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Biochemical Pharmacology 61 (2001) 1455–1462

Biochemical Pharmacology

Commentary

Polyunsaturated fatty acids, melatonin, and cancer prevention

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Abstract

Many nutritional, hormonal, and environmental factors affect carcinogenesis and growth of established tumors in rodents. In some cases, these factors may either enhance or attenuate the neoplastic process. Recent experiments performed in our laboratory using tissue-isolated rat hepatoma 7288CTC *in vivo* or during perfusion *in situ* have demonstrated new interactions among four of these factors. Two agents, dietary linoleic acid (C18:2n6) and "light at night," enhanced tumor growth, and two others, melatonin and n3 fatty acids, attenuated growth. Linoleic acid stimulated tumor growth because it is converted by hepatoma 7288CTC to the mitogen, 13-hydroxyoctadecadienoic acid (13-HODE). Melatonin, the neurohormone synthesized and secreted at night by the pineal gland, and dietary n3 fatty acids are potent antitumor agents. Both inhibited tumor linoleic acid uptake and 13-HODE formation. Artificial light, specifically "light at night," increased tumor growth because it suppressed melatonin synthesis and enhanced 13-HODE formation. Melatonin and n3 fatty acids acted via similar or identical G_i protein-coupled signal transduction pathways, except that melatonin receptors and putative n3 fatty acid receptors were used. The results link the four factors in a common mechanism and provide new insights into the roles of dietary n6 and n3 polyunsaturated fatty acid intake, "light at night," and melatonin in cancer prevention in humans.

Keywords: Linoleic acid; n3 Fatty acids; Melatonin; Tumor growth; Signal transduction; Cancer prevention

1. Introduction

Naturally occurring polyunsaturated fatty acids of the n6 and n3 types, the neurohormone melatonin, and diurnal light are known to have important functions in normal health, growth, and reproduction in laboratory animals and humans. Both laboratory and clinical studies have shown that these dietary, hormonal, and environmental factors also have roles in cancer promotion, progression, and cachexia. Several mechanisms have been proposed to explain these functions. In this commentary, we discuss recent experimental evidence that connects these four factors in a single new mechanism.

2. Polyunsaturated fatty acids

Linoleic acid (C18:2n6), an n6 polyunsaturated fatty acid, is abundant in the Western diet. It is the major fatty acid in safflower, sunflower, corn, soy bean, and cottonseed oils, accounting for greater than 50% of the total fatty acid content in these oils [1]. Although it is an essential fatty acid, consumption of modest amounts, equivalent to 1% of total calories, is adequate to protect against essential fatty acid deficiency [2]. Since the linoleic acid content of olive oil is 10.5%, male tumor-bearing Buffalo rats consuming 18 g/day of a 10% olive oil diet (17.3 kJ/g) ingested 0.13 g linoleic acid/day [3]. Increase in body weight was normal, and an established, implanted tissue-isolated hepatoma, 7288CTC, grew at a rate of 1.1 g/day. Diets containing 10% fat from mixtures of olive and corn oils showed incremental changes in host arterial blood plasma linoleic acid concentrations and increased rates of tumor linoleic acid uptake, 13-HODE formation, and tumor growth. The growth rate of hepatoma 7288CTC in rats fed a 10% corn oil diet was 2 g/day, 100% greater than, and significantly different from, tumor growth in rats fed the 10% olive oil diet [3]. The linoleic acid content of corn oil is 60%. In two solid murine transplantable tumors, the addition of 0.1% trilinolein to a fat-free diet increased tumor growth; added purified arachi-

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Abbreviations: 13-HODE, 13-hydroxyoctadecadienoic acid; NDGA, nordihydroguaiaretic acid; EGF, epidermal growth factor; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; 8-Br-cAMP, 8-bromo-cyclic adenosine monophosphate; FATP, fatty acid transport protein; cAMP, cyclic adenosine monophosphate; TGFα, tumor growth factor alpha; MAPK, mitogen-activated protein kinase; and FAT, fatty acid translocase.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00634-7

donic acid did not affect tumor growth [4]. Diets containing increased levels of linoleic acid also promoted faster growth rates in human breast [5, 6] and prostate [7] cancer xenografts in nude mice. In tissue-isolated hepatoma 7288CTC grown in essential fatty acid-deficient rats and perfused *in situ* with essential fatty acid-free blood containing added purified linoleic acid, maximal rates of [^{3}H]thymidine incorporation were observed when the plasma free linoleic acid concentration was 0.4 to 0.5 mM. Added purified arachidonic acid was only about one-third as effective [8]. Arterial blood plasma non-esterified linoleic acid concentrations of 0.4 to 0.5 mM are within the range observed in healthy, normal humans [9].

