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d-α-Tocopherol inhibition of vascular smooth muscle cell proliferation occurs at physiological concentrations, correlates with protein kinase C inhibition, and is independent of its antioxidant properties

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ABSTRACT ***d*-α-Tocopherol, but not *d*-β-tocopherol, negatively regulates proliferation of vascular smooth muscle cells at physiological concentrations. *d*-α-Tocopherol inhibits protein kinase C (PKC) activity, whereas *d*-β-tocopherol is ineffective. Furthermore *d*-β-tocopherol prevents the inhibition of cell growth and of PKC activity caused by *d*-α-tocopherol. The negative regulation by *d*-α-tocopherol of PKC activity appears to be the cause and not the effect of smooth muscle cell growth inhibition. *d*-α-Tocopherol does not act by binding to PKC directly but presumably by preventing PKC activation. It is concluded that, in vascular smooth muscle cells, *d*-α-tocopherol acts specifically through a nonantioxidant mechanism and exerts a negative control on a signal transduction pathway regulating cell proliferation.***

Vascular smooth muscle cell (vascular SMC) proliferation represents a significant event in a number of diseases such as arteriosclerosis and hypertension (1–4). Smooth muscle proliferation is controlled by growth factors released from blood cells (1, 2, 5), by inhibitors or stimulants produced by the vessel wall cells (6, 7), by tocopherols, and by active oxygen species (8, 9). Evidence indicates that experimental atherosclerosis and foam cell formation can be effectively retarded by antioxidants (10–12). In addition, supplementation of human subjects with antioxidants has been shown to increase the resistance of their low density lipoproteins to oxidation and to protect against arteriosclerosis (13–16). As antioxidants, tocopherols may stimulate in some cases cell proliferation by removing inhibitory lipid peroxides (17–22). However, *d*-α-tocopherol has also a direct effect as cell-growth inhibitor, and this effect is not obviously mediated by its reduction–oxidation properties (23–25).

PKC participates in one of the major signal transduction systems triggered by the external stimulation of cells by various ligands including hormones, neurotransmitters, and growth factors (26). Activation of PKC by phorbol esters may be responsible for their growth-promoting activity. *d*-α-Tocopherol has been shown to inhibit PKC activity in a number of cell lines and, in particular, in SMC. The mechanism of this inhibition has not yet been clarified (23–25).

In the present study PKC inhibition has been found to be the basis of the inhibition of cell proliferation by *d*-α-tocopherol. Moreover PKC inhibition has been found to be cell cycle dependent, a result inconsistent with a direct interaction between PKC and *d*-α-tocopherol. Finally, the inhibitory specificity of *d*-α-tocopherol versus *d*-β-tocopherol and their mutual competition suggest a nonantioxidant mechanism to be at the basis of its action.

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MATERIALS AND METHODS

Growth media and serum were from GIBCO; A7r5 rat aortic SMC were from the American Type Culture Collection; phorbol 12-myristate 13-acetate (PMA) and streptolysin-O (25,000 units) were from Sigma; calphostin C, calyculin A, and okadaic acid were from LC Services (Woburn, MA); [γ-^{32}P]ATP (30 Ci/mmol; 1 Ci = 37 GBq); and [methyl-^{3}H]thymidine (25 Ci/mmol) was from Amersham. *d*-α-Tocopherol and *d*-β-tocopherol were gifts from Henkel (La Grange, IL). The peptide PLSRTLSVAAKK, used as a substrate for the PKC assay, was synthesized by C. Servis (Epalinges, Switzerland). A fragment of bovine myelin basic protein, MBP-(1–14), was from Bachem.

Cell Culture. SMC were grown in Dulbecco's modified Eagle's medium (DMEM) containing antibiotics and 10% fetal calf serum (FCS). Cells between passage 7 and 14 were used for all experiments. Viability was determined by the trypan blue method. Cells in a subconfluent state were made quiescent by incubating them in a medium containing 0.2% FCS for at least 48 h. *d*-α-Tocopherol and *d*-β-tocopherol were added to the cultures as ethanol solutions or were adsorbed to FCS as described (27) at the indicated concentrations.

