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# Review Article

# 4-Hydroxynonenal As a Biological Signal: Molecular Basis and Pathophysiological Implications

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## ABSTRACT

**Reactive oxygen intermediates (ROI) and other pro-oxidant agents are known to elicit, *in vivo* and *in vitro*, oxidative decomposition of $\omega$-3 and $\omega$-6 polyunsaturated fatty acids of membrane phospholipids (*i.e.*, lipid peroxidation). This leads to the formation of a complex mixture of aldehydic end-products, including malonyldialdehyde (MDA), 4-hydroxy-2,3-nonenal (HNE), and other 4-hydroxy-2,3-alkenals (HAKs) of different chain length. These aldehydic molecules have been considered originally as ultimate mediators of toxic effects elicited by oxidative stress occurring in biological material. Experimental and clinical evidence coming from different laboratories now suggests that HNE and HAKs can also act as bioactive molecules in either physiological and pathological conditions. These aldehydic compounds can affect and modulate, at very low and nontoxic concentrations, several cell functions, including signal transduction, gene expression, cell proliferation, and, more generally, the response of the target cell(s). In this review article, we would like to offer an up-to-date review on this particular aspect of oxidative stress—dependent modulation of cellular functions—as well as to offer comments on the related pathophysiological implications, with special reference to human conditions of disease.** Antiox. Redox Signal. 1, 255–284.

## INTRODUCTION

REACTIVE OXYGEN INTERMEDIATES (ROI), pro-oxidant agents and, more generally, free radical species are known to elicit *in vivo* and *in vitro* oxidative decomposition of $\omega$-3 (22:6) and $\omega$-6 (18:2, 20:4) polyunsaturated fatty acids of membrane phospholipids, a process usually referred to as lipid peroxidation (Esterbauer *et al.*, 1991). This process involves the so called $\beta$-cleavage reaction of lipid hydroperoxides (*i.e.*, lipid alkoxy-radicals) and leads eventually to the formation of a very complex mixture of aldehydic end products, including malonyldialdehyde (MDA), *n*-alkanals, 2-alkenals, and 4-hydroxy-2,3-nonenal (HNE), and other 4-hydroxy-2,3-alkenals (HAKs) of different chain lengths (Esterbauer, 1985; Esterbauer *et al.*, 1988, 1991).

HAKs were discovered in the early 1960s as carcinostatic and cytotoxic substances present in autooxidized methyl-linoleate (Schauenstein *et al.*, 1964; Schauenstein, 1967). Extensive experimental research, mainly performed by the group of Erwin Schauenstein and Hermann Esterbauer in Graz, led to the identification of the mechanism of formation of these molecules. Their peculiar chemical reactivity was soon elucidated and reliable methods for detection and chemical synthesis of HAKs were developed (see Esterbauer *et al.*, 1991, Comporti,

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1998, and references therein for comprehensive review). However, the relevance of these achievements was not immediately appreciated by the scientific community.

A fundamental step has been represented by the discovery that diffusible cytotoxic aldehydes were produced during the course of lipid peroxidation of liver microsomes (Benedetti *et al.*, 1977, 1979a,b). HNE was soon recognized as a major cytotoxic aldehyde in a biological system undergoing oxidative stress (Benedetti *et al.*, 1980). These studies led to the proposal of a crucial concept: unlike short-lived free radical species, relatively long-lived, lipid-soluble, aldehydes such as HAKs were supposed to be able to diffuse from the site of origin (*i.e.*, cellular membranes) to reach and affect other intracellular and extracellular biological targets. As a consequence, HNE and related aldehydes were proposed as putative ultimate toxic messengers, potentially able to mediate oxidative stress-related injury at a molecular level (Dianzani, 1982; Slater, 1984; Comporti, 1985, 1998; Esterbauer *et al.*, 1991). HNE and HAKs were soon detected *in vitro* and *in vivo* in several experimental models of pro-oxidant-induced liver injury (Benedetti *et al.*, 1982, 1984a; Esterbauer *et al.*, 1982; Poli *et al.*, 1985). Interest in the role of these compounds has grown exponentially in the last decade, as reflected by the progressive and impressive increase in the number of scientific reports (Fig. 1).