Tumor incidence in chemically induced carcinogenesis in rat pancreas [10], mammary gland [11], and colon [12] also showed a dose–response relationship with dietary linoleic acid content. A plateau in tumor incidence was suggested at dietary linoleic acid contents (w/w) greater than 4 to 8.5% in pancreas [10], 4% in breast [11], and 0.6% in colon [12]. Quantitative analyses of plasma free or total linoleic or arachidonic acid concentrations were not reported in these studies. In hepatoma 7288CTC-bearing Buffalo rats fed a diet containing 4.5% linoleic acid (w/w), the mean 24-hr arterial blood plasma free linoleic acid concentration was about 0.5 mM [3]. Thus, the estimated arterial blood plasma linoleic acid concentration at the inflection point in the incidence of pancreas [10] and breast tumors [11] was probably very close to 0.5 mM, the plasma free linoleic acid concentration at the inflection point for maximal rates of [^{3}H]thymidine incorporation in hepatoma 7288CTC [8]. Studies that tested the effects of different amounts of linoleic acid-containing dietary oils on tumorigenesis and growth of established rodent tumors or human cancer xenografts were unable to exclude arachidonic acid as the fatty acid required for active tumor growth. Despite this, these studies provided strong *in vivo* evidence for saturable dose–response relationships in: dietary linoleic acid intake; plasma linoleic and arachidonic acid concentrations; tumor promotion in rodent pancreas, mammary gland, and colon; and growth of hepatoma 7288CTC and human breast and prostate cancer xenografts.

Of the linoleic acid taken up by hepatoma 7288CTC *in vivo* and during perfusion *in situ*, 1–10% was converted to 13-HODE by lipoxygenase activity [3, 13]. [^{14}C]Linoleic acid added to the arterial blood was recovered as [^{14}C]13-HODE in the tumor venous blood [3]. Addition of a lipoxygenase inhibitor, NDGA, to the drinking water inhibited the formation of 13-HODE in the tumor and caused regression of growth *in vivo* but did not change the rate of tumor linoleic acid uptake. Addition of NDGA to the arterial blood during perfusion of hepatoma 7288CTC *in situ* did not affect tumor linoleic acid uptake but inhibited the formation of 13-HODE and decreased [^{3}H]thymidine incorporation by the tumor [13]. Most importantly, addition of 13-HODE to arterial blood containing NDGA restored [^{3}H]thymidine incorporation to pre-NDGA levels (Table 1). Although it has not been determined in hepatoma 7288CTC, evidence from other cell types indicates that 13-HODE augments the mitogenic effects of EGF [14]. We concluded from these experiments that neither arachidonic acid nor linoleic acid, itself, is an important mitogen. Rather, linoleic acid serves as a substrate and 13-HODE, the product of tumor lipoxygenase activity, is the mitogen responsible for linoleic acid-dependent tumor growth [13].

Table 1
Effects of NDGA, melatonin, and EPA on [^{3}H]thymidine incorporation in hepatoma 7288CTC

Treatment	[^{3}H]Thymidine incorporation (dpm/μg tumor DNA)
Control[a,b]	42 ± 5
NDGA (10 μM)[a]	21 ± 3
NDGA + 13-HODE[a,c]	570 ± 200
Control[b,d]	42 ± 5
Melatonin (1 nM)[d]	24 ± 3
Melatonin + 13-HODE[c,d]	470 ± 31
Control[e,f]	416 ± 89
EPA (0.45 ± 0.02 mM)[e]	23 ± 5
EPA + 13-HODE[c,e]	563 ± 77

Tumors were perfused *in situ* for 150 min in the absence (control) or presence of the agents indicated. The EPA concentration is plasma free fatty acid. [^{3}H]Thymidine (2 μCi/g estimated tumor weight) was injected into the arterial blood catheter leading to the tumor 20 min before the end of the perfusion. The [^{3}H]thymidine made one pass through the tumor. Values are means ± SD for 3 perfusions.

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[b] Tumors and donor arterial blood were obtained from fed rats.

[c] The mean 13-HODE concentration in the perfusions was 0.43 ± 0.2 μM.

