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α-tocopherol succinate, but not α-tocopherol or other vitamin E analogs, stimulates prolactin release from rat anterior pituitary cells in vitro

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We have previously reported the isolation of a vitamin E analog (α-tocopherol succinate) from a green barley leaf extract, which stimulates the release of prolactin and growth hormone from rat anterior pituitary cells in vitro. In the present study, we tested the commercially available forms of α-tocopherol and also succinic acid. Normal anterior pituitary cells were treated with different commercially available forms of tocopherol (100 μg/ml) and succinic acid (50 to 100 μg/ml). Only α-tocopherol succinate caused a significant increase ($P < 0.01$) in prolactin release. In addition, α-tocopherol, δ-tocopherol, α-tocopherol acetate, α-tocopherol nicotinate, succinic acid disodium salt, and succinic acid butenedoic acid did not affect the prolactin release in vitro. The data demonstrate that of all the biologically active forms of vitamin E, only α-tocopherol succinate possesses the ability to enhance anterior pituitary hormone release in vitro. (J. Nutr. Biochem. 6:340–344, 1995.)

Keywords: green barley leaf extract; prolactin; α-tocopherol succinate; vitamin E

Introduction

α-tocopherol succinate, but not α-tocopherol and its analogs, has been found to have several unique biochemical properties in vitro and in vivo. In vitro studies have shown that α-tocopherol succinate induces differentiation and growth inhibition in certain animal and human tumor cells in culture, whereas α-tocopherol, α-tocopherol acetate, and α-tocopherol nicotinate were ineffective. In other studies, α-tocopherol succinate reduced basal and ligand stimulated adenylate cyclase activity and expression of c-*myc* and *ras* oncogenes in certain tumor cells in culture.[1] Heat alone or in combination with radiation and chemicals has been extensively used in the treatment of animal and human tumors. Vitamin E succinate, when combined with heat treatment, reduces the survival of melanoma cells more than that produced by vitamin E succinate alone.[2] It has been shown that α-tocopherol succinate inhibits growth and induces morphological differentiation in melanoma cells.[3,4] α-tocopherol succinate also inhibits HL-60 human promyelocytic leukemia cell proliferation and induces HL-60 cells to differentiate toward a functionally deficient macrophage-like cell. This suggests a role of α-tocopherol succinate as an antitumor proliferative agent and as a modifier of human leukemia cell differentiation.[5] α-tocopherol succinate was also demonstrated to be a potent in vitro modulator of retrovirus-induced immune abnormalities.[6] Kline and Sanders investigated the effect of α-tocopherol succinate on lectin-induced chicken T-cell proliferation. Their results indicated that growth-inhibiting properties of α-tocopherol succinate are unique and may not involve antioxidant properties.[7] Studies have also demonstrated that α-tocopherol succi-

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nate inhibits the proliferation of estrogen receptor–positive and estrogen receptor–negative human breast cancer cell lines in a dose-dependent manner in vitro.[8]

The role of free radical–mediated reactions such as lipid peroxidation in a wide range of tissue injuries is a subject of great current interest. Carini et al.[9] reported that α-tocopherol succinate requires esterase activity to yield free α-tocopherol in order to show antioxidant activity in microsomal fractions of rat liver. However, α-tocopherol succinate exerted a more effective antioxidant protection than an equivalent amount of directly added free α-tocopherol. The authors believed that the apparent lack of activity of directly added free α-tocopherol may be due in some way to its extreme hydrophobicity. Alfonso et al.[10] have shown that α-tocopherol succinate enhances the effect of γ-irradiation on neuroblastoma cells in culture through antioxidation mechanisms. Farriss also reported the unique cytoprotective properties of α-tocopherol succinate in hepatocytes. His results indicated that the exogenous administration of α-tocopherol succinate completely protected hepatocytes from cadmium-induced injury and lipid peroxidation. However, hepatocytes exposed to tocopherol were not protected from the toxic manifestations of cadmium.[11]

Green barley leaf extract, a dried extract of young green barley leaves, is widely used in Japan and other countries as a nutritional supplement. We have recently reported the isolation of a vitamin E analog from green barley leaf extract that stimulates the release of prolactin and growth hormone from rat anterior pituitary (AP) cells in vitro.[12] This molecule was identified as α-tocopherol succinate, or vitamin E. The objective of the present study was to identify the form(s) of α-tocopherol capable of the stimulation of prolactin from rat anterior pituitary cells in vitro. Therefore, we have tested the commercially available forms of α-tocopherol and also succinic acid. Our results suggest that only α-tocopherol succinate, but not α-tocopherol or other vitamin Es or succinic acid, can stimulate prolactin release from rat anterior pituitary cells in vitro. This is the first evidence for a novel biochemical action of a vitamin E succinate at the level of the pituitary.

