13-Hydroxyoctadecadienoic Acid Is the Mitogenic Signal for Linoleic Acid-dependent Growth in Rat Hepatoma 7288CTC in Vivo

Leonard A. Sauer,² Robert T. Dauchy,² David E. Blask,³,² Brenda J. Armstrong, and Simone Scalici
Laboratory of Experimental Neuroendocrinology/Oncology, Bassett Research Institute, Cooperstown, New York 13326

ABSTRACT

Growth of hepatoma 7288CTC in male Buffalo rats is directly dependent on uptake of linoleic acid (LA) from the arterial blood. One to 5% of the LA taken up is converted to 13-hydroxyoctadecadienoic acid (HODE), an agent that enhances epidermal growth factor-dependent mitogenesis. The role of 13-HODE in LA-dependent growth of solid tumors is not known. In this study, we examined LA uptake and 13-HODE formation on growth of tissue-isolated hepatoma 7288CTC in vivo and on [3H]thymidine incorporation and DNA content during perfusion in situ. Fatty acid uptake and metabolite release were determined from arteriovenous difference measurements. Tumor-bearing and blood donor rats were fed either LA-sufficient or -deficient diets. Hepatoma 7288CTC removed LA from the arterial blood and released 13-HODE [and a small amount of 13-keto-octadecadienoic acid (KODE)] into the venous blood both in vivo and during perfusion. Treatment with the lipoxygenase inhibitor norhydroguaiaretic acid (10 μM) did not affect tumor LA uptake, but inhibited release of 13-HODE and 13-KODE in vivo and during perfusion, suppressed growth in vivo, and inhibited [3H]thymidine incorporation during perfusion. The addition of 13-HODE to the nordihydroguaiaretic acid-containing whole blood perfusate increased the rate of [3H]thymidine incorporation 10 times and nearly doubled tumor DNA content; the LA taken up is converted to 13-hydroxyoctadecadienoic acid (HODE), which fulfills an important role in viability and renewal in neoplastic cells in vitro. This mechanism through which dietary LA affects carcinogenesis and tumor growth is not yet clear. LA is an EFA that may be metabolized in the host body and tumor to arachidonic acid and cellular lipids and to lipid mediators derived from both LA and arachidonic acid. One or more of these agents, formed either by host and/or tumor tissues, could have a growth stimulative effect on initiated tumor cells and established tumors. Research in our laboratory has been aimed at defining the mechanism through which LA influences growth in hepatoma 7288CTC, a transplantable rat tumor that has an active growth response to increased dietary LA (5). Experiments performed in vivo and during perfusion in situ using tissue-isolated preparations of this tumor showed direct associations between dietary LA intake, host arterial blood LA concentrations, and tumor LA intake and growth rate in vivo (5). In perfused tumors the incorporation of [3H]thymidine into tumor DNA (6) was directly related to the ambient arterial plasma LA concentration and tumor LA uptake. Increased concentrations of plasma arachidonic acid were about one-fourth as effective as LA (6), and oleic acid, which stimulates tumor cell growth in vitro (7, 8), was ineffective. Hepatoma 7288CTC converted 1–5% of the LA removed from the arterial blood to 13-HODE in vivo and during perfusion in situ; formation and release of 13-HODE into the tumor venous blood was directly dependent on tumor LA uptake (5). Lesser amounts of 13-KODE and 9-HODE were also released, but no hydroxylated products of arachidonic acid metabolism were detected. These data suggested that 13-HODE formation is required for LA-dependent growth in hepatoma 7288CTC in vivo. In this study, we examined the role of LA uptake and 13-HODE release on growth in vivo and the effect of 13-HODE on [3H]thymidine incorporation during perfusion in situ. The results, which showed a requirement of 13-HODE for both activities, provide evidence that 13-HODE is the mitogenic factor in LA-dependent growth in hepatoma 7288CTC. This is the first demonstration of a direct mitogenic effect of 13-HODE in a solid tumor.