Determination of the *d*-α- and *d*-β-Tocopherol Content in A7r5 Cells. Cells were incubated with either 50 μM *d*-α-tocopherol or 50 μM *d*-β-tocopherol (added as ethanol solution; final ethanol concentration, 0.1%). To control cells only ethanol was added. After three washes with phosphate-buffered saline (PBS) containing 1% bovine serum albumin, tocopherol content was measured by reverse-phase HPLC essentially as described (28). The amounts of *d*-α-tocopherol and *d*-β-tocopherol found in SMC were 1.4 ± 0.4 and 2.0 ± 0.4 nmol/mg of protein, respectively. In the absence of added tocopherols, the amounts were <0.1 nmol/mg of protein. The same results were essentially obtained in PMA-stimulated cells.

Determination of PKC Activity in Permeabilized Cells. Activity of PKC in permeabilized SMC was assayed by the procedure of Alexander *et al.* (29) with minor modifications. Quiescent A7r5 cells were subjected to different treatments as indicated. During the last hour of the preincubation period, cells were treated with 100 nM PMA. Then, cells were washed twice with PBS, resuspended in intracellular buffer (5.2 mM $MgCl_2$/94 mM KCl/12.5 mM Hepes/12.5 mM EGTA/8.2 mM $CaCl_2$, pH 7.4) and divided in 220-μl portions (1.5×10^5 cells). Assays were started by adding [γ-^{32}P]ATP (9 cpm/pmol; final concentration, 250 μM), peptide substrate (final concentration, 70 μM), and streptolysin-O (0.3 unit). The reaction mixtures were incubated at 37°C for 10 min, and the reaction

Abbreviations: FCS, fetal calf serum; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; SMC, smooth muscle cell(s); MBP, myelin basic protein.

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was stopped by addition of 100 μl of 25% (wt/vol) trichloroacetic acid in 2 M acetic acid. Aliquots (10-μl) were spotted onto P81 paper (Whatman). This was washed twice for 10 min with 30% (vol/vol) acetic acid containing 1% phosphoric acid and once with ethanol, dried, and cut, and radioactivity was assayed. Basal phosphorylation in the absence of the peptide was subtracted from the experimental data. The absolute values of PKC activity changed depending of the number of passages of the cells, ranging from 63 pmol of phosphate per min per 10^6 cells at passage 5 to 215 pmol of phosphate per min per 10^6 cells at passage 20. Only cells from passages 7–14 were used in this study, having PKC activities from 75 to 150 pmol of phosphate per min per 10^6 cells, but the inhibitory effect of *d*-α-tocopherol was always consistent.

[³H]Thymidine Incorporation. To measure DNA synthesis, cells were pulsed with [³H]thymidine (1 μCi per well) at the indicated times after restimulation with FCS. After labeling, cells were washed twice with PBS, fixed for 20 min with ice-cold 5% trichloroacetic acid, and solubilized in 0.1 M NaOH/2% Na_2CO_3/1% SDS. The radioactivity incorporated into the acid-insoluble material was determined in a liquid scintillation analyzer.

Flow Cytometry Analysis. The A7r5 cell cycle was monitored by flow cytofluorometry. Cells at different stages of the cycle after stimulation with FCS were washed three times with PBS, fixed with cold 70% ethanol, and, after 48 h, stained with a solution of propidium iodide (50 μg/ml; Molecular Probes) containing RNase (40 μg/ml; Boehringer Mannheim) according to standard methods (30). The samples were then analyzed in an EPICS Profile II instrument using a software package from Coulter.