Methods and materials

Thyrotropin-releasing hormone (TRH), α-tocopherol, α-tocopherol succinate, α-tocopherol acetate, α-tocopherol nicotinate, δ-tocopherol, succinic acid butenedoic acid, and succinic acid disodium salt were all obtained from Sigma (St. Louis, MO USA). All buffers and solvents were of HPLC grade (Fisher Scientific, Pittsburgh, PA USA).

Anterior pituitary cell culture

Anterior pituitary cells from normal adult female or male Sprague–Dawley rats were dispersed as described by Spangelo et al.[13] Normal cells were seeded into 96- or 24-well tissue culture plates (Falcon, Oxnard, CA USA) at a density of 0.1 or 0.4 × 10^6 viable cells/well in 0.25 mL or 1.5 mL of RPMI-1640 medium supplemented with 2.5% fetal calf serum, 7.5% horse serum, and 7.5 μg/mL of fungizone (Gibco, Grand Island, NY USA). The cells were allowed to attach to the wells in a humidified atmosphere of 5% CO_2 and 95% air at 37°C for a minimum period of 4 days before an experiment was performed. On the day of an experiment, the cells were rinsed twice (1 hr each) with serum-free RPMI-1640 medium containing antibiotics. Test substances were placed in the wells at varying concentrations and, following 30 min incubations, the media were quickly removed and saved for radioimmunoassay (RIA).

Prolactin was determined by standard RIA techniques using materials and protocols supplied by the NIDDK Rat Pituitary Hormone Distribution Program. Inter- and intraassay variations for prolactin was less than 8%. All samples were assayed in duplicate with results expressed in terms of NIDDK standards (rat prolactin RP-3). The variability within a set of quadruplicate values was 5 to 10%.

Statistical analysis

The data were expressed as the mean ± SEM of groups consisting of at least four wells. Experiments were performed independently at least two or three times with representative results reported. The Fisher protected least significant difference test for multiple comparisons was used for the statistical evaluation of these data. A P value of less than 0.05 was considered significant.

Results

Figure 1 shows the concentration-dependent influence of α-tocopherol succinate on prolactin release from rat anterior