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[e] Reprinted with permission from Cancer Res 2000;60:5289–95 [Copyright (2000) American Association for Cancer Research, Inc. (Ref. 22)].

[f] Tumors and donor arterial blood were obtained from fasted rats.

The tumor growth-promoting action of dietary linoleic acid contrasts with the effect of dietary n3 polyunsaturated fatty acids. In rodents, ingestion of diets containing n3 fatty acids from plant oils, predominantly α-linoleic acid (C18:3n3), and marine fish oils, predominantly EPA (C20:5n3) and DHA (C22:6n3), consistently inhibited the growth of transplantable rodent mammary gland tumors [15] and human breast cancer xenografts [16, 17] and carcinogenesis in rat colon [18] and mammary gland [19]. α-Linolenic acid differs from linoleic acid by only an additional double bond in the n3 position, and it has been difficult to relate this apparent small structural difference to the large functional differences between n6 and n3 fatty acids. In 1992, we showed that α-linolenic acid and EPA inhibited both linoleic acid uptake and [^{3}H]thymidine incorporation in hepatoma 7288CTC perfused *in situ.* At an arterial blood plasma free linoleic acid concentration of 0.5 mM, the effects of the n3 fatty acids were clearly dose-dependent. K_i values for inhibition by α-linolenic acid of linoleic acid uptake and

[^{3}H]thymidine incorporation were 0.18 and 0.25 mM, respectively [8]. The results suggested that n3 fatty acids competed with other plasma fatty acids for uptake. However, mechanisms for fatty acid transport into cells were controversial [20, 21], and no clear experimental approach seemed possible in a solid tumor. Recent experiments now provide evidence that specific fatty acid transporters are present in hepatoma 7288CTC. As shown in Fig. 1A, addition of EPA inhibited uptake of fatty acids and formation of 13-HODE during perfusion *in situ*, and the inhibition was reversed by the addition of 8-Br-cAMP to the arterial blood [22]. Treatment with α-linolenic acid resulted in identical inhibitions of tumor fatty acid uptake and 13-HODE release *in vivo* and during perfusion *in situ.* Pertussis toxin or forskolin also reversed the inhibitory effects of n3 fatty acids [22]. Although the rates of n3 fatty acid uptake were low, they were not affected by either the complete block in uptake of plasma saturated, monounsaturated, and n6 polyunsaturated fatty acids or relief of this block by 8-Br-cAMP (Fig. 1A). This finding suggested that uptake of n3 polyunsaturated fatty acids occurred via a fatty acid transporter different from the carrier required for transport of plasma saturated and mono- and n6 polyunsaturated fatty acids. mRNA levels for FATP [23] measured in hepatoma 7288CTC were overexpressed relative to mRNA levels in normal Buffalo rat liver [24].

The inhibition of [^{3}H]thymidine incorporation into the DNA of hepatoma 7288CTC induced by EPA [8, 22] was reversed by the addition of 13-HODE to the arterial blood (Table 1). 13-HODE had no effect on tumor fatty acid uptake. Addition of pertussis toxin, forskolin, or 8-Br-cAMP to the arterial blood [22], however, effectively reversed the inhibitions of fatty acid uptake, 13-HODE formation, and [^{3}H]thymidine incorporation. Preliminary experiments using MCF-7 human breast cancer xenografts perfused *in situ* in nude rats also showed that EPA inhibited fatty acid uptake, 13-HODE release, and the incorporation of [^{3}H]thymidine in tumor DNA [unpublished results]. As in hepatoma 7288CTC, these inhibitions were reversed by pertussis toxin, forskolin, and 8-Br-cAMP. 13-HODE restored the rate of [^{3}H]thymidine incorporation but did not affect fatty acid uptake in MCF-7 xenografts. Thus, uptake of saturated, monounsaturated, and n6 polyunsaturated fatty acids from host arterial blood in hepatoma 7288CTC and MCF-7 xenografts required cAMP. n3 Fatty acids inhibited fatty acid uptake via a G_i protein-coupled signal transduction pathway that attenuated cAMP formation. Tumor uptake of linoleic acid was necessary for the formation of 13-HODE, the mitogen required for linoleic acid-dependent growth in rat hepatoma 7288CTC and MCF-7 human breast cancer xenografts.