RESULTS

Inhibition by *d*-α-Tocopherol of PKC Activity and of Proliferation Are Parallel Events. The effect of *d*-α-tocopherol on SMC proliferation and on PKC is shown in Fig. 1. A parallel inhibition of PKC activity and of proliferation has been observed to occur at concentrations of *d*-α-tocopherol close to those measured in healthy adults (14, 31).

Inhibition by *d*-α-Tocopherol of PKC and SMC Proliferation Is Prevented by *d*-β-Tocopherol. The effect of *d*-α- and *d*-β-tocopherol on the proliferation stimulated by FCS is shown in Fig. 2 *Upper*. *d*-α-Tocopherol (50 μM) inhibited cell growth ≈65%. However, *d*-β-tocopherol, an analogue of *d*-α-tocopherol lacking a methyl group in position 7 of the chromanol ring and having very similar antioxidant properties (25), did not show any significant inhibition of cell proliferation. The amount of *d*-α- and *d*-β-tocopherol present in the cells, as measured after 24 h of incubation, was not significantly different, indicating that the lack of inhibition is not due to a different uptake of *d*-β-tocopherol (23). Moreover *d*-α-tocopherol and *d*-β-tocopherol do not compete with each other for cellular uptake (not shown). The prevention by *d*-β-tocopherol of the proliferation inhibition by *d*-α-tocopherol suggests a site-directed event at the basis of *d*-α-tocopherol inhibition rather than a general radical scavenging reaction.

In SMC permeabilized with streptolysin-O (Fig. 2 *Lower*), *d*-α-tocopherol inhibited PKC activity, whereas β-tocopherol was ineffective. When both were present, *d*-β-tocopherol prevented the inhibitory effect of *d*-α-tocopherol. The inhibition by *d*-α-tocopherol and the lack of inhibition by *d*-β-tocopherol of cell proliferation and PKC activity shows that the mechanism involved is not related to the radical scavenging properties of these two molecules, which are essentially equal (25).

SMC Cycle and *d*-α-Tocopherol Inhibition. The length of the SMC cycle was analyzed by cytofluorimetry (Fig. 3 *Upper*). SMC, ≈10 h after activation by FCS, began to undergo a

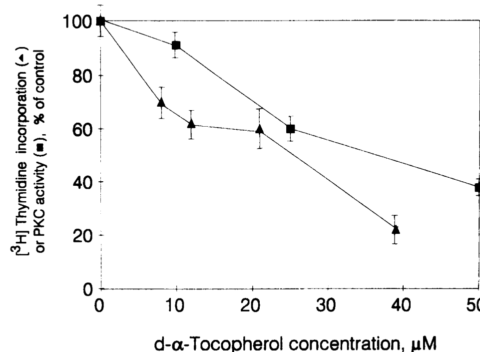

FIG. 1. Parallel inhibition of PKC activity and of proliferation in SMC. Quiescent cells were restimulated to grow by FCS in the presence or absence of *d*-α-tocopherol. After 7 h (cells in G_1 phase), PKC was measured. The control value in the absence of *d*-α-tocopherol (100%) corresponds to 124.0 ± 19.8 pmol/min per 10^6 cells. For DNA synthesis determination, a 3-h pulse of 0.5 μCi of [³H]thymidine per ml was given to the cells in S phase (15 h after the release from G_0 arrest). The control represents 84,332 ± 5150 cpm. Results are expressed as the mean ± SD of three independent experiments.

transition indicated by an increase of the DNA content, which reached a maximum at 20 h. At about 12 h (Fig. 3 *Lower Right*), an increase of [³H]thymidine incorporation became observable, which reached a maximum at 16 h. Thus, the length of the G_0 to G_2 phase can be set at ≈20 h and that of the G_1 phase at about 10–12 h.