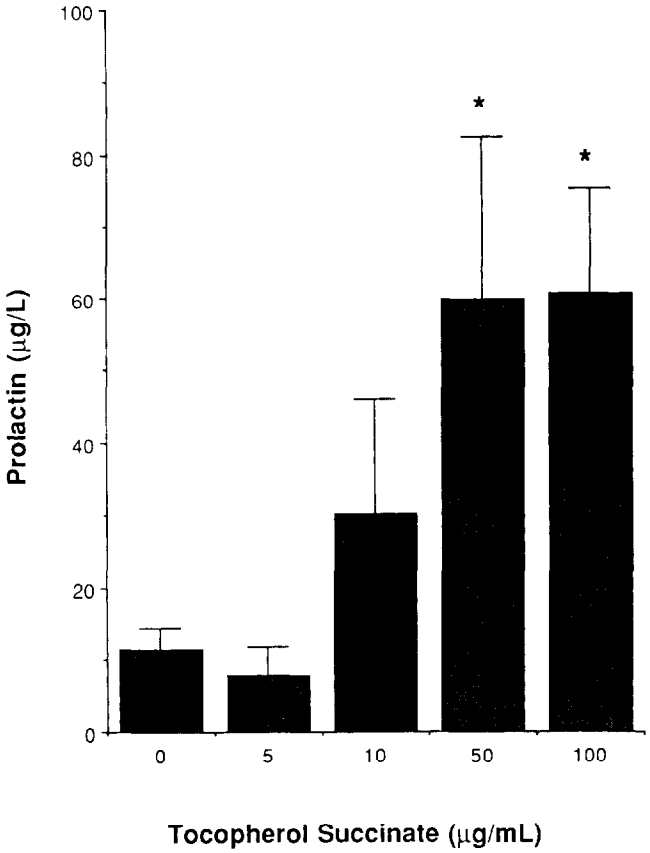


Figure 1 Concentration-dependent influence of α-tocopherol succinate on prolactin release from female rat AP cells in vitro. AP cells were cultured for 6 days and subsequently exposed to vehicle or α-tocopherol succinate for 30 min. α-tocopherol succinate significantly stimulated prolactin release from AP cells in a concentration-responsive manner (vehicle vs. 50 and 100 μg/mL; $P < 0.05$). The data are expressed as the mean ± SE of groups consisting of four observations. *$P \leq 0.01$ vs. vehicle control.

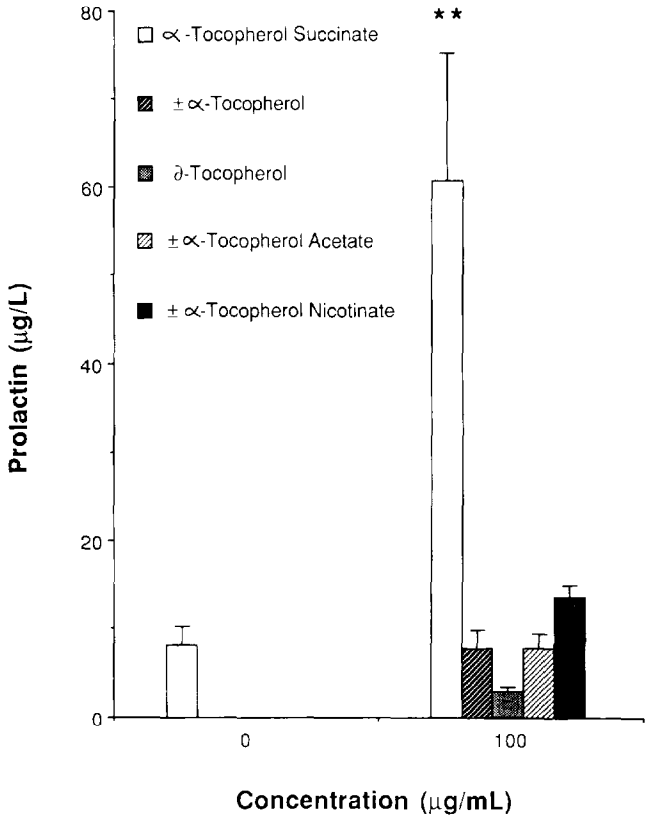


Figure 2 Effects of α-tocopherol analogs on prolactin release from female rat AP cells in vitro. AP cells were cultured for 6 days and subsequently exposed to vehicle, α-tocopherol succinate, ±α-tocopherol, δ-tocopherol, α-tocopherol acetate, or α-tocopherol nicotinate for 30 min. α-tocopherol succinate significantly stimulated prolactin release from AP cells (vehicle vs. 100 μg/mL; $P < 0.01$). However, no other form of tocopherol had any effect. The data are expressed as the mean ± SE of groups consisting of four observations. $^{**}P \leq 0.01$ vs. vehicle control

