Signaling and cytotoxic functions of 4-hydroxyalkenals

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Riahi Y, Cohen G, Shamni O, Sasson S. Signaling and cytotoxic functions of 4-hydroxyalkenals. Am J Physiol Endocrinol Metab 299: E879–E886, 2010. First published September 21, 2010; doi:10.1152/ajpendo.00508.2010.—The peroxidation of n-3 and n-6 polyunsaturated fatty acids (PUFAs) and of their hydroperoxy metabolites is a complex process. It is initiated by free oxygen radical-induced abstraction of a hydrogen atom from the lipid molecule followed by a series of nonenzymatic reactions that ultimately generate the reactive aldehyde species 4-hydroxyalkenals. The molecule 4-hydroxy-2E-hexenal (4-HHE) is generated by peroxidation of n-3 PUFAs, such as linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. The aldehyde product 4-hydroxy-2E-nonenal (4-HNE) is the peroxidation product of n-6 PUFAs, such as arachidonic and linoleic acids and their 15-lipoxygenase metabolites, namely 15-hydroperoxyeicosatetraenoic acid (15-HpETE) and 13-hydroperoxyoctadecadienoic acid (13-HpODE). Another reactive peroxidation product is 4-hydroxy-2E,6Z-dodecadienal (4-HDDE), which is derived from 12-hydroperoxyeicosatetraenoic acid (12-HpETE), the 12-lipoxygenase metabolite of arachidonic acid. Hydroxyalkenals, notably 4-HNE, have been implicated in various pathophysiological interactions due to their chemical reactivity and the formation of covalent adducts with macromolecules. The progressive accumulation of these adducts alters normal cell functions that can lead to cell death. The lipophilicity of these aldehydes positively correlates to their chemical reactivity. Nonetheless, at low and noncytotoxic concentrations, these molecules may function as signaling molecules in cells. This has been shown mostly for 4-HNE and to some extent for 4-HHE. The capacity of 4-HDDE to generate such “mixed signals” in cells has received less attention. This review addresses the origin and cellular functions of 4-hydroxyalkenals.

lipid peroxidation; diabetes; reactive oxygen species; 4-hydroxyhexenal; 4-hydroxynonenal; 4-hydroxydodecadienal; lipoxygenase; polyunsaturated fatty acids

THE TERM LIPID PEROXIDATION encompasses varied complex enzymatic and nonenzymatic reactions that generate myriad products with diverse physiological and pathophysiological functions. Among such peroxidation products of polyunsaturated fatty acids (PUFAs) the group of reactive aldehydes, known as 4-hydroxyalkenals, has been shown to exert dose-dependent regulatory roles as well as detrimental effects in various cell types and organs. The process of peroxidation of PUFAs is intensified in cells both in vitro and in vivo upon exposure to reactive oxygen species. The peroxidation of enzymatic and nonenzymatic oxidation products of n-3- and n-6-PUFAs is complex, and several alternative chemical mechanisms for the generation of 4-hydroxyalkenals have been proposed (71, 77, 87, 88, 98, 101). Niki (70) has recently summarized and reviewed this topic. Briefly, the critical initiation step is mediated by hydroxyl radical- and lipid peroxyl radical-mediated abstraction of bisallylic hydrogen atom from n-3 and n-6 unsaturated fatty acids or their oxidized hydroperoxy metabolites. This abstraction forms conjugated diene lipid hydroperoxides, which further undergo intramolecular rearrangement and chain-breaking reactions to generate a large number of saturated and unsaturated aldehydes. Among these products, unsaturated 4-hydroxyalkenals have been shown to possess important biological and cytotoxic effects.