The cytofluorimetric analysis of Fig. 3 *Lower Left* shows that *d*-α-tocopherol produced an inhibition of the $G_1 \rightarrow S$ cell transition, which resulted (Fig. 3 *Lower Right*) in a lesser [³H]thymidine incorporation into DNA without delaying the entry into the S phase. Fig. 3 *Lower Left* shows also that

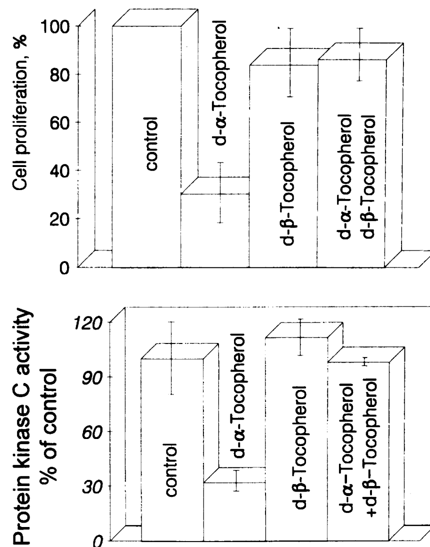

FIG. 2. (*Upper*) Different effect of *d*-α-tocopherol and *d*-β-tocopherol on SMC proliferation. Serum-deprived quiescent cells were restimulated to grow by FCS in the presence of 50 μM *d*-α-tocopherol and/or 50 μM *d*-β-tocopherol. After completion of the cell cycle (30 h), cells were counted with a hemocytometer. Viability was >95%. Control (100%) represents 289 ± 12 × 10^3 cells. Results are expressed as the mean ± SD of three independent experiments. (*Lower*) Effect of *d*-α-tocopherol and *d*-β-tocopherol on PKC activity. Quiescent cells were restimulated with 10% FCS in the presence or absence of 50 μM *d*-α-tocopherol or 50 μM *d*-β-tocopherol. After 7 h, cells were permeabilized and PKC was measured. Only the PMA-stimulated activity was considered. Control (100%) corresponds to 115.1 ± 24.0 pmol/min per 10^6 cells. Results are expressed as the mean ± SD of three independent experiments.

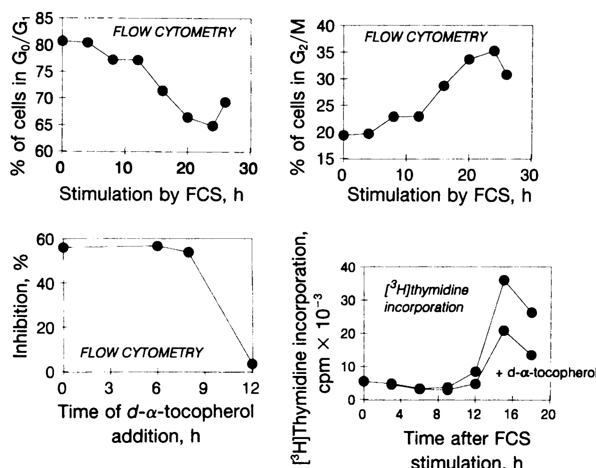

FIG. 3. SMC cycle analysis of *d*-α-tocopherol inhibition. (*Upper*) Quiescent cells were stimulated with FCS, and, at the indicated times, they were trypsinized, washed, and fixed by addition of 70% (vol/vol) ethanol. Cells were stained as indicated in text. Percentage of cells in G_0/G_1 (*Left*) and G_2/M (*Right*) phases were calculated from the corresponding histograms. (*Lower Left*) Quiescent cells were stimulated with FCS and, at the indicated times, treated with 50 μM *d*-α-tocopherol. At 18 h after stimulation, cells were collected and analyzed as previously described. Percent inhibition indicates the reduction in the fraction of cells that progress through the G_2/M phase. (*Lower Right*) Quiescent cells were stimulated with FCS in the presence or absence of 50 μM *d*-α-tocopherol and, at the indicated times, were pulsed with [^{3}H]thymidine (1 μCi per well) for 1 h. Data are from a representative experiment done in triplicate. Similar results were obtained in an additional separate experiment.

d-α-tocopherol was effective when added at any time during the initial 9 h of the cell cycle. Addition of *d*-α-tocopherol after this point did not result in inhibition of the cell cycle progression. [^{3}H]Thymidine incorporation also was not affected by *d*-α-tocopherol when its addition was made after 8–9 h from the beginning of phase G_1 (not shown). The data suggest that a *d*-α-tocopherol restriction point exists in the cell cycle, located in the late G_1 phase.