Sensitive methods for detecting *in vivo* generation of HAKs have been developed (see Comporti, 1998) and applied extensively to biological samples in normal and pathological conditions, leading to the identification of the presence of these compounds (mainly HNE) in an impressive number of human and animal conditions of disease (Tables 1–5).

Most of the available data are dedicated to the description of cytotoxic and mutagenic effects displayed by these aldehydes and to the metabolism of these compounds in different tissues and cells. Usually these reports have been obtained by using relatively high concentrations of HNE and HAKs (range 0.01–1 m*M*) and are devoted mainly to analyzing the mechanisms leading to the dose-dependent toxic and mutagenic effects displayed in isolated or cultured normal and neoplastic mammalian cells in relation to defined human or experimental conditions of disease. Published studies range from simple analyses of cell viability or cell proliferation in different cells to more specific investigations that try to elucidate the action of these compounds at subcellular and molecular levels. This includes the effects exerted by HAKs on specific functions associated with defined organelles (for example, mitochondrial functions), defined metabolic pathways, as well as the action on defined enzymes, proteins, or nucleic acids. The interested reader can refer to several excellent reviews published

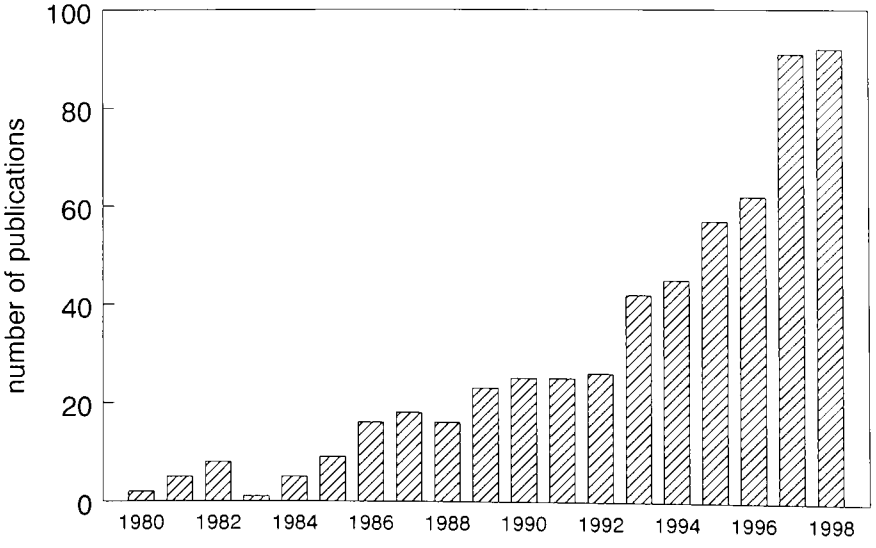


**FIG. 1. Number of published scientific articles and reviews on international journals with peer reviewing concerning 4-hydroxynonenal in the period 1980–1998.** Source: *MEDLINE* search ("4-hydroxynonenal" as search term).

TABLE 1. HNE AND HNE PROTEIN ADDUCTS IN NERVOUS SYSTEM

| *Human disease* | *Detection site* | *Detection methods* | *References* |
|---|---|---|---|
| Parkinson disease | Nigral neurons | IHC | Yoritaka *et al.* (1996) |
| | Cerebrospinal fluid (CSF) | MS | Selley (1998) |
| Sporadic amyotrophic lateral sclerosis | CSF | HPLC | Smith *et al.* (1998a) |
| | Lumbar spynal cord (Ventral horn motor neurons) | IHC | Pedersen *et al.* (1998) |
| Alzheimer's disease | Neurofibrillary tangles Dystrophic neurites | IHC | Sayre *et al.* (1997) |
| | Neurofibrillary tangles | IHC | Montine *et al.* (1997a) |
| | Neurofibrillary tangles | IHC | Montine *et al.* (1997b) |
| | Ventricular fluid | HPLC WB | Lovell *et al.* (1997) |
| | Amigdala | | Markesbery and Lovell (1998) |
| | Hippocampus | HPLC | |
| | Parahippocampus gyrus | WB | |
| | Hippocampus | IHC | |
| | Entorinal cortex | IHC | Montine *et al.* (1998) |
| | Temporal cortex | IHC | |
| | Cytoplasm of pyramidal neurons | IHC | |
| | Amyloid deposits | IHC | Ando *et al.* (1998) |
| Familial polyneuropathy amyloidotic | Amyloid deposits | IHC | Ando *et al.* (1997) |

| *Related animal model* | *Detection site* | *Detection methods* | *References* |
|---|---|---|---|
| Alzheimer's transgenic mice | Cerebral amyloid deposits | IHC | Smith *et al.* (1998b) |

