c-AMP acting as the second messenger [10].

E_2-L and lipoidal derivatives of C_{19}- and C_{21}-steroids occur in human blood [11, 12] and it is known that E_2-stearate [13], as well as other synthetic E_2-L [14], function as long-acting estrogens in the rat. This estrogenic action results from slow release of E_2 from these esters [13, 15]. However, the precise function of long chain fatty acid esters of steroid hormones, synthesized within target tissue cells, remains unknown. In order to examine possible functions of these esters formed within cells, we have followed up earlier leads which showed that both the rates of accumulation and the relative compositions of the E_2-L fraction, or the lipoidal fraction formed from 5-androstene-3β, 17β-diol, differed between ER positive and negative breast cancer cell lines [3–6]. We have now compared the rates of E_2-L formation at very early times following exposure of these cell lines to [^{3}H]E_2 and have also determined the rate of saturation of ER with ligand. Evidence is provided that E_2-L does not function as a direct means of transport of E_2 to the nucleus, but rather may be involved in maintaining occupancy of ER by free E_2 liberated from E_2-L. Possible hormonal control of E_2-L metabolism has also been investigated.

EXPERIMENTAL

Materials

[2,4,6,7-^{3}H]E_2 (96 Ci/mmol) was obtained from Amersham (U.K.). Unlabelled steroids, calcitonin, dibutyryl c-AMP and 3-isobutyl-1-methylxanthine were purchased from Sigma (St Louis, MO). Hormone sensitive lipase ex adipose tissue was kindly supplied by Dr S. J. Yeaman. [^{3}H]E_2-L were prepared as described previously [16] but to a higher specific activity by employing 40 μCi of [^{3}H]E_2 and 1.2 mg of unlabelled E_2. Purification of the labelled monoesters was carried out by TLC on 0.2 mm silica gel sheets in chloroform–acetone (37:3 vol/vol) containing butylated hydroxytoluene to prevent oxidation of polyunsaturated fatty acid esters. Rechromatography and radioactivity scanning of samples showed they were free of E_2 and diester. The sources of the human mammary cancer cell lines were as described previously [3] and these were routinely maintained in RPM1-1640 (Hepes buffered, phenol red present) medium containing 10% fetal calf serum and 10 μg/ml insulin.

[^{3}H]E_2-L assays at early time intervals

Cells were grown in 12-well plates (6×10^4 cells/well) in RPM1-1640 medium containing 10% dextran coated charcoal-stripped foetal calf serum and 0.5 μg/ml insulin, in the absence of phenol red. Medium was changed after 3 days and at the end of 6 days 5 nM [^{3}H]E_2 was added in fresh medium. At various time intervals, medium was removed and the cells quickly washed ($\times 2$) with 0.9% (w/v) NaCl containing 100 nM E_2 and ethanol (1 ml) added. After standing at 25°C for 1 h and overnight at 4°C, E_2-17β-oleate (5 μg in 5 μl acetone) was added and the ethanol extract transferred to centrifuge tubes. After centrifugation, the supernatant was collected, dried under N_2 and partitioned ($\times 2$) between hexane and 70% aqueous methanol. The hexane fractions were combined and back washed with 70% aqueous methanol saturated with hexane. An aliquot (1 ml) was taken for liquid scintillation counting, counts at zero time were subtracted, and net counts converted to pmol. It was previously demonstrated that only 0.11 ± 0.07 (SD)% of total counts from [^{3}H]E_2 partitioned into hexane under the conditions described above, whereas 99.8 ± 2.1 (SD)% of [^{3}H]E_2-17β-oleate partitioned into hexane [16].

Specific uptake of [^{3}H]E_2 into ER

Cells were grown in 12-well plates as above and in parallel with the cultures used for [^{3}H]E_2-L assays. Specific binding [^{3}H]E_2 by the intact cells was measured at time intervals after exposure to 5 nM [^{3}H]$E_2 \pm 5$ μM E_2, as described by Gyling and Lerclercq [17]. DNA was measured in 6 separate wells, following the recommended procedure of these authors.