INTRODUCTION

In rodents, an increased dietary intake of LA acts as a promoter of chemical carcinogenesis in colon, pancreas, and breast and as a growth stimulant for transplanted tumors derived from these and other tissues (1). Growth of human breast (2) and prostate cancer (3, 4) xenografts in immunodeficient rodents is also stimulated by increased dietary LA intake, suggesting that this fatty acid fulfills an important role in viability and renewal in neoplastic cells in vitro. The mechanism through which dietary LA affects carcinogenesis and tumor growth is not yet clear. LA is an EFA that may be metabolized in the host body and tumor to arachidonic acid and cellular lipids and to lipid mediators derived from both LA and arachidonic acid. One or more of these agents, formed either by host and/or tumor tissues, could have a

MATERIALS AND METHODS

Materials. Ingredients used in preparation of the EFA-free diet were purchased from US Biochemical (Cleveland, OH). Methyl esters of rapeseed oil and fatty acid standards were purchased from Supelco (Bellevfante, PA). NDGA, boron trifluoride-methanol, 15-hydroxy-9-oxoprostanetioic acid, and general biochemical supplies were purchased from Sigma Chemical Co (St. Louis, MO). 9- and 13-HODE and 5-HETE (each as the racemic mixture) and 13-KODE were obtained from Cayman Chemicals (Ann Arbor, MI). [Methyl-3H]thymidine (6.7 Ci/mmol) was purchased from Research Products International (Mt. Prospect, IL). Heptane (HPLC grade), chloroform, methanol (HPLC grade), ethanol, and C18−PrepSep columns were products of Fisher Chemical Co.

Animals, Diets, and Tumor Implantation and Growth. Male Buffalo (BUF/NCR) rats (4–5 weeks of age) weighing 35–75 g were purchased from Charles River Laboratories (Kingston, NY). Male Sprague Dawley rats weighing 150–200 g were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Except for two rat groups described below, all animals were given free access to water and an EFA-replete diet (Prolab rat, mouse, hamster 1000 formula; Agway, Syracuse, NY). Duplicate determinations of six separate batches showed that this diet contained 4.1 g of total fatty acids/100 g diet; 1.3% myristic acid (14:0), 22.7% palmitic acid (16:0), 2.6% palmitoleic acid (16:1), 12.3% stearic acid (18:0), 33.1% oleic acid (18:1n9), 25.7% LA (C18:2n6), and 0.2% arachidonic acid (C20:4n6). Trace amounts of other unidentified fatty acids accounted for 2.1%. Two groups of Buffalo and Sprague Dawley rats were given free access to water and a semipurified, fat-free, EFA-deficient diet that contained 0.03 g of LA/100 g diet and small amounts of non-EFAs derived from the corn starch component of the diet (5). All rats were subjected to alternate 12-h periods of dark and light (6:00
a.m.–6:00 p.m.) throughout the experiments. There was no light contamination during the dark phase. Temperature and humidity were maintained at 23°C and 40–60%, respectively.

Tissue-isolated Morris hepatoma 7288CTC was implanted in male Buffalo rats on the tip of a vascular stalk formed from the superficial epigastric artery and vein, as described previously (9). The tumor implant and vascular stalk were enclosed within a parafilm envelope, placed in the inguinal fossa, and the skin incision was closed. Vascularization of the implant was limited to new vessel connections with the epigastric artery and vein; subsequent tumor growth was s.c. The latent period from implantation to first evidence of tumor growth was recorded, and subsequent tumor size was estimated every 2–3 days from measurements made through the skin (10). These data were converted to weights, as described previously (10), and growth rates (g/day) were calculated by linear regression. Tumor-bearing rats were treated with NDGA (10 μM) supplied in the drinking water.

Tumor Arteriovenous Difference Measurements in Vivo and during Perfusion In Situ. Arteriovenous difference measurements across tissue-isolated tumors in vivo were performed when the estimated tumor weight was 4–6 g. Experiments were scheduled between 8:00 a.m. and 10:30 a.m. after a normal nocturnal feeding period. Procedures for anesthesia and heparinization of the host rat, for surgical preparation of the tumor and for collection of arterial and tumor venous blood samples, were performed as described previously (5, 6, 9). Anesthetized host rats were breathing air unassisted, and body temperature was maintained at 37°C with a heating pad (below) and a heat lamp (above). Blood flow from the tumor vein, which typically ranged from 0.09–0.12 ml/min, was collected passively. Both arterial and tumor venous blood samples were collected into tubes chilled in ice. Separate samples were collected simultaneously for measurement of hemocrit and for analysis of blood gases and pH.