3. Melatonin and “light at night”

The neurohormone melatonin is synthesized in the pineal gland from the amino acid tryptophan [25]. Synthesis and release of melatonin into the blood exhibit a prominent circadian rhythm, occurring during darkness, and depending upon regular periods of light and total darkness. Retinal photoreceptors sensitive to specific wavelengths of visible light entrain an endogenous clock in the suprachiasmatic nucleus of the hypothalamus [26, 27]. This oscillating biological clock drives the circadian rhythms of the body [27]. From the suprachiasmatic nucleus, neural information about light is transmitted, via a complex multisynaptic pathway, to the pineal gland for regulation of melatonin synthesis [25]. An increase in the length of the dark phase prolongs the duration of melatonin synthesis and secretion. On the other hand, light introduced during the dark phase suppresses melatonin production [28]. The blue-green portion of the visible spectrum has the greatest suppressive effect [29], and exposure to a low light intensity, equivalent to that occurring during twilight, is sufficient to suppress melatonin secretion in rats [30] and humans [31].

Melatonin has an important role in normal and abnormal physiological processes, including regulation of circadian rhythms in metabolism in organs and cells and circadian stage-dependent inhibition [32] of chemically induced tumorigenesis in rats [33, 34] and growth of rat hepatoma 7288CTC [24]. A normal circadian rhythm in plasma melatonin concentrations in systemic blood [24] was observed in Buffalo rats bearing tissue-isolated hepatoma 7288CTC exposed to alternate 12 hr of bright light and total darkness. Associated with the melatonin circadian rhythm were remarkable metabolic changes in the tumor. Active tumor fatty acid uptake and 13-HODE release occurred during the day and were inhibited during the night. The periods of suppressed and active fatty acid uptake and 13-HODE formation coincided exactly and directly with the circadian rhythm of peaks and troughs in the plasma melatonin concentration. Exposure of the tumor-bearing rats to either constant light [24, 35] or light contamination during the dark period [35] suppressed melatonin secretion, increased tumor growth, and increased linoleic acid uptake and 13-HODE formation *in vivo.*

In hepatoma 7288CTC perfused *in situ*, the addition of 1 nM melatonin to the arterial blood inhibited tumor fatty acid uptake and the release of 13-HODE into the venous blood (Fig. 1B). The perfusion was performed with whole blood collected between 8:00 and 9:00 a.m. from fasted donor rats; melatonin is low to undetectable in blood collected in the morning [24, 35] and fasting increased the concentration of plasma lipids. This response was identical to that observed *in vivo* [24], except that the onset of the *in vivo* tumor response, which occurred in the evening, was gradual, reflecting the gradual increase in endogenous melatonin concentration. Addition of S20928 [*N*-(2-(1-naphthyl)ethyl)cyclobutanecarboxamide], a melatonin receptor antagonist [36], reversed the inhibition by melatonin, providing strong evidence that the tumor response *in vivo* resulted from changes in endogenous melatonin secretion. Addition of pertussis toxin, forskolin, or 8-Br-cAMP was as effective as