Figure 1A presents the general nonenzymatic peroxidation pathway of n-3 and n-6 PUFAs, which generates 4-hydroxy-2E-hexenal (4-HHE) and 4-hydroxy-2E-nonenal (4-HNE), respectively. Figure 1B depicts the enzymatically regulated peroxidation pathways that ultimately produce 4-HNE and 4-hydroxy-2E,6Z-dodecadienal (4-HDDE). The initial step in this pathway is the conversion of n-6 PUFAs to their respective hydroperoxy metabolites by the action of various isotypes of lipoxygenases (12-LO and 15-LO). The peroxidation product 4-HDDE is exclusively derived from 12-hydroperoxyeicosatetraenoic acid (12-HpETE), the 12-LO metabolite of arachidonic acid; 4-HNE is the end peroxidation product of 15-LO metabolites of arachidonic acid, linoleic acid, and other n-6 PUFAs (93). The flux of PUFAs through this pathway is significantly enhanced in the presence of free radicals due to two independent processes. The first is the aforementioned radical-induced abstraction of the hydrogen atom from the hydroperoxy metabolites of PUFAs. The second is a free radical-mediated inactivation of the enzyme glutathione per-
oxidase (GPx). Normally, this enzyme transforms 12-LO-derived hydroperoxy metabolites of arachidonic acid to their respective hydroxy metabolites [e.g., 12-hydroxyeicosatetraenoic acid (12-HETE)] (6). Once GPx is inactivated, hydroperoxy metabolites of lipoxygenases accumulate and become readily subjected to peroxidation (14).

The LO-dependent pathway of lipid peroxidation (Fig. 1B) implies a cell-specific generation of 4-HNE and 4-HDDE, consistent with the particular expression of the various types of lipoxygenases. Indeed, Guichardant et al. (7, 39) found that a rat’s retina, which expresses 15-LO, generates predominantly 4-HNE. Similarly, the major 4-hydroxyalkenal generated in 3T3-L1 preadipocytes is 4-HNE, due to the expression of 15-LO (26). Conversely, bovine aortic endothelial cells that predominantly express 12-LO under hyperglycemic conditions generate excessive amounts of 4-HDDE but not 4-HNE (2, 3, 80). Bacot et al. (7) also suggest a similar correlation between the cell-specific expression of 15- and 12-LO and the generation of the end-aldehyde peroxidation products 4-HDDE and 4-HNE, respectively.

Notably, the essential linoleic acid (LA) and α-linolenic (ALA) acid are converted in mammals by a series of desaturation and elongation reactions to various PUFAs. LA gives rise to n-6 arachidonic and adrenic acids and ALA generate n-3 eicosapentaenoic and docosahexaenoic acids. The dietary sources of these two latter n-3 PUFAs are fish and shellfish. Other aspects on the availability and abundance of the various lipid precursors of 4-hydroxyalkenals are well described in a recent review by Russo (84). The inherent instability of 4-hydroxyalkenals due to their rapid nonenzymatic oxidation and/or their chemical tendency to form stable adducts with macromolecules greatly limits their dietary availability in their aldehyde reactive form.

The relative rate of production and abundance of 4-HHE, 4-HNE, and 4-HDDE differs significantly in mammals (Fig. 2): 4-HHE is the most abundant in plasma and urine of human subjects and in rodents, followed by 4-HNE, whereas 4-HDDE is the least abundant (39). Van Kuijk et al. (102) showed that the main source of 4-HHE is docosahexaenoic acid, which is the abundant n-3 PUFA in most tissues (92). Because the rate of production of 4-HNE and 4-HDDE greatly depends on cell-specific expression and function of 15- or 12-LO in cells, the significantly higher levels of 4-HNE compared with 4-HDDE indicate a ubiquitous expression of 15-LO compared with a more limited expression pattern of 12-LO. Moreover, the larger repertoire of n-6 PUFAs that are substrates to 15-LO also contribute to the increased generation of 4-HNE compared with 4-HDDE, which is preferentially derived from arachidonic acid. Figure 2 also depicts the diverse lipophilicity of these compounds, as reflected by their varied Log P (octanol/water partition coefficient) values: 0.89 for 4-HHE, 2.45 for 4-HNE, and 3.48 for 4-HDDE. There is also a clear, positive correlation between these Log P values and the chemical reactivity of the different molecules in forming covalent adducts with macromolecules (Fig. 2). These data, derived from Bacot et al. (7), show the capacity of the three compounds to form stable covalent adducts with ethanolamine phospholipid subclasses. Noteworthy is the negative correlation between the chemical reactivity and the plasma and urine content of these molecules: 4-HDDE is the least abundant but most active, 4-HHE is the most abundant and the least reactive, and HNE is intermediately placed between the two.