Detailed descriptions of the surgical and technical procedures for the perfusion of tissue-isolated tumors in situ were described previously (6, 11–13). Donor blood (about 50 ml) for perfusion was collected between 8:00 a.m. and 10:00 a.m. from heparinized adult male Sprague Dawley rats weighing 250–300 g and was filtered through cheesecloth and stored, under mineral oil, in a chilled, stirred reservoir packed in ice. The whole blood perfusate was pumped from the reservoir using a peristaltic pump and passed through a heat-exchanger that warmed the blood to 37°C and then an artificial lung that chilled, stirred reservoir packed in ice. The whole blood perfusate was pumped from the reservoir using a peristaltic pump and passed through a heat-exchanger that warmed the blood to 37°C and then an artificial lung that chilled, stirred reservoir packed in ice. The whole blood perfusate was pumped from the reservoir using a peristaltic pump and passed through a heat-exchanger that warmed the blood to 37°C and then an artificial lung that chilled, stirred reservoir packed in ice. The whole blood perfusate was pumped from the reservoir using a peristaltic pump and passed through a heat-exchanger that warmed the blood to 37°C and then an artificial lung that chilled, stirred reservoir packed in ice. The whole blood perfusate was pumped from the reservoir using a peristaltic pump and passed through a heat-exchanger that warmed the blood to 37°C. The eluent was monitored at 235 nm and 279 nm using an ISCO variable wavelength detector, and the peaks were integrated and quantified using the ISCO ChemResearch software program. The extinction coefficients used were: 9- and 13-HODE, 23,000; 13-KODE, 22,300; and 5-HETE, 27,000. Ultraviolet absorption spectra of samples and standards were recorded using a Hewlett-Packard (Palo Alto, CA) diode array spectrophotometer with methanol as solvent and blank.

Statistical Analysis. Results are expressed as mean ± SD. The effects of NDGA, 13-HODE, and 13-KODE alone and in combination on incorporation of [3H]thymidine into tumor DNA were examined by one-way ANOVA. Differences were considered to be significant at P < 0.05. When differences were detected, the means were compared using Student-Newman-Keuls multiple comparison test (16).

RESULTS

Fatty Acid Uptake, 13-HODE Release, and Growth of Hepatoma 7288CTC in Vivo: Effect of NDGA. A-V differences for total plasma lipid fatty acids and 13-HODE were measured across 12 tissue-isolated hepatomas 7288CTC growing in male Buffalo rats. All animals were given free access to laboratory chow and water and were subjected to a 12-h light:12-h dark photoperiod. Tumors in animal group 1 (n = 6) received no treatment. At the time of harvest (19 days after implantation), the mean tumor growth rate was 0.7 ± 0.1 g/day and weight was 5.6 ± 0.4 g. A-V differences measured across the tumors showed an uptake of total arterial plasma fatty acids and LA of 3.81 ± 1.4 and 0.73 ± 0.3 μg/min/g, respectively, and a release of 13-HODE (36 ± 5.7 ng/min/g) into the tumor venous blood equivalent to 4.6% of the LA used. Rats in group 2 (n = 6) were treated with NDGA (10 μM), a lipooxygenase inhibitor, that was added to the drinking water. The mean tumor growth rate in rat group 2 was 0.8 ± 0.1 g/day before NDGA treatment, and the estimated tumor weight at the time of treatment was 6.0 ± 0.4 g/day. NDGA caused a marked regression of tumor growth. During the 6 days of NDGA...
treatment the tumors regressed (mean growth rate $= -0.71 \pm 0.1$ g/day) to a final mean tumor weight of $1.8 \pm 0.6$ g at the time of harvest. Total fatty acid and LA uptakes were $4.6 \pm 1.5$ and $1.0 \pm 0.3$ $\mu g/min/g$, respectively, and there was no detectable release of 13-HODE, indicating that tumor fatty acid uptake was not influenced by the presence of NDGA or absence of 13-HODE. These data suggested that formation of 13-HODE from LA was the likely critical event in LA-dependent tumor growth and that uptake of LA itself was a necessary, but penultimate, step.

Fatty Acid Uptake and 13-HODE Release in Hepatoma 7288CTC during Perfusion in Situ: Effects of NDGA. The techniques for tumor perfusion in situ described in “Methods” were designed to reproduce the in vivo condition as closely as possible. Tumor-bearing and donor rats were given free access to laboratory chow and water, and all experiments were performed between 8:00 a.m. and 10:30 a.m. after a normal overnight feeding period. Perfusions typically lasted 120–150 min. Fig. 1 shows the steady-state rates of LA and total fatty acid uptake observed during perfusion in situ of a single hepatoma 7288CTC. Uptakes of total fatty acids and of LA alone were constant during the 150-min perfusion. Release of 13-HODE into the tumor venous blood was steady until the addition of NDGA. 13-HODE is unmeasureably low in arterial blood and is not generated in the perfusion apparatus in the absence of a tumor. The addition of the lipooxygenase inhibitor NDGA (10 $\mu M$) to the reservoir donor blood at 60 min abolished 13-HODE release but affected neither LA nor total fatty acid uptake. About 30 min were required for the NDGA-containing reservoir blood to pass through the 37°C water bath, artificial lung, and the tumor.