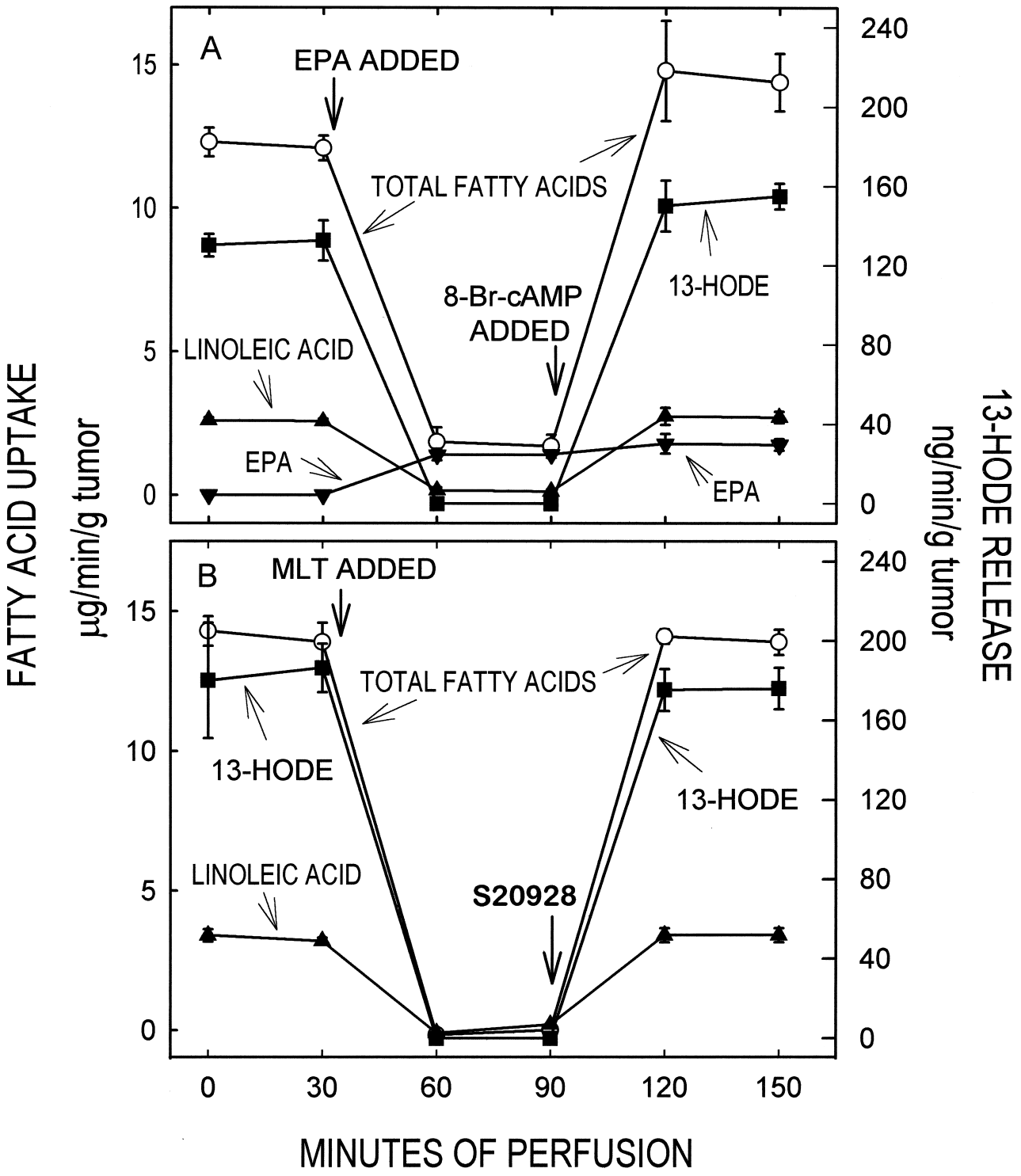

Fig. 1. Effects of EPA and 8-Br-cAMP (A) and melatonin and S20928 (B) on fatty acid uptake and 13-HODE release in hepatoma 7288CTC perfused *in situ*. Arterial and tumor venous blood samples were collected at zero time and at 30-min intervals. Each point represents the mean ± SD for three perfusions. Host and blood donor rats were fasted for 48 hr to increase blood lipid levels. Tumor blood flow was 0.11 to 0.13 mL/min. (A) EPA (0.68 ± 0.02 mM in arterial blood plasma) was added at 36 min, and 8-Br-cAMP (10 μM) was added at 96 min. Mean tumor weight was 5.8 ± 0.4 g. Reprinted with permission from Cancer Res 2000;60:5289–95 [Copyright [2000] American Association for Cancer Research, Inc. (Ref. 22)]. (B) Melatonin (MLT, 1 nM) was added at 36 min, and S20928 (1 nM) was added at 96 min. Mean tumor weight was 5.9 ± 0.6 g. Reprinted with permission from Cancer Res 1999;59:4693–701 [Copyright (1999) American Association for Cancer Research, Inc. (Ref. 24)].

S20928 in restoring tumor fatty acid uptake and 13-HODE formation to pre-melatonin levels [24]. S20928 had no effect on the inhibition of tumor fatty acid uptake caused by EPA [22]. Melatonin also inhibited the incorporation of [^{3}H]thymidine in hepatoma 7288CTC during perfusion *in situ* (Table 1). However, 13-HODE, in the presence of melatonin, restored tumor [^{3}H]thymidine incorporation to control levels but did not restore fatty acid uptake. Identical effects of melatonin on tumor fatty acid uptake, 13-HODE formation, and [^{3}H]thymidine incorporation were observed in MCF-7 human breast cancer xenografts perfused *in situ* [unpublished results]. We concluded from these results that melatonin and n3 polyunsaturated fatty acids act to inhibit growth of hepatoma 7288CTC and MCF-7 human breast cancer xenografts via very similar or identical G_i protein signal transduction pathways, except that the melatonin receptors and the putative n3 polyunsaturated fatty acid receptors are different.