The chemical interactions underlying the tendency of 4-hydroxyalkenals to form covalent adducts with macromolecules have been recently reviewed by Poli et al. (76), Petersen and Doorn (74), and Negre-Salvayre et al. (69). Briefly, the pres-
ene of α,β-unsaturated carbonyl in 4-hydroxyalkenals catalyzes interactions with nucleophiles via Michael addition reactions and Schiff base formation with proteins, phospholipids, and DNA. Cysteine, histidine, and lysine moieties in proteins readily react with 4-HNE; the formed histidine adducts are more stable than the conjugates of the two other amino acid moieties (32, 67, 74, 86). Bacot et al. (6) have studied the covalent binding interactions of 4-hydroxyalkenals with ethanolamine phospholipid subclasses. The high lipophilicity of 4-hydroxyalkenals, in particular 4-HNE and 4-HDDE, favors their accumulation in the lipid bilayer compartment of membranes. Consequently, membrane phospholipids and membrane proteins (i.e., receptors, adaptor proteins, transporters, and channels) are prime targets for these molecules. The combined covalent interactions with membrane phospholipids and proteins can alter membranes’ structure, fluidity and functions (53, 76). Nair et al. (68) have proposed that the chemical interactions of 4-hydroxyalkenals with DNA involve the formation of etheno (ε) adducts with deoxyribonucleotide moieties (i.e., 1,N6-ethenodeoxyadenosine; 3,N4-ethenodeoxycytosine; 1,N2- or 3,N2-ethenodeoxyguanosine). Despite this potent chemical reactivity of 4-hydroxyalkenals, not all types of cells are equally susceptible to their damage due to the expression of hydroxyalkenyl neutralizing enzymes (see below and Refs. 65 and 90).

It should be noted that at physiological and nontoxic levels these 4-hydroxyalkenals have been shown to function as signaling molecules and regulate various cell functions and interactions (19, 29, 34). Of peculiar interest are the observations that at nontoxic levels these molecules may even provide protection against oxidative damage in cells (19, 29, 31, 70, 76).

4-HHE

This molecule is most abundant among the 4-hydroxyalkenals. However, due to its low lipophilicity and reduced chemical reactivity, 4-HHE is considered less physiologically active and less damaging than 4-HNE and 4-HDDE (32, 50). Indeed, little is known about direct regulatory and signaling functions of 4-HHE under physiological conditions. The precursors of 4-HHE are n-3 PUFAs, such as eicosapentaenoic and docosahexaenoic acids. These two unsaturated fatty acids have endowed pleiotropic beneficial roles in humans, including cardioprotective, anti-inflammatory, anti-hyperlipidimic, and anticancer effects (63, 81). Of remarkable interest is the fat-1 mouse model, which lends support to this view; unlike wild-type mice and other mammals, this mouse can convert n-6- to n-3 PUFAs due to a transgenic expression of the Caenorhabditis elegans 6-desaturase (fat-1) gene (51). Ample studies have shown improved cellular and organ functions in these mice, including prevention of neoplasia (37), improved glucose tolerance and insulin secretion (105), and anti-inflammatory effects (106). Hitherto, it has not been established to what extent the increased production of 4-HHE following peroxidation of n-3 PUFAs in these mice may contribute or perhaps compromise these positive outcomes of the increased n-3 PUFAs abundance. Enhanced peroxidation of n-3 PUFAs to 4-HHE may occur in the fat-1 mice upon the induction of metabolic or environmentally induced oxidative stressful conditions. It was shown before that intraperitoneally or intravenously administered high doses of 4-HHE caused severe peritonitis and liver damage, respectively (89). A few studies have recorded negative effects of an increased generation of 4-HHE from n-3 PUFAs in fat-1 mice. Bonilla et al. (13) reported that these mice were immunologically compromised and exhibited impaired resistance to tuberculosis. Tanito et al. (97) induced phototoxic retinal damage in these mice and found a positive correlation between the level of the docosahexaenoic acid, the extent of lipid peroxidation and generation of 4-HHE, and the severity of the retinal damage. Other detrimental effect of 4-HHE were observed in other clinical and experimental models: Long et al. (60) associated the elevated levels of docosahexaenoic acid in the CNS of patients with Alzheimer’s disease to radical-induced formation of 4-HHE. Lee et al. (55) suggested that 4-HHE-activated NF-κB in vascular endothelial cells increases inducible nitric oxide (iNOS) gene expression and leads to cell dysfunction due to an excessive generation of the nitric oxide radical. Je et al. (48) have associated NF-κB-mediated inflammatory effects in endothelial cells to 4-HHE-dependent activation of IκB kinase/NF-κB-inducing kinase pathway. These interactions may also explain the findings of Lee at al. (56) on 4-HHE-induced apoptosis in endothelial cells.