Effects of 13-HODE on $[^{3}H]$Thymidine Incorporation and DNA Content of Hepatoma 7288CTC Perfused in Situ. The role of 13-HODE in the incorporation of $[^{3}H]$thymidine into tumor DNA and on the DNA content of tumors perfused with EFA-replete arterial blood was examined in the presence (control perfusions) and the absence (perfusions containing NDGA) of endogenous 13-HODE formation. Examples of both of these conditions were shown in Fig. 1. $[^{3}H]$thymidine incorporation and DNA contents of control and NDGA-treated tumors grown in and perfused with donor blood from EFA-replete rats and the effects of exogenous 13-HODE, 13-KODE, or 9-HODE are shown in Fig. 2, A and B. Each experimental group contained three perfused tumors and is designated according to the type of treatment. Perfusions were for 2 h. Tumor uptake of total plasma fatty acids and LA from the arterial blood perfusates among the seven groups were not significantly different, were comparable with those measured in vivo in Buffalo rats given free access to laboratory chow (5, 15, 17), and were unaffected by the different treatments. The rate of 13-HODE release into the venous blood in the control group accounted for about 2% of LA uptake. Perfusion for 2 h with arterial blood containing exogenous 13-HODE increased the rate of $[^{3}H]$thymidine incorporation about 10 times and nearly doubled the DNA content ($P < 0.001$) the rate of release of 13-KODE (11.9 $\pm 2.0$ ng/min/g), no 13-KODE uptake; 13-HODE uptake (143.0 $\pm 12.0$ ng/min/g), no 13-KODE uptake; 13-HODE uptake and uptake (0.48 $\pm 0.06$ and 0.25 $\pm 0.05$ ng/min/g, respectively) 13-KODE supply and uptake (140.3 $\pm 2.0$ ng/min/g), no 13-KODE uptake; 13-HODE supply and uptake, (0.55 $\pm 0.05$ and 0.29 $\pm 0.04$ ng/min/g), 13-KODE supply (11.9 $\pm 2.0$ ng/min/g), no 13-KODE uptake, 13-HODE release (41.5 $\pm 3.1$ ng/min/g), no 13-KODE uptake, 13-HODE supply and uptake (0.48 $\pm 0.06$ and 0.25 $\pm 0.05$ ng/min/g, respectively) 13-KODE supply and uptake (140.3 $\pm 12.0$ ng/min/g), no 13-KODE uptake.

Fig. 1. Effect of NDGA on LA and total fatty acid uptake and 13-HODE release in hepatoma 7288CTC during perfusion in sit. Tumor weight was 5.4 g. Host and blood donor rats were fed an EFA-sufficient diet and were fasted for 24 h before the start of the experiment to increase blood lipid levels. Arterial blood flow was 0.13 ml/min. The mean rates of supply of total fatty acids and LA to the tumor were $30.2 \pm 0.8$, and $6.4 \pm 0.2$ $\mu g/min/g$, respectively. NDGA, sufficient to give a 10 $\mu g$ concentration in the whole blood perfusate, was added to the reservoir arterial blood at 60 min.