Effects of dibutyryl c-AMP and calcitonin on E_2-L metabolism

ZR-75-1 cells were grown for 7 days in T-25 flasks in the same medium used to maintain cell cultures (see above). The medium was then

replaced with medium lacking insulin and containing 10 nM [^{3}H]E_2. Dibutyryl c-AMP (0.5 mM final concentration) was then added in phosphate buffered saline (PBS)-control flasks receiving PBS only. Any departures from these conditions are indicated in figure legends. Cells were harvested at time intervals and washed (×3) with PBS. Pelleted cells were then extracted overnight at 4°C with 2 ml of acetone–ethanol (1:1 vol/vol). [^{3}H]E_2-L was then isolated from the dried extract after partitioning between 70% aqueous methanol and hexane as described above.

Lipoidal estrogen esterase activity

Human mammary carcinoma tissue. In agreement with the report of Katz *et al.* [18], esterase activity towards [^{3}H]E_2-L contained in the 28,000 *g* supernatant of MCF-7 cells, was found to be quite low. Comparative rates of hydrolysis of a group of the above esters was then sought by employing pooled human breast carcinoma tissue. Tissue which was completely free of fat was chosen and 3 g of tissue from 4 separate tumours (stored at −20°C for 1 month) was homogenized (Ultraturrax instrument) with 7 ml of 0.2 M sodium phosphate buffer, pH 7.0, containing 1 mM dithiothreitol and 1 mM EDTA. After centrifuging for 30 min at 28,000 *g*, the supernatant was removed and defatted bovine serum albumin (Sigma) added to a final concentration of 1%. The [^{3}H]E_2-L were prepared by sonication in the presence of phospholipid as described previously [9]. Each incubation contained 0.1 ml of the sonicated substrate and 0.3 ml of 28,000 *g* supernatant; the final concentration of substrate was 19 μM. Control incubations employed supernatant which had been heated at 80°C for 20 min [18]. After 18 h at 37°C, methanol containing 50 μg E_2/ml was added to a concentration of 70% vol/vol and the mixture extracted (×3) with hexane. An aliquot (300 μl) of the aqueous methanol was transferred to a separate tube, an equal volume of methanol added, and the mixture allowed to stand at 4°C overnight. Precipitated protein was removed by centrifugation. This step was essential in order to reduce blank values caused by absorption of [^{3}H]E_2-L onto colloidal protein. The clear supernatant was removed, water added to adjust the methanol content to 70%, and a final extraction made with hexane saturated with 70% methanol. An aliquot of the 70% methanol, containing liberated [^{3}H]E_2, was removed for counting. After subtraction of counts in the blanks employing heated enzyme, net counts were converted to pmol E_2 from a knowledge of the specific activity of the labelled ester.

Mammary cancer cell lines. MCF-7 and MDA-MB-231 cells were grown in T-150 flasks in RPM1-1640 medium containing 5% dextran charcoal-stripped foetal calf serum and 1 μg/ml insulin. Cells were washed (×2) with PBS and the cell contents of 2 flasks combined and centrifuged. The resulting pellet was homogenized (Ultraturrax instrument) with 3 ml of 0.2 M sodium phosphate buffer, pH 7.0, containing 1 mM dithiothreitol and 1 mM EDTA. After centrifugation at 28,000 *g* for 30 min, defatted bovine serum albumin was added, as above, and 0.3 ml mixed with 0.1 ml of sonicated [^{3}H]E_2-17β-oleate to give a final substrate concentration of 19 μM. The procedure described above was then followed.

Miscellaneous

Protein was determined by the method of Lowry *et al.* [19] and DNA by the method of Burton [20]. Liquid scintillation counting was carried out on a Beckman LS 3801 instrument equipped with external standardization. Statistical evaluation was carried out by Student's *t*–test.

RESULTS

Pulse labelling of E_2-L in MCF-7 (ER positive) and MDA-MB-231 (ER negative) human mammary cancer cells

Although previous studies demonstrated the gradual accumulation of [^{3}H]E_2-L in these cell lines during exposure to [^{3}H]E_2, this was performed over a 0.5 to 2 h period [3]. [^{3}H]E_2-L accumulation has now been examined at much shorter time intervals (Fig. 1). In MCF-7 cells, an initial rapid rate of formation of [^{3}H]E_2-L occurred followed by a decrease at 6 min and a further very slow increase up to 30 min. In MDA-MB-231 cells, a similar early phase was observed but the rate of increase after 6 min was much greater.