Collectively, these studies indicate that, despite the low chemical reactivity and lipid solubility, high levels of 4-HHE can compromise the favorable effects attributed to n-3 PUFAs, especially under conditions that favor the peroxidation of these fatty acids to 4-HHE.

4-HNE

Since its discovery as a peroxidation product of n-6 PUFAs, 4-HNE has reigned in the field of hydroxyalkenal research. The principal reason for this attention is its high chemical reactivity and lipophilicity and its commercial availability for biological and chemical research. Many excellent reviews have been published over the years, summarizing both signaling and cytotoxic effects of this molecule (19, 28, 29, 32, 38, 70, 74, 76, 101, 110). Among the various signaling targets of 4-HNE, the following are particularly interesting: c-Jun NH2-terminal kinase (JNK) (11), p38 mitogen-activated protein kinases (p38 MAPK) (111), cell cycle regulators (8), and protein kinase-C and -δ (PKCδ and PKC5) (16, 23). However, the underlying notion that 4-HNE acts like a classical ligand that interacts with binding/catalytic sites in proteins is in most cases unfounded. In fact, most studies suggest that these “signaling” effects of 4-HNE result primarily from its adduct-forming capacity with macromolecules and not by functioning as an activating or allosteric ligand of regulatory proteins in signal transduction pathways. These 4-HNE-induced modifications evoke numerous cellular responses, ranging from an adaptive protection against oxidative stress to irreversible cytotoxic injuries and cell death. Niki and colleagues (20, 21, 70) have reported that nontoxic levels of 4-HNE induce the expression of antioxidant enzymes through the activation of NF-E2-related transcription factor-2 (Nrf2) in PC12 cells. In contrast, Poli et al. (76) have listed various enzymes (oxidoreductases, transffereases, hydrodases, lyses, and isomerases), membrane proteins (carriers, receptors, ion channels, transporters) and cytoskeletal proteins that are inhibited or inactivated by targeted 4-HNE adduction. For instance, 4-HNE modification of thioredoxin-1 compro-
mises the antioxidant function of the latter and leads to an increased production of reactive oxygen species (36). The conjugation of 4-HNE to glucose transporter 3 (GLUT3) in the hippocampus of diabetic rats has been associated with decreased neural glucose utilization (78). The formation of 4-HNE adducts with a histidine residue in extracellular signal-regulated kinase (ERK1/2) inhibits downstream signaling of the latter in hepatocytes (85). In vitro addition of 4-HNE to an e-amo group of a lysine residue of glucose-6-phosphate dehydrogenase inactivates the enzyme (95). It has also been shown that 4-HNE induces β-cell apoptosis by a direct interaction with caspase-3 (33). Awashti et al. (5) have comprehensively reviewed the role of 4-HNE in mediating stress-induced apoptosis. Interestingly, platelet-derived growth factor receptor-β (PDGFRβ) activity in smooth muscle cells is transiently activated following a covalent interaction with 4-HNE but further deteriorates to inhibition of phosphorylation and to inactivation of the receptor (103). It has been proposed that 4-HNE-induced activation of NF-κB mediates its proinflammatory effects (57, 58). Nonetheless, others have reported that 4-HNE inactivates NF-κB by inhibition of IκB phosphorylation (41, 73).