Fig. 2. Effect of 13-HODE, 13-KODE, 13-HODE + 13-KODE, NDGA, NDGA + 13-HODE, and NDGA + 9-HODE on tumor $[^{3}H]$thymidine incorporation (A) and DNA content (B) in hepatoma 7288CTC perfused in situ. Each bar represents the mean $\pm SD$ for three tumors perfused for 2 h under the treatments indicated. Rates of tumor supply and uptake of total fatty acids (19.1 $\pm 1.3$ and 7.1 $\pm 0.9$ ng/min/g, respectively) and LA (5.0 $\pm 0.4$ and 2.0 $\pm 0.3$ ng/min/g, respectively) were not different among the seven groups (n = 21). Results for 13-HODE and 13-KODE releases, supplies, and uptakes for individual tumors were 13-HODE uptake (143.0 $\pm 12.0$ ng/min/g), no 13-HODE uptake; 13-HODE release (30.7 $\pm 1.9$ and 6.7 $\pm 2.7$ ng/min/g); 13-HODE supply and uptake (1.3 $\pm 0.6$ and 1.0 $\pm 0.2$ ng/min/g, respectively) 13-KODE supply and uptake (142.3 $\pm 14.2$ ng/min/g), no 13-KODE uptake; 13-KODE supply and uptake (0.48 $\pm 0.06$ and 0.25 $\pm 0.05$ ng/min/g, respectively) 13-KODE supply and uptake (140.3 $\pm 12.0$ ng/min/g), no 13-KODE uptake; 13-KODE supply and uptake, (0.35 $\pm 0.05$ and 0.29 $\pm 0.04$ ng/min/g), 13-KODE supply (11.9 $\pm 2.0$ ng/min/g), no 13-KODE uptake, 13-HODE supply and uptake (0.48 $\pm 0.05$ and 0.1 $\pm 0.05$ ng/min/g). A, asterisks indicate statistical differences from control, 13-KODE, NDGA, and NDGA + 9-HODE ($P < 0.001$). B, bars with different asterisk designations are statistically different ($P < 0.001$).
Because EFAD tumors contain only small amounts of LA and the LA concentration in EFAD donor blood is nearly undetectable (6), these data provide strong evidence for the direct mitogenic effect of 13-HODE.

Fig. 3A shows that the rates of \(^{3}H\)thymidine incorporation (19.5 ± 4.1 dpm/µg DNA) in untreated EFAD tumors (absence of endogenous LA) were identical to those observed in NDGA-treated tumors grown in rats fed an EFA-replete diet (21.2 ± 3.2 dpm/µg DNA; Fig. 2A). Therefore, an inhibition of 13-HODE formation resulting either from an absence of LA in the arterial blood or from treatment of the tumor with a lipoxygenase inhibitor decreased \(^{3}H\)thymidine incorporation to the same extent. LA was present during the 2 h of perfusion in the NDGA-treated tumors, but 13-HODE was not, indicating that 13-HODE not LA itself is required for tumor growth in vivo.

DISCUSSION

The results reported here provide strong evidence that the mitogenic factor responsible for LA-dependent growth in hepatoma 7288CTC is 13-HODE, a product of tumor lipoxygenase activity. Formation of 13-HODE was a critical event in tumor growth; LA itself, 13-KODE (a metabolite of 13-HODE) and 9-HODE were inactive. The data shown in Fig. 2 and the dose-response curves shown in Fig. 3A and B, provide a biochemical explanation for the relationships among increased LA uptake, 13-HODE release into the tumor venous blood (5), \(^{3}H\)thymidine incorporation and DNA content during perfusion in situ (6), and increased growth of hepatoma 7288CTC in vivo (5). The time required for the response in \(^{3}H\)thymidine incorporation to exogenous 13-HODE was surprisingly brief; both NDGA-treated and LA-deficient tumors showed a robust increase in the rate of \(^{3}H\)thymidine incorporation following a 2-h perfusion with 13-HODE. This fast response time is in good agreement with earlier data showing that perfused tumors exposed to a new, higher steady-state concentration of LA in arterial blood shifted to a new faster rate of \(^{3}H\)thymidine incorporation after about 3 h of perfusion (12). Similarly, perfused tumors exposed to a new, but lower steady-state arterial blood LA concentration required about 3 h of perfusion before the new, slower rate of \(^{3}H\)thymidine incorporation was established. These 3-h periods included time for changes in LA availability, lipoxygenase activity, and intracellular 13-HODE concentrations. Half-times for activation (or deactivation) of 1–1.5 h for DNA synthesis in a solid tumor in vivo are short and undoubtedly explain why increases in \(^{3}H\)thymidine incorporation and DNA content were discernible in vivo a few hours after the plasma lipid concentrations were increased by an acute fast (17) or induction of acute streptozotocin-induced diabetes (18). Considering the complex steps, many of which are unknown, between LA uptake at the cell membrane, 13-HODE production in the cytoplasm, and DNA synthesis in the nucleus, the pathway for LA-dependent growth in hepatoma 7288CTC (and presumably for LA-dependent growth in other rodent and human tumors) is highly efficient, developed, and controlled (see Ref. 19).