EPA and α-linolenic acid [22] and melatonin [37] were also found to inhibit both the entry and exit of fatty acids in inguinal fat pads *in vivo* and during perfusion *in situ.* Fatty acid transport during the lipolysis induced by fasting and during fatty acid uptake induced by feeding was inhibited by EPA and melatonin; the rates of fatty acid release and uptake were restored by 8-Br-cAMP, forskolin, or pertussis toxin. The inhibitory effects of melatonin were reversed by S20928, but S20928 did not affect the inhibition due to EPA. These findings raised an important question: are the protective, anticachectic effects of EPA [38] and melatonin [39] on host white adipose tissue fat stores dependent upon inhibition of fatty acid transport? Cancer cachexia, the debilitating loss of appetite and protein and fat stores that may occur in rodents and humans with cancer, has been implicated as the immediate cause of death in many cancer patients [40]. Tisdale and coworkers [38, 41, 42] have shown that EPA reduced the rate of tumor growth and inhibited the loss of fat stores in the murine MAC 16 colon cancer model [41]. Lipolysis in epididymal adipocytes isolated from MAC 16-bearing mice was stimulated *in vitro* by a tumor-produced lipid-mobilizing factor [41] purified from the MAC 16 colon carcinoma. This lipolytic effect of the factor was attenuated by EPA in a pertussis toxin-sensitive pathway that inhibited adenylyl cyclase activity and decreased the intracellular cAMP concentration [42]; a G_i protein-coupled signal transduction pathway mediated by a putative n3 fatty acid receptor was proposed [42].

4. Discussion

Two schematic diagrams that depict the pathways for linoleic acid activation and n3 fatty acid- and melatonin-dependent suppression of tumor growth are shown in Fig. 2. The signaling pathways proposed were based upon results from studies of rodent tumors and human cancer xenografts in immunodeficient rodents and from other pertinent literature. Maximal rates of tumor growth (upper diagram, solid arrows) were predicted to occur in animals that were either pinealectomized or exposed to constant light [24] and fed a diet high in linoleic acid [3]. EGF present in host arterial blood maintained EGF-dependent mitogenesis. Entry of plasma saturated, monounsaturated, and n6 polyunsaturated fatty acids into the tumor cells was facilitated by FATP. A portion of the linoleic acid that entered was converted to 13-HODE by a 15-lipoxygenase. The only constraints on the rate of tumor growth were the rates of linoleic acid supply and uptake and 13-HODE formation because plasma melatonin and n3 fatty acid concentrations were absent or very low. The melatonin receptors and the putative n3 (omega-3) fatty acid receptors and transporters were unoccupied. The lower diagram in Fig. 2 depicts the signaling pathways (dashed arrows) for tumor growth suppressed by diurnal light and ingestion of dietary n3 fatty acids. Melatonin, which was secreted in darkness, reached a peak plasma concentration about 1:00 to 2:00 a.m. In rats ingesting n3 fatty acids, the circulating plasma concentrations were present continually, but the highest concentration occurred following ingestion. Melatonin and n3 fatty acids occupied the ligand sites exposed on the membrane melatonin receptors [43] and putative n3 fatty acid receptors [42], respectively. Plasma n3 fatty acids entered the tumor cell via putative specific transporters. The interactions of each ligand with its receptor released inhibitory α_i subunits from the associated G_i proteins, adenylyl cyclase activity was inhibited, and the intracellular cAMP level was decreased [42, 43]. Entry of plasma saturated, mono-, and n6 polyunsaturated fatty acids via FATP was reduced or abolished by the decreased cAMP concentration, and 13-HODE formation decreased because linoleic acid was less available to the lipoxygenase. Entry of the n3 fatty acids was unaffected and continued. The extent of the control of tumor growth by melatonin and n3 fatty acids depended upon the robustness of the circadian rhythm for melatonin secretion and the n3 fatty acid concentrations in the diet and arterial blood.