Another target for 4-HNE is the mitochondrion. Roede and Jones (83) have recently reviewed the role of 4-HNE in mitochondrial dysfunction. Excessive and uncontrolled reactive oxygen species production by the mitochondria can aberrantly mediate peroxidation of unsaturated fatty acids and subsequently large 4-HNE production. Several groups have demonstrated that key proteins in the mitochondrial respiratory chain [e.g., the adenine nucleotide translocator (ANT) cytochrome c oxidase] and enzymes in the TCA cycle (α-ketoglutarate dehydrogenase and isocitrate dehydrogenase) are particularly susceptible to 4-HNE-induced inactivation (9, 17, 18, 30, 45).

Membrane phospholipids, cholesterol, and LDL are also targets for 4-HNE-induced modifications. Bacot et al. (6, 7, 10) characterized these interactions with different subclasses of phospholipids in vitro and in vivo experimental models. Their studies demonstrate that exposure to high levels of 4-hydroxyalkenals, including 4-HNE, distorts the normal lipid bilayer structure of membranes, their integrity, and consequently their function. The role of oxidized-LDL in macrophage activation, foam cell formation, and atherosclerosis is well recognized (46, 47, 104). Oxidative modification of LDL is mediated by radical-induced formation of hydroperoxides and reactive aldehydes, of which 4-HNE plays a central role (43, 44, 59). This aberrant species of LDL accumulates in the intima of blood vessels, where it is taken up by macrophages via scavenger receptors (i.e., CD36, SR-A) and participates in the transformation of macrophages to foam cells (109). Therefore, 4-HNE may also be considered an atherogenic factor.

One of the main reasons for 4-HNE-induced cell dysfunction is a decline in general protein turnover in cells as a result of altered proteasomal activity and the subsequent accumulation of HNE-crosslinked proteins. Okada et al. (72) and Grune and colleagues (38, 49) have suggested that the accumulation of such aggregates of these modified proteins progressively reduces the proteasomal complex activity and consequently impairs protein turnover in cells.

Clinical studies have also associated various pathologies to increased levels of free 4-HNE in plasma and biological fluids. For instance, it was about 2.5-fold higher in sera and ventricular fluid samples of Alzheimer’s disease patients (61, 64). Similar increased levels were detected in serum samples of sporadic amyotrophic lateral sclerosis (ALS) patients compared with the levels measured in normal controls (91). A striking increase of 4-HNE level was found in the plasma of patients with alcoholic liver disease ranging from 2.8-fold in the early stage of liver insult to 8.4-fold at the cirrhotic stage (1). More examples of other human diseases in which 4-HNE levels have been detected are discussed in more details by Poli et al. (76).

We have recently reviewed the roles of 4-hydroxyalkenals in diabetes (69). In short, hyperglycemia is a strong trigger of lipid peroxidation due to the overproduction of glucose-derived free radicals. In addition, hyperglycemia also augments the expression of 12- and 15-LO, leading to an elevated rate of production of 12- and 15-HpETE, the precursors of 4-HNE and 4-HDDE, respectively. These high-glucose-induced effects are closely related to the clinical observation of a marked increase in serum levels of 4-HNE-modified albumin in type 2 diabetic patients (99). The elevated production of 4-hydroxyalkenals in diabetic patients and in animal models of diabetes has been associated with β-cell dysfunction and the development of macrovascular disease and the accelerated deterioration to atherosclerosis (69).