The data on the cell cycle-dependent inhibition by *d*-α-tocopherol reported in the experiments of Fig. 3 could also be interpreted in terms of a need of a given incubation time for *d*-α-tocopherol to penetrate the cells and reach its target. This possibility was excluded by the following experiments, which have also shown that a mechanism involving a direct interaction between PKC and *d*-α-tocopherol is not compatible with the experimental data.

Inhibition by *d*-α-Tocopherol Is Not Caused by a Direct Interaction with PKC. The data in Fig. 4 and previous data from this laboratory (23) show that PKC activity increased during the cell cycle progression, reaching a maximum in the late G_1 phase. The time of addition of *d*-α-tocopherol during the cell cycle determined the extent of PKC inhibition. When *d*-α-tocopherol was added in the G_0 phase of the cycle and incubated for 7 h in the absence of FCS, no inhibition was observed. When FCS was added together with *d*-α-tocopherol and cells were stimulated to enter in G_1 phase, inhibition of PKC by *d*-α-tocopherol was observed after 7 h of incubation (Fig. 4). When *d*-α-tocopherol was added in the G_1 phase (and PKC activity was measured 7 h later), *d*-α-tocopherol showed no inhibition. To keep cells in the G_0/G_1 phase, cells were washed and reincubated with a serum-free medium after reaching the G_1 phase, and, as shown in Fig. 4 *Lower*, they did not progress to the S-phase of the cycle.

PKC Inhibition by *d*-α-Tocopherol Appears To Be the Cause and Not the Result of SMC Growth Inhibition. To establish if PKC inhibition could be at the basis of the negative regulation of cell growth by *d*-α-tocopherol, the experiment shown in Fig. 5 *Upper* was carried out. A highly specific inhibitor of PKC, calphostin C (32, 33) appeared to potently inhibit FCS-induced proliferation of SMC. Thus, PKC inhibition results in cell growth inhibition. Alternatively the question can be asked whether inhibition of cell proliferation may result in PKC inhibition. In the experiment reported in Figure 5 *Lower*, quiescent cells were stimulated to grow by adding FCS for different times; the data points represent the time of exposure of the cells to FCS. At these intermediate times, FCS-containing medium was removed and replaced with a buffer devoid of FCS. After 12 h from the addition of FCS to the cells, PKC activity and the incorporation of [^{3}H]thymidine were measured. The experiment shows that serum deprivation of SMC in the first 6 h after their entry into the G_1 phase resulted in inhibition of [^{3}H]thymidine incorporation in DNA or in other words, in an arrest of the cycle. Under these conditions, however, PKC activity continuously increased, indicating that inhibition of cell proliferation does not cause inhibition of PKC activity. Moreover, no increase in PKC amount was observed (not shown), suggesting that the increase in activity observed during the cell cycle progression may be due to a postranslational modification of the enzyme.