Portions of the proposed signaling pathways depicted in Fig. 2 are supported by experimental evidence from cell lines and solid tumors. 13-HODE is known to have an important role in sustaining EGF-dependent mitogenesis in murine fibroblasts and Syrian hamster embryo cells *in vitro* [see Ref. 44 for review]. EGF stimulated the formation of 13-HODE in both cell lines, and inhibition of 13-HODE formation attenuated mitogenesis. EGF and TGFα stimulated DNA synthesis and the conversion of linoleic acid to 13-HODE in BT-20 human breast carcinoma cells *in vitro* [45]. BT-20 cells overexpress the EGF receptor and the erbB-2 oncogene product; the lipoxygenase inhibitor NDGA blocked 13-HODE formation and EGF- and TGFα-dependent DNA synthesis. Because tyrosine phosphorylation of the EGF receptor increased 13-HODE formation, a close association between the EGF receptor and the 15-lipoxygenase was proposed [14]. Most importantly, 13-

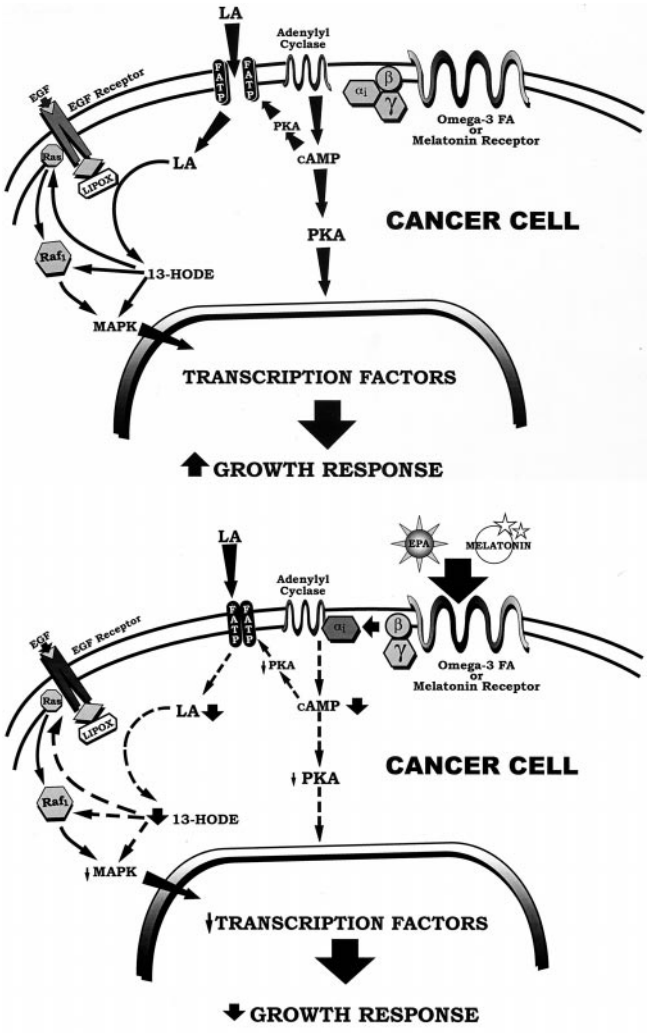

Fig. 2. Provisional diagrams for the proposed signal transduction pathways that mediate control by n3 fatty acids and melatonin of fatty acid uptake and linoleic acid-dependent growth in hepatoma 7288CTC. Growth stimulation is shown in the upper diagram and growth inhibition by n3 fatty acids and melatonin in the lower diagram. See text for discussion.

HODE altered the balance between kinase and phosphatase activities to favor a tyrosine phosphorylation state in the EGF pathway from the EGF receptor to MAPK activity [14]. Consequently, a decrease in intracellular 13-HODE shifted the balance between kinase and phosphatase activities toward dephosphorylation of the EGF receptor, deactivation of the downstream MAPK pathway, and decreased rates of cell proliferation. Thus, 13-HODE appears to be an important mitogen in several rodent cell lines *in vitro,* in human breast cancer cell lines *in vitro,* and in rat hepatoma 7288CTC in Buffalo rats and MCF-7 human breast cancer xenografts in nude rats. However, the mitogenic effects of 13-HODE may be tissue- or organ-specific; 13-HODE has been reported to have an antiproliferative effect in skin [46, 47] and human colon carcinoma cell lines [48].

The binding of melatonin to melatonin receptors is known to inhibit cAMP formation via G_i protein-coupled signal transduction pathways [43]. The sensitivities of melatonin inhibition of fatty acid transport to S20928, pertussis toxin, forskolin, or 8-Br-cAMP in hepatoma 7288CTC [24] and in MCF-7 human breast cancer xenografts indicate the presence of a melatonin receptor-mediated, G_i protein-coupled pathway in these tumors. While there is evidence that n3 fatty acids mediate a G_i-coupled pathway for fatty acid transport in hepatoma 7288CTC and inguinal fat pads in Buffalo rats [22] and during lipolysis in isolated murine adipocytes [42], there is as yet no evidence for specific n3 fatty acid receptors.