Several recent in vitro studies have shown that lipoxygenase-derived metabolites of LA are important lipid mediators in signaling pathways for cell proliferation. Glasgow and Eling (20) and Cowlen and Eling (21) have reported that 13-HODE is a potent stimulator of EGF-dependent mitogenesis in BALB/c 3T3 fibroblasts (20) and Syrian hamster embryo fibroblasts (21). EGF also stimulated the metabolism of LA to 13-HODE in these cell lines. The active LA metabolites formed by lipoxygenase activity in syrian hamster embryo fibroblasts were identified as the (S) enantiomers of 13-HPODE and 13-HODE (22). The (13R)-enantiomers were inactive, as were the

13-HODE, which occurred in the presence of NDGA, reestablished the rate of \(^{3}H\)thymidine incorporation and DNA content. 9-HODE, a metabolite of LA present in tumor venous blood in trace amounts (5), was ineffective in reversing the inhibitory effects of NDGA.

Dose-Response Effects of 13-HODE on \(^{3}H\)Thymidine Incorporation and DNA Content in Tumors Grown in EFAD Rats.

13-HODE is not released from tumors grown in EFAD rats. The growth rate of hepatoma 7288CTC in vivo in EFAD rats is limited by inadequate amounts of EFA, mainly LA (5, 6), which severely limits tumor 13-HODE release (5). Perfusion of EFAD tumors in situ with whole blood perfusates from donor EFAD rats, which contained increasing concentrations of exogenous LA, caused dose-dependent increases in the rates of incorporation of \(^{3}H\)thymidine into tumor DNA and in DNA content (6). \(^{3}H\)Thymidine incorporation and the DNA content of tumors grown in EFAD rats that were exposed to increased concentrations of exogenous 13-HODE during the perfusion also responded with increased rates of 13-HODE uptake (Fig. 3A) and \(^{3}H\)thymidine incorporation (Fig. 3A) and DNA contents (Fig. 3B).
arachidonate metabolites, (15S)-hydroperoxyeicosatetraenoic acid, and (15S)-HETE (22). Lipoxygenase inhibitors 5,8,11,14-eicosatetraenoic acid and NGDA effectively blocked mitogenesis and the conversion of LA to (13S)-HPODE and (13S)-HODE (20, 21). Reddy et al. (23) reported that [3H]thymidine incorporation in human breast carcinoma BT-20 cells in culture was increased by either transforming growth factors or EGF. Both of these growth factors increased production of 13-HODE from added LA, and NGDA blocked 13-HODE formation and [3H]thymidine incorporation. Arachidonic acid metabolites of prostaglandin H synthetase were formed in low amounts; 13-HODE formation was not affected by the addition of indomethacin. The effect of 13-HODE on [3H]thymidine incorporation in BT-20 cells in the presence of NGDA was not described (23). In a recent study, Glasgow et al. (24) showed that (13S)-HPODE and (13S)HODE increased EGF-dependent mitogenesis and up-regulated EGF-dependent tyrosine phosphorylation by inhibiting the dephosphorylation of EGF receptor.

As judged from comparisons of results from the fibroblast and human BT-20 cell lines used in the in vitro experiments described above and results shown in Figs. 1–3, it seems possible that 13-HODE production in hepatoma 7288CTC serves to augment EGF-dependent mitogenesis. EGF receptor is frequently overexpressed in animal and human neoplastic cells (25), and EGF-stimulated DNA synthesis in primary cultures of human and rat hepatocytes (26) and in two human hepatoma cell lines (27). However, 13(S)-HODE has also been reported to have additional activities. The activation of protein kinase C-α mediated by 12(S)-HETE in murine B16 melanomata cells (28) and activation of protein kinase C in mouse epidermis (29) are inhibited by (13S)-HODE. Both 9- and 13-HODE were identified as components of oxidized low-density lipoprotein in young and advanced human atherosclerotic lesions (30) and were shown recently to be endogenous activators and ligands for peroxisome proliferator-activated receptor-γ in macrophage foam cells (31).

A research goal of this laboratory is to understand the mechanisms through which hormonal, nutritional, and environmental factors control LA-dependent tumor growth. We believe that experiments performed with solid rodent and human (grown in immunodeficient rodents) tumors in vivo or during perfusion in situ will provide biochemical and physiological insights that cannot be duplicated in vitro. Recently, we demonstrated that exposure of tumor-bearing rats to constant light or to low levels of light contamination during the dark phase decreased secretion of the pineal hormone melatonin. Animals Sci., 56: 678–681, 1986.


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