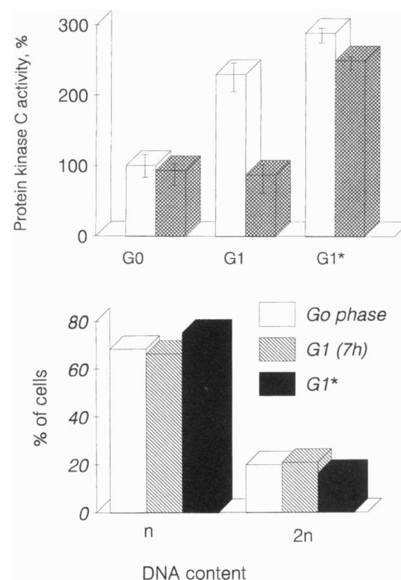

FIG. 4. (*Upper*) Cycle-dependence of PKC inhibition by *d*-α-tocopherol. Quiescent cells (G_0), stimulated with FCS for 7 h (G_1) or stimulated with FCS for 7 h and then incubated in serum-free medium for additional 7 h (G_1^*) were used. G_0 cells were preincubated with 50 μM *d*-α-tocopherol for 7 h; G_1 cells received 50 μM *d*-α-tocopherol at the time of restimulation and were further incubated for 7 h; G_1^* cells were treated for 7 h with 50 μM *d*-α-tocopherol after removal of the serum-containing medium. PMA-stimulated PKC activity was measured as described in text; 100% corresponds to 60.9 ± 12.5 pmol/min per 10^6 cells. Data are from three independent experiments and are expressed as the percentage (mean ± SD) for each condition relative to G_0. □, Control; ▩, *d*-α-tocopherol. (*Lower*) Cell cycle analysis. Cells were analyzed by flow cytometry as described in Fig. 3. n, DNA content before S-phase; 2n, after DNA synthesis.

Protein Phosphatase 2A and the Inhibition of PKC by *d*-α-Tocopherol. The inhibition of PKC activity observed only after addition of *d*-α-tocopherol to cells in the G_0 phase of the cycle (and not associated with a diminution of the amount of expressed protein) prompted us to investigate if a phosphatase could be responsible for the deactivation of PKC during the cycle. To eliminate the possibility that the inhibition caused by *d*-α-tocopherol could be brought about by an increased dephosphorylation of the phosphorylated peptide used as substrate, the following experiments were carried out. The MBP-(4–14) fragment whose phosphorylation is phosphatase insensitive was used as substrate (34). Under our experimental

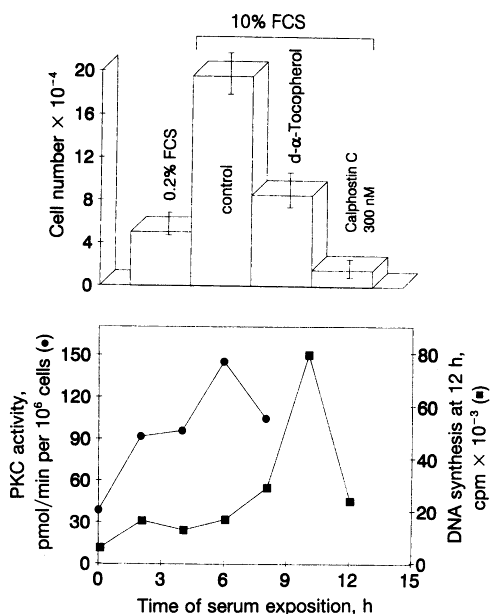

FIG. 5. (*Upper*) Inhibition of PKC by calphostin C is associated with inhibition of cell proliferation. Quiescent cells were restimulated with 10% FCS in the presence of 50 μM *d*-α-tocopherol or 300 nM calphostin C. Cells were counted after completion of the cell cycle (30 h). Results are expressed as the mean ± SEM from two separate experiments. (*Lower*) Arrest of SMC in the G_1 phase is not associated with inhibition of PKC activity. Quiescent cells were exposed for a limited period to 10% FCS. After the indicated times, the medium was removed, and the cells were washed and further incubated in serum-free medium. At 12 h after the addition of FCS, PKC and [^{3}H]thymidine incorporation (after a 1-h pulse with [^{3}H]thymidine at 1 μC per well) were determined. Results are representative of a typical experiment. Similar results were obtained in two independent experiments.