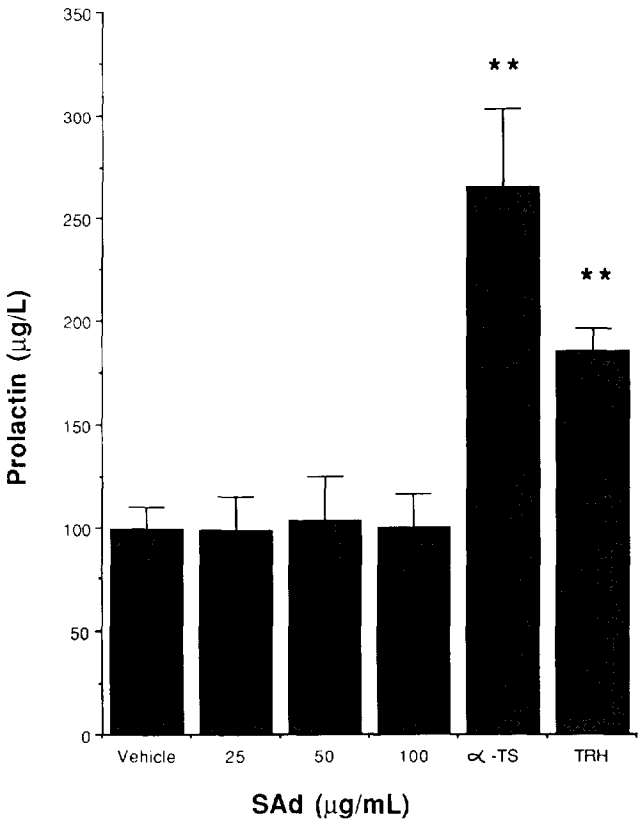


Figure 3 Concentration-dependent influence of succinic acid disodium salt (SAd) on prolactin release from male rat AP cells in vitro. AP cells were cultured for 5 days and subsequently exposed to vehicle, α-tocopherol succinate (α-TS), SAd, or TRH for 30 min. α-TS significantly stimulated prolactin release from AP cells (vehicle vs. 100 μg/mL; $P < 0.01$). The prolactin releasing factor TRH (100 nM) also significantly stimulated prolactin release from AP cells (vehicle vs. 100 nM, $P < 0.01$). However, 25 to 100 μg/mL of SAd had no effect. The data are expressed as the mean ± SE of groups consisting of four observations. $^{**}P \leq 0.01$ vs. vehicle control.

pituitary cells in vitro. α-tocopherol succinate at a concentration of 50 to 100 μg/mL caused a significant increase ($P < 0.01$) in prolactin release.

The effects of different α-tocopherol analogs on prolactin release from pituitary cells in vitro are shown in *Figure 2*. Treatment of normal anterior pituitary cells with different forms of tocopherol (100 μg/mL) caused significant increase ($P < 0.01$) in prolactin release in only α-tocopherol succinate-treated cells. However, α-tocopherol δ-tocopherol, α-tocopherol acetate, and α-tocopherol nicotinate did not affect the prolactin release in vitro.

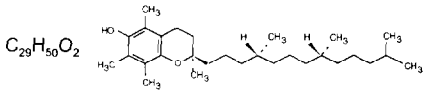
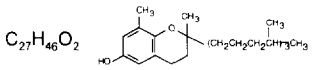
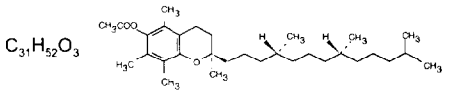
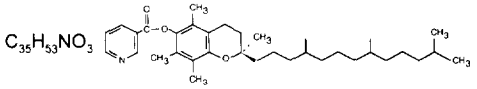
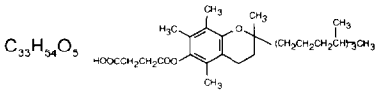
Table 1 shows the chemical structure of the tocopherol analogs that had been tested on prolactin release from pituitary cells in vitro. The only difference in the chemical structure of the α-tocopherol and α-tocopherol succinate is the existence of a succinate molecule on the benzene ring. To investigate if the succinate molecule is responsible for the stimulation of prolactin release in vitro, the effect of succinic acid disodium salt and succinic acid butenedoic acid were studied. Treatment of normal anterior pituitary cells with different forms of succinic acid (25–100 μg/mL) did not affect the prolactin release (*Figures 3* and *4*). Thus, α-tocopherol succinate enhanced the release of prolactin beyond that achievable with TRH alone.

Discussion

We have previously reported that α-tocopherol succinate stimulates the release of prolactin and growth hormone from rat anterior pituitary cells in vitro.[12] In the present study we report that only α-tocopherol succinate but not α-tocopherol or other vitamin E analogs stimulate prolactin release from rat anterior pituitary cells in vitro. Our data using different commercially available forms of α-tocopherol and also succinic acid indicate that the prolactin releasing activity of α-tocopherol succinate does not reside only in the succinate molecule of the α-tocopherol succinate. This finding indicates that α-tocopherol succinate may have an important hitherto unknown role in regulating neuroendocrine responses.