Comparative rates of binding of [^{3}H]E_2 to ER and [^{3}H]E_2-L formation in ER positive cell lines

Rates of saturation of ER were measured in whole cells by incubation with 5 nM [^{3}H]E_2 in the presence and absence of 5 μM unlabelled E_2. [^{3}H]E_2-L was measured in cells exposed to 5 nM [^{3}H]E_2 for the same time periods. Results of a

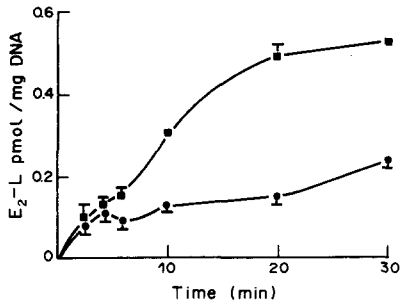

Fig. 1. Rate of synthesis of E_2-L in MCF-7 cells (●) and MDA-MB-231 cells (■) after exposure to 5 nM [^{3}H]E_2. Cells were grown in 12 well plates and values represent means ± SE for 3 wells.

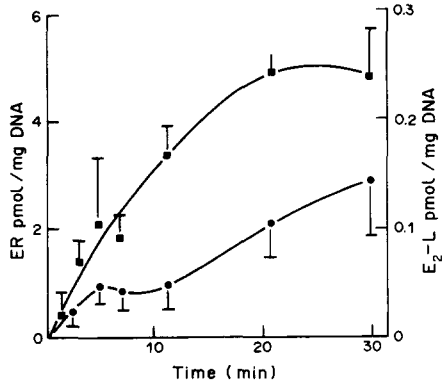

Fig. 2. Comparison of the rate of specific binding of E_2 to ER (■) with that of E_2-L formation (●) in MCF-7 cells exposed to 5 nM [^{3}H]E_2. For each time point, assays were the means of 3 wells and the values shown are means ± SE for 4 separate experiments.

number of such experiments with MCF-7 cells are shown in Fig. 2. It can be seen that saturation of ER by E_2 occurred after some 20 min to reach a maximum value of 5 pmol/mg DNA. By contrast, the maximum amount of [^{3}H]E_2-L formed was only 0.15 pmol/mg DNA at 30 min. At no stage did the formation of E_2-L exceed, or indeed approach the level of E_2 specifically bound to ER. Very similar results were obtained with ZR-75-1 cells (Fig. 3).

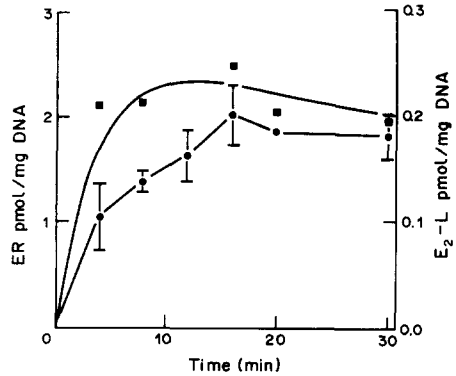

Fig. 3. Comparison of the rate of specific binding of E_2 to ER (■) with that of E_2-L (●) formation in ZR-75-1 cells exposed to 5 nM [^{3}H]E_2. Values are means ± SE for 6 separate wells for E_2-L and means of duplicate wells for ER.

Lipoidal estrogen esterase activity in human mammary cancer tissue and cell lines

It had been established previously that purified bovine hormone sensitive lipase hydrolysed E_2-17β-oleate and a number of other steroid fatty acid esters [9]. Furthermore, comparative rates of hydrolysis of these esters by hormone sensitive lipase were similar to that of a partially purified esterase derived from bovine placenta—a tissue which had been chosen to study E_2 esterification and de-esterification reactions [8, 9]. Before carrying out studies on the esterase in human mammary cancer cells, preliminary data on comparative rates of hydrolysis of a number of E_2-L was obtained using purified hormone sensitive lipase. Apparent K_m values for such esters (palmitate, palmitoleate, stearate, linoleate, linolinate and oleate) ranged from 5–27 μM (mean 9.0 ± 8.9 SD). The enzyme present in the 28,000 *g* supernatant [18] of pooled human mammary cancer tissue was then examined with a group of E_2-L at a concentration of 19 μM. Results are shown in Table 1. In these assays defatted bovine serum albumin was present to absorb liberated fatty acid and

Table 1. Lipoidal estrogen esterase activities in human mammary cancer tissue and ER positive and negative cell lines