conditions the MBP-(4–14) substrate, after phosphorylation, was not hydrolyzed, as indicated by its very slow concentration decay after the kinase had been inhibited by staurosporine (A.T., unpublished data). Fig. 6 shows that the phosphorylation of the MBP-(4–14) substrate was inhibited by *d*-α-tocopherol. However, in the presence of calyculin A, a potent inhibitor of protein phosphatases 1 and 2A (35, 36), the effect of *d*-α-tocopherol was largely prevented. Similar experiments carried out with okadaic acid, a more specific inhibitor of protein phosphatase 2A, gave similar results (Fig. 6). The data suggest a mechanism of protein kinase C deactivation by *d*-α-tocopherol involving a protein phosphatase 2A type.

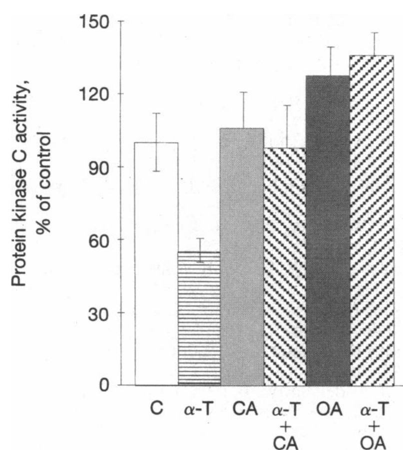

FIG. 6. Protein phosphatase 2A and the inhibition of PKC by *d*-α-tocopherol. Cells were restimulated for 7 h with 10% FCS in the presence or absence of 50 μM *d*-α-tocopherol (α-T) as indicated. Calyculin A (CA; 2 nM), or okadaic acid (OA; 2 nM) was added 1 h before the activity measurement. All the values correspond to PMA-stimulated PKC activity, which was determined by using the phosphatase-insensitive peptide MBP-(4–14) as substrate. Control cells (100%) corresponds to 97.5 ± 12.0 pmol/min per 10^6 cells. Data are expressed as the mean ± SD of four independent experiments. C, control.

DISCUSSION

The inhibition of SMC proliferation by *d*-α-tocopherol at physiological concentrations may explain the notion that *in vivo* SMC are quiescent and that they multiply only under conditions of stress (2, 37). Depletion of *d*-α-tocopherol may occur locally or generally as a consequence of oxidative stress (38–40), and such a condition would result in cell-growth stimulation. A dietary or oxidative diminution of *d*-α-tocopherol can thus play a role in the onset and development of arteriosclerosis as indicated by the protection exerted by vitamin E diet supplementation (15, 16). Antioxidants have been found to be beneficial in inhibiting smooth muscle proliferation and neointima formation in animal models (41, 42), and *d*-α-tocopherol itself, associated with ascorbic acid, has been shown also to be effective. Since many antioxidants (ascorbic acid, lipoic acid, glutathione, tocopherols, etc.) interact with each other (43–45), their effect may be to spare *d*-α-tocopherol, which has the additional and more complex function of controlling cell proliferation. Such a cell-specific function does not exclude other effects such as the protection against oxidation of low density lipoproteins (46–48).

A specific inhibition of cell proliferation and PKC activity by *d*-α-tocopherol relative to *d*-β-tocopherol may be caused by several factors. The two molecules are very similar, and it has been shown (25) that their antioxidant activity is essentially the same. Uptake of *d*-α-tocopherol and *d*-β-tocopherol in SMC is very similar, and they do not compete with each other for transport. The fact that *d*-β-tocopherol penetrates into the cells and where it provides a function is shown by the experiments in which *d*-β-tocopherol prevented the effects of *d*-α-tocopherol. These data suggest two main conclusions: (*i*) the effect of *d*-α-tocopherol and *d*-β-tocopherol cannot be reconciled with their antioxidant activity; and (*ii*) there may be a common site where the two compounds bind in a mutually exclusive way, *d*-α-tocopherol acting as an agonist and *d*-β-tocopherol acting as an antagonist.