A significant gap in the understanding of the pathways shown in Fig. 2 is the absence of information on the mechanism of fatty acid transport in tumors. Fatty acids may enter cells by simple diffusion [20], but evidence is growing that specific transporters are necessary [21]. Presently, three fatty acid transporters have been identified and characterized [49]. Two are integral membrane proteins: FAT, the rat homolog of human CD36 [50], and FATP [23]. The plasma membrane-bound fatty acid binding protein is a peripheral membrane protein similar or identical to mitochondrial aspartate aminotransferase [49, 51]. Each of these proteins increases fatty acid uptake when expressed in various cell lines [23, 50, 51]. FATP is a member of a family of 5–6 related isoforms represented among several tissues within a species and with homologues in different species [52]. FATP is highly expressed in tissues with high rates of fatty acid oxidation and metabolism, such as heart, adipose tissue, and skeletal muscle [52]. Relative to normal liver, FATP is overexpressed in hepatoma 7288CTC [24] and presumably is the major fatty acid transport protein in this tumor. The murine FATP gene has an insulin response element, and expression of FATP mRNA levels in 3T3-L1 adipocytes is down-regulated by insulin and up-regulated by nutrient depletion [53]. A recent report indicates that short-term regulation of fatty acid transport by FAT in rat muscle is controlled by cellular redistribution among endosomal and cell membrane locations [54]. At the present time, the kinetic mechanism through which the melatonin- and n3 fatty acid-induced changes in intracellular cAMP concentrations alter fatty acid transport activity in hepatoma 7288CTC and inguinal fat pads in Buffalo rats and MCF-7 human breast cancer xenografts in nude rats is unknown.

A number of studies have proposed mechanisms to explain the growth-inhibitory effects of melatonin and n3 fatty acids on tumorigenesis and the growth of established tumors. Investigations in chemically induced carcinogenesis indicated that melatonin inhibited, and pinealectomy enhanced, the carcinogenic process [34, 55]. Experiments performed *in vitro* suggested that a major action of melatonin was to block the growth stimulatory effects of hormones and growth factors [34, 56, 57]. Proposed mechanisms for the tumor growth-inhibitory effects of n3 fatty acids have included inhibition of growth-promoting prostaglandins [15, 16, 58], alteration in the ratio of n6/n3 fatty acids in cell membranes [18], and peroxidation of n3 fatty acids incorporated into tumor membranes [17]. Dietary n3 fatty acids

were also shown to inhibit the azoxymethane-induced expression of *ras*-p21 in the colon of male F344 rats [59]. Recently, it was reported that EPA inhibited cell division in a number of human cancer cell lines as a result of its ability to: release Ca^{2+} from intracellular stores; prevent replenishment of the stores; activate protein kinase R; and inhibit protein synthesis at the initiation of translation [60]. In addition, DHA was shown recently to inhibit the growth of cultured metastatic melanoma cells [61]. The growth inhibition was correlated with an increase in hypophosphorylated retinoblastoma protein, suggesting interactions among polyunsaturated fatty acid metabolism and the retinoblastoma pathway. Further research will be required to determine if these actions of n3 fatty acids [59–61] occur upstream or downstream of the n3 fatty acid-mediated inhibition of cAMP-dependent fatty acid transport [22].

Over the last several years, scientific and lay publications have emphasized: (a) the increased cancer risks associated with a "Western" type high-fat diet that is enriched in n6 fatty acids; (b) the decreased cancer risks associated with "Eastern and Mediterranean" type low-fat diets containing n3 fatty acids and n9 monounsaturated fatty acids; and (c) the increased cancer risks of "light at night" and suppression of the normal melatonin circadian rhythm. It is remarkable that these seemingly unrelated nutritional and environmental factors act as cancer risks or benefits by increasing or reducing, respectively, the availability of linoleic acid to the cancer cell. We believe these findings and future studies will provide a convincing scientific rationale for the development of new stronger recommendations for cancer prevention that consider dietary intake of linoleic and n3 fatty acids and melatonin supplementation and/or photoperiodic alterations.

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