	Specific activity (nmol E_2/mg protein/18 h) ± SD			
Substrate	Pooled tissue	MCF-7 (C)[a]	MCF-7 (E_2)	MDA-MB-231
E_2-oleate	0.16 ± 0.005	0.40 ± 0.04	0.38 ± 0.02	0.93 ± 0.26[b]
E_2-linoleate	0.16 ± 0.006			
E_2-linolenate	0.27 ± 0.01			
E_2-stearate	0.038 ± 0.001			

For pooled cancer tissue, the 28,000 *g* supernatant was incubated with [^{3}H]E_2-L at a concentration of 19 μM added as a stabilized sonicated preparation and in the presence of defatted bovine serum albumin (see Methods). Values are means ± SD of triplicate assays. Cells were cultured in T-150 flasks using RPMI-1640 medium (no phenol red) supplemented with 6 ng/ml insulin and 5% dextran coated charcoal-stripped foetal calf serum. MCF-7 cell cultures received 5 nM E_2 or vehicle (ethanol at a final concentration of 0.1%) for the last 4 days of growth. At confluence, 2 flasks were pooled for each determination, the cells collected and esterase measured in the 28,000 *g* supernatant. Values are means ± SD of 3 such determinations.

[a]C = Control; E_2 = cells treated with hormone. [b]Significantly higher ($P < 0.05$) than MCF-7 (C).

the substrates were presented as colloidal preparations (see Methods). Also shown in Table 1 are the specific activities of the esterase in MCF-7 and MDA-MB-231 cell 28,000 *g* supernatants, employing E_2-17β-oleate as substrate. Significantly higher activity was found in the MDA-MB-231 cells. In the case of MCF-7 cells, exposure of cultures to 5 nM E_2 for 4 days prior to harvesting, did not alter the specific activity of the esterase.

Influence of dibutyryl c-AMP and calcitonin on E_2-L metabolism

It has been mentioned that the composition of the total E_2-L fraction formed in mammary cancer cell cultures changes with time of exposure to E_2, showing that the system is in a dynamic state. Partially purified lipoidal estrogen esterase from bovine placenta was found to react with [^{3}H] diisopropylfluorophosphate to yield a major labelled species of M_r 84,000 on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. This enzyme was also activated by c-AMP dependent protein kinase and in such reactions behaved similarly to bovine hormone sensitive lipase [9]. Potential activation of lipoidal estrogen esterase in human mammary cancer cells was then sought by comparing the rate of accumulation of E_2-L in ZR-75-1 cells cultured in the presence and absence of dibutyryl c-AMP. As shown in Fig. 4, the nucleotide had no effect on this accumulation, nor on the breakdown of E_2-L following a period of accumulation and subsequent transfer to medium lacking E_2 (Fig. 4).

Since insulin is known to promote the dephosphorylation (and therefore the deactivation) of hormone sensitive lipase [10], experiments were carried out in which cells were transferred to medium lacking insulin for 2 days prior to addition of [^{3}H]E_2. E_2-L was then allowed to accumulate for 16 h, and its rate of breakdown measured after transfer to medium lacking [^{3}H]E_2. No differences in this rate were observed (data not shown) compared to the data shown in Fig. 4 wherein insulin was removed at the time of [^{3}H]E_2 addition.

Calcitonin has been reported to elevate c-AMP and mediate the activation of c-AMP-dependent protein kinase isoenzymes in MCF-7 cells [21]. Accordingly, the possible influence of calcitonin on the rate of breakdown of E_2-L accumulated in MCF-7 cells, was investigated. However, neither calcitonin nor dibutyryl c-AMP, were found to influence this rate (Fig. 5).

In order to cover the possibility that dibutyryl c-AMP may have been degraded by c-AMP diphosphoesterase, an inhibitor of this enzyme (3-isobutyl-1-methylxanthine) was added to cell cultures in a similar manner to that described for the use of this inhibitor in c-AMP activation of cholesteryl ester hydrolase in macrophage cultures [22]. Rates of breakdown were reduced by the inhibitor itself, but addition of dibutyryl c-AMP produced no further change in rate (Fig. 6).