PKC inhibition appears to be sufficient to cause inhibition of SMC proliferation *in vitro*. On the other hand, it was also shown that inhibition of cell cycle progression by serum removal in the G_1 phase does not cause inhibition of PKC activity (Fig. 5). The molecular events by which PKC activity is affected by *d*-α-tocopherol are not yet fully clarified, although several possible mechanisms can be excluded. A direct interaction of *d*-α-tocopherol with PKC is improbable, since such interaction should be the same all through the cell cycle. On the contrary, a cell cycle-dependent inhibition (Fig. 4) can be observed. The possibility that *d*-α-tocopherol inhibits the expression of PKC has been also investigated, but throughout the cycle and in the presence and absence of *d*-α-tocopherol, the amount of PKC as measured by immunoblot analysis remains constant (experiments not reported).

The measurement of PKC activity in streptolysin O-permeabilized cells was carried out in the presence of PMA, which brings the enzyme activity to its maximum, irrespective of the concentration of diacylglycerol, Ca^{2+}, and phosphatidylserine (for a review, see ref. 26). Thus, a possible mechanism of *d*-α-tocopherol action implying inhibition of the production of the physiological activators of PKC can be eliminated, as well as activatory binding to a receptor protein (49, 50). Since the activity measurements were carried out in the presence of PMA, a possible primary role of *d*-α-tocopherol

as an inhibitor of PKC translocation (51, 52) can also be excluded.

PKC becomes active as a consequence of phosphorylation at discrete sites, brought about by a PKC kinase and by autophosphorylation. Since the inhibition of PKC activity by d-α-tocopherol is not caused by a direct interaction with the enzyme or by a diminution of its synthesis, the following hypothesis may be considered: (*i*) a phosphatase activated by d-α-tocopherol diminishes the level of PKC-phosphorylated peptide [this possibility has been excluded by the use of a phosphatase-insensitive peptide substrate MBP-(4–14)]; (*ii*) PKC activity itself is diminished because of inhibition of its phosphorylation by d-α-tocopherol (53, 54); and (*iii*) PKC activity is diminished because of activation of its dephosphorylation by d-α-tocopherol (55). To establish whether d-α-tocopherol interfered with the process of phosphorylation or dephosphorylation of PKC, the experiment of Fig. 6 was carried out. Two inhibitors of protein phosphatases (okadaic acid and calyculin A) were used. If d-α-tocopherol inhibited the phosphorylation of PKC, these compounds should not have interfered with the effect of d-α-tocopherol. In other words, inhibition by d-α-tocopherol of PKC kinase would result in inhibition of PKC activity despite the presence of okadaic acid or calyculin A. On the other hand, if d-α-tocopherol activated (or increased the expression of) a phosphatase responsible for PKC inactivation, such an event would have been blocked by calyculin A or okadaic acid. The latter compounds would, in fact, prevent activation of a phosphatase, or, in the event more enzyme had been induced, they would keep it inactive. In the presence of these phosphatase inhibitors, d-α-tocopherol does not have an effect on PKC activity, indicating that an active phosphatase is necessary for d-α-tocopherol to exhibit its inhibitory properties. Thus, it appears that the inhibition of PKC by d-α-tocopherol is mediated by deactivation of the enzyme by a protein phosphatase, possibly of the type 2A, which is sensitive to okadaic acid.

On the basis of the comparison presented here between d-α-tocopherol and d-β-tocopherol and of a previous analysis of several other tocopherols and similar compounds (23, 56), a correlation between inhibition of proliferation and of PKC, and antioxidant properties of the compound cannot be verified. The specificity of the inhibition of both proliferation and PKC activity of d-α-tocopherol versus d-β-tocopherol, due to the strong similarity between the two molecules, implies a site-directed recognition mechanism. The involvement of protein phosphatase 2A appears to be at the basis of the inhibition of cell proliferation by d-α-tocopherol.

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