The mechanism by which α-tocopherol succinate stimulates hormone release is not known. Thyrotropin Releasing Hormone (TRH) stimulation of prolactin release is mediated through increased hydrolysis of polyphosphoinositide.[14] Coincubation of α-tocopherol succinate with an optimal amount of TRH causes an additive increase in prolactin

Table 1 Commercially available forms of tocopherol

Form	Molecular weight	Emperical formula	Chemical structure
(±) α-tocopherol (vitamin E)	430.7	$C_{29}H_{50}O_2$	
(+) δ-tocopherol	402.7	$C_{27}H_{46}O_2$	
(±) α-tocopherol acetate	472.8	$C_{31}H_{52}O_3$	
(±) α-tocopherol nicotinate	535.8	$C_{35}H_{53}NO_3$	
(±) α-tocopherol succinate	530.8	$C_{33}H_{54}O_5$	

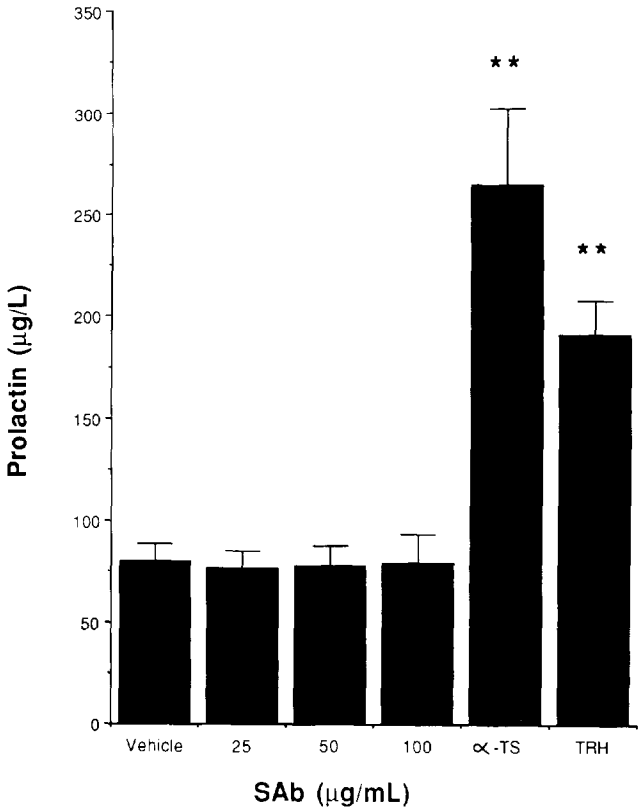


Figure 4 Concentration-dependent influence of succinic acid butenedoic acid (SAb) on prolactin release from male rat AP cells in vitro. AP cells were cultured for 5 days and subsequently exposed to vehicle, α-tocopherol succinate (α-TS), or SAb for 30 min. α-TS significantly stimulated prolactin release from AP cells (vehicle vs. 100 μg/mL; $P < 0.01$). The prolactin releasing factor TRH (100 nM) also significantly stimulated prolactin release from AP cells (vehicle vs. 100 nM; $P < 0.01$). However 25 to 100 μg/mL of SAb had no effect. The data are expressed as the mean ± SE of groups consisting of four observations. $^{**}P \leq 0.01$ vehicle control.

release compared with the effect of α-tocopherol succinate and TRH alone. These results suggest that α-tocopherol succinate may act via a mechanism(s) different from that of TRH. In addition, our previously reported data indicate that α-tocopherol succinate has no effect on anterior pituitary intracellular cAMP accumulation. The mechanism by which α-tocopherol succinate stimulates hormone release, therefore, does not appear to be dependent on changes in cAMP. This finding was also confirmed by several other investigators. Torrel et al.[15] reported that vitamin E succinate treatment increases cAMP-dependent protein kinase activity in the cytosolic fraction of B-16 melanoma cells without altering the cellular cAMP level.

Finally, α-tocopherol succinate, but not α-tocopherol, has been found to have several unique biochemical properties in vitro and in vivo. Our finding defines a new and unique biochemical property for α-tocopherol succinate. The difference in hormone releasing activity observed for α-tocopherol and α-tocopherol succinate may be due to the hydrophilic nature of this molecule in contrast to α-tocopherol, which is hydrophobic. Our finding indicates an important previously unknown role of α-tocopherol succinate in regulating neuroendocrine responses.

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