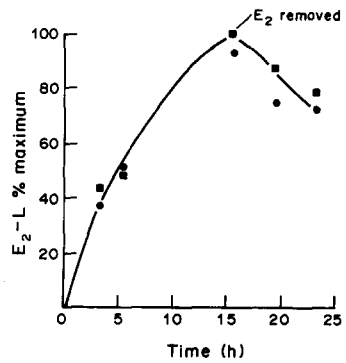

Fig. 4. Effects of dibutyryl c-AMP on the rate of accumulation of E_2-L in the presence of E_2 and the rate of breakdown of E_2-L following subsequent transfer to medium lacking E_2. ZR-75-1 cells were grown in T-25 flasks for 7 days in the same medium used to maintain the cell cultures (see Methods). Medium was then replaced with this medium but lacking insulin and containing 10 nM [^{3}H]E_2 in the presence (●) and absence (■) of 0.5 mM dibutyryl c-AMP. In one set of flasks, cells were transferred to medium lacking [^{3}H]E_2 after a 16 h period of exposure to the labelled hormone. Values represent means of duplicate flasks.

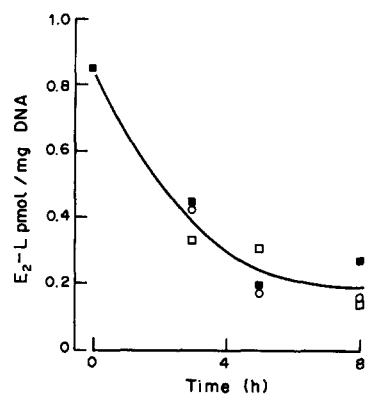

Fig. 5. Effect of dibutyryl c-AMP and calcitonin on the rate of breakdown of E_2-L after prior exposure of cells to 5 nM [^{3}H]E_2 for 16 h. MCF-7 cells were grown in T-25 flasks in normal medium for 3 days. After a further 4 days in this medium but lacking insulin, cells were exposed to 5 nM [^{3}H]E_2 for 16 h and transferred to medium lacking insulin and E_2, but containing 12 μg/ml calcitonin (○), 1 mM dibutyryl c-AMP (□), or vehicle (PBS) (■). Values represent means of duplicate flasks.

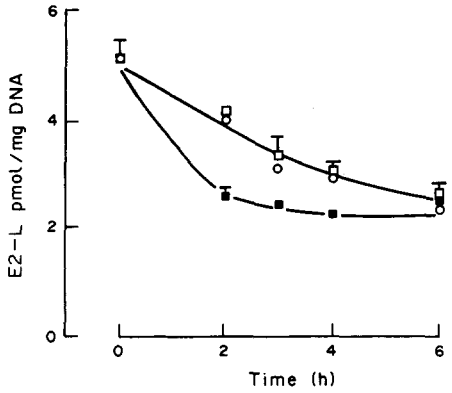

Fig. 6. Effect of dibutyryl c-AMP on breakdown of E_2-L in the presence of a c-AMP diphosphoesterase inhibitor. ZR-75-1 cells were grown in 12-well plates for 8 days using the normal medium used to maintain cell cultures. This medium was then replaced with that lacking insulin but containing 10 nM [^{3}H]E_2. After 16 h, the medium was then removed and replaced with medium lacking insulin but containing 0.5 mM 3-isobutyl-1-methylxanthine (○), or the latter plus 1 mM dibutyryl c-AMP (□). Controls (■) received vehicle of dilute ethanol; the final ethanol concentration being 0.2% in each case. Cells were harvested with a micro-policeman after addition of an excess of ethanol and transfer to centrifuge tubes. After centrifugation, E_2-L was measured in the supernatant and DNA in the pellet. Values are means ± SE for 3 wells.

Although lipoidal estrogen esterase in ZR-75-1 cells was unaffected by c-AMP, nevertheless the enzyme belonged to the serine esterase group as seen by susceptibility to diisopropyl fluorophosphate. Cells were grown in medium containing 10% dextran coated charcoal stripped medium, 0.5 μg insulin/ml, and then exposed to 5 nM [^{3}H]E_2 in the same medium for 16 h. Cells were then transferred to medium lacking [^{3}H]E_2, but containing 0.1 mM diisopropyl fluorophosphate in 0.1% dimethylsulphoxide, or vehicle alone, and E_2-L measured after 5 h. In the presence of the serine esterase inhibitor, the levels of E_2-L were 2.5 fold higher than in controls ($P < 0.005$). Katz *et al.* [18] have reported that the esterase in MCF-7 cells is inhibited by diisopropyl fluorophosphate, but not by sodium fluoride or iodoacetic acid.