It should be emphasized that, despite the fact that some of the studies cited above distinguish between signaling functions and cytotoxic interactions of 4-HNE, both processes result in fact from the 4-HNE tendency to conjugate with macromolecules and modify their function and structure. These studies have not identified selective targets for binding or allosteric interactions of 4-HNE in the pharmacological sense.

4-HDDE

The term “the stepchild in the 4-hydroxyalkenal family” suits well this important molecule. Although this is the most chemically reactive member of the family, it has never gained the same research interest as 4-HNE. The major setback has been the lack of commercial availability of this molecule. Few studies have been performed with 4-HDDE, which was synthesized by Bacot et al. (6). This group has shown in a series of studies that the chemical potency of 4-HDDE is significantly higher than that of 4-HNE and 4-HDME (6, 7, 39, 40). The plasma and urine levels of 4-HDDE and its metabolites are the lowest compared with the other two hydroxyalkenals. Nevertheless, 4-HDDE levels in cells that express 12-LO do reach a high effective level, especially when the cells are subjected to an oxidative stress. One such example is vascular endothelial cells: the expression of 12-LO in these cells is regulated by the ambient glucose. Increasing glucose concentrations augment 12-LO expression and 4-HDDE, but not 4-HNE generation (2, 80). Similarly, other cells that exclusively express 12-LO may also produce high levels of 4-HDDE when exposed to free radicals. For instance, Bleich et al. (12) have implicated insulin-secreting β-cell dysfunction to cytokine-induced expression of 12-LO. It will be interesting to determine whether 4-HDDE adducts mediate these detrimental effects. Similarly, 4-HDDE may be the 12-LO metabolite implicated in inhibition of renin release from the juxtaglomerular apparatus in the kidney (4). It remains to be investigated whether 4-HDDE...
regulates cell functions in other cells that express 12-LO, such as leukocytes, platelets and epidermal cells, prostate cancer cells, and selected neural cells (107).

4-Hydroxyalkenals Are Endogenous Ligands for Peroxisome Proliferator-Activated Receptor-δ

Yu et al. (108) used cell-based transactivation assays to determine the capacity of various eicosanoids to activate, directly or indirectly, various peroxisome proliferator-activated receptors (PPARs); some prostaglandins and various HETEs were tested positive in this assay. It was not clear, however, whether these compounds or their peroxidation metabolites interacted directly with PPARs. The first indication that PPARs could be a target for 4-HNE came from the study of Pizzimenti et al. (75), who reported synergistic effects of 4-HNE with pharmacological PPAR ligands. Coleman et al. (26) later proved that 4-HNE was an intracellular agonist of PPARδ in 3T3-L1 preadipocytes. An in silico analysis of the ligand-binding domain (LBD) of PPARδ suggested specific interactions between 4-HNE and His413 in the binding pocket. Interestingly, this study found no evidence for a covalent interaction between the two in the PPARδ LBD despite the well-established capacity of 4-HNE to interact stably with histidine moieties. The structural and chemical constraints that obstruct this nucleophilic interaction in PPARδ LBD have not been determined yet.

We have recently proven that 4-HDDE is an endogenous ligand for PPARδ in vascular endothelial cells (80). In this project, we followed the transactivation of PPAR response element (PPRE)-driven luciferase expression by the different human PPAR isotypes (α, γ1, γ2, and δ) in the presence of 4-HDDE. Only PPARδ significantly augmented luciferase expression in the presence of 4-HDDE. We obtained similar results when such transfected cells were treated with 4-HNE (unpublished data). The generation of 4-HDDE increases significantly in endothelial cells exposed to high glucose levels. Our study has correlated this interaction with PPARδ-induced expression of the protein calreticulin, which interacts with and destabilizes GLUT1 mRNA molecules. Subsequently, the expression of this glucose transporter is reduced in the cells, and their capacity to take up glucose is reduced in the face of hyperglycemia. We suggest that this mechanism protects the cells against deleterious effects of glucose under hyperglycemic conditions.