IHC, Immunohistochemistry; MS, mass spectometry; WB, western blot; HPLC, high-pressure liquid chromatography.

in the 1990s that deal mostly with these toxicological aspects of HNE and HAKs action (see Esterbauer *et al.*, 1991; Burcham, 1998; Comporti, 1998; Dianzani, 1993, 1998; and references therein). In this connection, a growing area of interest is now represented by the involvement of these aldehydes and oxidative stress in the pathogenesis of chronic degenerative diseases of the nervous system, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Markesbery, 1997; Keller and Mattson, 1998). Moreover, HNE has been recently shown to induce neuronal apoptosis in cultured PC-12 cells (Kruman *et al.*, 1997), a report that was later confirmed in a different experimental model (Compton *et al.*, 1998).

In this review, we would like to turn the attention of the reader to a fascinating emerging concept: at very low (*i.e.*, nontoxic) concentrations, HNE and HAKs may act as potential biological signals able to modulate signal transduction, gene expression, cell proliferation, and, more generally, the response of target cells in normal and pathological conditions. Moreover, we will try to suggest possible pathophysiological implications of HNE and HAK generation in conditions of disease, with special reference to human conditions.

## HNE METABOLISM, CHEMICAL REACTIVITY, AND HNE-ADDUCT FORMATION IN TISSUES AND CELLS

Intracellular steady-state levels of HNE are essentially the result of an equilibrium between its generation and metabolism. Rate and efficiency of HNE metabolism in a defined type of cell can result in complete disposal of this compound, and then in the prevention of major cell injury or response, or in an incomplete removal

TABLE 2. HNE AND HNE PROTEIN ADDUCTS IN CHRONIC LIVER DISEASES

| *Human disease* | *Detection site* | *Detection methods* | *References* |
|---|---|---|---|
| Chronic hepatitis C | Hepatocytes | IHC | Paradis *et al.* (1997a) |
| Genetic hemochromatosis | Hepatocytes | IHC | Paradis *et al.* (1997b) |
| Wilson's disease | Hepatocytes | IHC | Paradis *et al.* (1997b) |
| Primary biliary cirrhosis | Biliary Epithelial Cells | IHC | Paradis *et al.* (1997b) |
| Alcoholic liver disease | Hepatocytes (cytoplasm) | IHC | Paradis *et al.* (1997b) |
| | Hepatocytes (cytoplasm) | IHC | Ohira *et al.* (1998) |
| | Plasma | GC/MS | Aleynik *et al.* (1998) |
| *Related animal model* | *Detection site* | *Detection methods* | *References* |
| Chronic ethanol consumption (rat) | Liver mitochondria and microsomes | HPLC | Kamimura *et al.* (1992) |
| | Plasma | FA | French *et al.* (1993) |
| | Liver extract | WB | Li *et al.* (1997) |
| | Parenchyma and sinusoids | IHC | Niemela *et al.* (1998) |
| Chronic ethanol consumption (micropig) | Parenchyma | IHC | Niemela *et al.* (1995) |
| Iron overload (rat) | Hepatocytes | IHC | Houglum *et al.* (1990) |
| | Plasma | WB | |
| Alcohol plus iron (rat) | Parenchyma | IHC | Tsukamoto *et al.* (1995) |
| Chronic cholestasis (rat) | Liver extracts | HPLC | Parola *et al.* (1996) |
| | Plasma | HPLC | |
| Chronic $CCl_4$ administration (HAK's) (rat) | Liver | HPLC | Parola *et al.* (1992a) |

IHC, Immunohistochemistry; WB, Western blot; HPLC, high-pressure liquid chromatography; FA, fluorimetric assay.