DISCUSSION

Both ER positive and negative human mammary cancer cell lines showed an initial rise in E_2-L formation, accompanied by a trough or inflection at around 6 min following exposure to 5 nM E_2 (Fig. 1). This would be explained by esterase action and confirms previous data, based on a changing composition of the total E_2-L fraction, that the system is in a dynamic state involving esterification and de-esterification systems [4–6]. The more rapid rate of accumulation of E_2-L in MDA-MB-231 cells at these early times following exposure to E_2, is also in keeping with previous comparative data on ER positive and negative cell lines collected over longer time periods [3, 5].

When assessment of the rate of saturation of ER by ligand in human mammary cancer cells was made in direct comparison with the rate of E_2-L formation, then ~37% (MCF-7) and ~62% (ZR-75-1) saturation of ER was attained before appreciable accumulation of E_2-L occurred at around 4 min. This data then does not support the concept that E_2-L may act in the direct transport of hormone in esterified form to the nuclear receptor for subsequent reconversion to free E_2. It would rather seem that esterification of E_2 serves to retain the hormone in an exclusively intracellular form [3], which by slow release of E_2 by esterase action, would help maintain occupancy of ER necessary for promotion of DNA synthesis.

Whilst the same esterifying enzyme is apparently involved in the acylation of E_2 and other steroids such as dehydroepiandrosterone and 5-androstene-3β, 17β-diol [4, 5], it is not known whether one or more enzymes are involved in the deacylation process. It is known that the lipoidal estrogen esterase, partially purified from bovine placenta, will hydrolyse E_2-17β-oleate and dehydroepiandrosterone oleate—as will purified hormone sensitive lipase [9]. There are further indications that one enzyme may be involved since the composition of E_2-L, and the 3β-monoester and 17β-monoester lipoidal derivatives of 5-androstene-3β, 17β-diol, formed by human mammary cancer cells, are all very similar [4, 6]. Furthermore, significantly higher proportions of E_2-17β-stearate, or the 3β- and 17β-stearates of 5-androstene-3β, 17β-diol were formed in ER negative compared to ER positive human mammary cancer cells [4, 6]. As shown in Table 1, E_2-17β-stearate was hydrolysed at a low rate compared to other E_2-L by the esterase present in pooled human mammary cancer tissue. When coupled to the higher esterase activity in MDA-MB-231 cells compared to MCF-7 cells (Table 1), this could explain the proportionally higher level of the stearate esters found in ER negative cell lines.

No evidence was obtained that the lipoidal estrogen esterase was under estrogen control (Table 1), which confirms the experience of Katz *et al.* [18] for the esterase in MCF-7 cells acting on E_2-17β-valerate or E_2-17β-stearate. However,

these authors found that a separate esterase, which hydrolysed E_2-17β-acetate, was under positive control of estrogen. In view of the fact that the partially purified lipoidal estrogen esterase from bovine placenta was very similar, if not identical, to hormone sensitive lipase [9], it was anticipated that the esterase in human mammary cancer cells might be activated by a mechanism involving c-AMP as second messenger. However, addition of dibutyryl c-AMP was without effect, either during the accumulation of E_2-L following exposure of cells to E_2, or in the subsequent phase following accelerated E_2-L breakdown when E_2 was removed from the cultures. E_2-L breakdown was not increased when hydrolysis of dibutyryl c-AMP was prevented by a diphosphoesterase inhibitor, or when calcitonin, which increases c-AMP levels in MCF-7 cells, was present. Thus the lipoidal estrogen esterase may be different to that previously examined in bovine placenta. In this regard, it may be significant that although the compositions of the total E_2-L fraction formed by bovine endometrium and placenta were almost identical [16], they were quite distinct from that formed by human breast cancer tissue or breast cancer cells in culture; E_2-L from bovine tissues contained a high percentage of unsaturated and essential fatty acids [2, 16] in marked contrast to that derived from mammary cancer cells and tissue [4, 23]. From the above evidence, it could be concluded that the lipoidal estrogen esterase in human mammary cancer cells is a serine esterase which is not regulated by a c-AMP-dependent protein kinase. There does, however, remain the possibility that such a system may be involved, but under the conditions of culturing cells *in vitro*, the enzyme could exist in an extensively phosphorylated form.

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