The discovery that 4-HNE and 4-HDDE are endogenous activating ligands of PPARδ is particularly important in view of the remarkable metabolic and regulatory functions this nuclear receptor mediates. For instance, selective PPARδ ligands possess anti-inflammatory properties (52). Other reports have shown that PPARδ regulates the oxidative capacity of the mitochondrion, switches fuel preference from glucose to fatty acids, increases cholesterol export and represses inflammatory gene expression in macrophages (35), augments fatty acid oxidation (22, 82), and regulates glucose metabolism and insulin sensitivity (54). These diverse metabolic regulatory functions of PPARδ have led to the idea that it may serve as a prime therapeutic target in metabolic diseases (24, 62, 79, 96). Among other classes of selective PPARδ agonists, 4-hydroxyalkenal derivatives, devoid of the covalent binding property, may serve as prototype molecules for the development of such therapeutic agents.

Cellular Defense Mechanisms Against 4-Hydroxyalkenals

Not all cells are equally susceptible to 4-hydroxyalkenal-induced damage. We, for instance, have found that the LD50 of 4-HNE in β-cells in rat isolated islets of Langerhans is 110 µmol/l, whereas the corresponding LD50 in the insulin-secreting INS-1E cell line is nearly fourfold lower (unpublished data). This disparate sensitivity of the various β-cells to 4-HNE-induced damage may depend on their relative expression of the 4-hydroxyalkenal-neutralizing enzymes fatty aldehyde dehydrogenase (FALDH), which transforms the aldehyde group to a carboxylic moiety, and of glutathione-S-transferase (GST), which hydrolyzes 4-hydroxyalkenal-thiol adducts (15, 27, 94). Various studies have convincingly established the role of these neutralizing systems of reactive aldehydes species in various cells and tissues, such as liver cells (62), the heart (94), the retina (25, 42), or cultured adipocytes (27). In this last study, 3T3-L1 adipocytes became resistant to 4-HNE-induced insulin resistance upon ectopic expression of FALDH. It has been proposed that aldehyde dehydrogenase-1B1, expressed in mitochondria, may protect these organelles by metabolizing reactive aldehydes such as 4-HNE. Noteworthy, prolonged exposure of cells to 4-HNE can progressively deactivate these enzymes and render the cells more vulnerable to cytotoxic 4-HNE adducts. Indeed, Traverso et al. (100) have shown that 4-HNE inactivated this neutralizing system in livers of diabetic rats.

The acidic derivatives of the three 4-hydroxyalkenals are 4-hydroxy-2E-hexenoic acid (4-HHA), 4-hydroxy-2E-nonenoi acid (4-HNA), and 4-hydroxy-2E,6Z-dodecadienoic acid (4-HDDA) (39). Few have investigated the potential biological function of these acidic metabolites. Echtay et al. (30) have proposed that 4-HNA uncouples proton conductance in mitochondria, whereas Murphy et al. (66) identified the γ-butyrate receptor in neurons as target for the same molecule.

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Summary

The research on the physiological and pathological effects of the various types of free radical species has been intense, and numerous molecules involved in these interactions have been identified and investigated. Lipid peroxidation of PUFA's and the resulting reactive aldehyde species have also attracted considerable attention. These molecules have been particularly interesting due to their relative chemical stability and long-term effects. This short review summarizes the alternative pathways of 4-hydroxyalkenal production and their chemical and biological properties, as highlighted in the model in Fig. 3. 4-HDDE emerges as a potent and interesting peroxidation product, which deserves attention and thorough investigation. The identification of PPARδ as a real cellular signaling target to 4-hydroxyalkenals may open a new direction in the research on the biological functions of 4-HHE, 4-HNE, and 4-HDDE.

DISCLOSURES

No conflicts of interest are declared by the authors.

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**Echinocladium Rhipidophyllum**