TABLE 3. HNE AND HNE–PROTEIN ADDUCTS IN HUMAN PLASMA LDL AND ATHEROSCLEROSIS

| *Detection site* | *Detection methods* | *References* |
|---|---|---|
| In autooxidized human plasma LDL | FL | Quehenberger *et al.* (1987)<br>Esterbauer *et al.* (1987) |
| In human plasma LDL artificially oxidized or modified | FL | Quehensberger *et al.* (1988)<br>Jürgens *et al.* (1990)<br>Chen *et al.* (1992)<br>Van Kuijk *et al.* (1995)<br>Requena *et al.* (1997) |
| In oxidized LDL in atherosclerotic lesions | WB | Yla-Herttuala *et al.* (1989)<br>Yla-Herttuala *et al.* (1990) |
| In LDL oxidized by advanced glycosilation-end products | MS | Al-Abed *et al.* (1996) |
| In oxidized LDL accumulating in the intima (early lesions) | IHC | Napoli *et al.* (1997) |
| *Related findings in experimental models* | | |
| *Detection site* | *Detection methods* | *References* |
| In vivo oxidation of LDL (rabbit) | FL | Palinski *et al.* (1989) |
| In oxidized LDL in atherosclerotic lesions (rabbit) | WB | Yla-Hertuala *et al.* (1989) |
| HNE adducts in atherosclerotic lesions (rabbit) | IHC | Rosenfield *et al.* (1990) |
| In oxidized LDL of Apo-E deficient mice | IHC | Palinsky *et al.* (1994) |
| In plasma of hyperlipideic Watanabe rabbits | GC/MS | Kinter *et al.* (1994) |

IHC, Immunohistochemistry; MS, mass spectometry; WB, Western blot; HPLC, high-pressure liquid chromatography; FL, fluorescence; GC, gas chromatography; MS, mass spectrometry.

TABLE 4. HNE AND HNE–PROTEIN ADDUCTS IN VARIOUS DISEASES

| *Human disease* | *Detection site* | *References* |
|---|---|---|
| Rheumatoid arthritis and osteoarthritis | Plasma<br>Synovial fluid | Selley *et al.* (1992) |
| Perinatal hypoxia | Blood from umbilical arterial cord | Schmidt *et al.* (1996) |
| Adult respiratory distress syndrome (ARDS) | Plasma | Quinlan *et al.* (1996) |
| Children systemic Lupus erythematosus | Plasma | Michel *et al.* (1997)<br>Grune *et al.* (1997a) |
| Normal and preeclamptic trophoblast cells in placenta | Placenta | Morikawa *et al.* (1997)<br>Casasco *et al.* (1997) |
| HIV-1 positive patients | Plasma | Fuchs *et al.* (1995) |
| Cardiomiopathy with cataracts and complex I deficiency | Skin fibroblasts | Luo *et al.* (1997) |
| *Animal model* | *Detection site* | *References* |
| Passive Heymann nephritis (rat) | Glomerular epithelial cells<br>Glomerular basement membrane<br>Immune deposits | Neale *et al.* (1994) |
| Diabetic rats | Plasma | Traverso *et al.* (1998) |
| Acute pancreatitis (rat) | Pancreas | Reinheckel *et al.* (1998) |

that will allow more relevant HNE-dependent intracellular lesions or responses to occur.

HNE can be removed or detoxified by a number of different pathways, including direct interaction with glutathione (GSH), as observed in different cells and summarized in Figure 2 (Canuto *et al.*, 1994; Ullrich *et al.*, 1994, 1997; Hartley *et al.*, 1995; Grune *et al.*, 1997a,b; Siems *et al.*, 1997a, 1998; Srivastava *et al.*, 1998; Petras *et al.*, 1999). HNE can be transformed by aldehyde dehydrogenase (ALDH) isoforms into the major metabolite 4-hydroxy-nonenoic acid and by alcohol dehydrogenase (ADH) isoforms into 1,4-dihydroxynonene. In addition, several glutathione S-transferase (GST) isoforms can catalyze the formation of GSH–HNE conjugate (Alin *et al.*, 1985; Singhal *et al.*, 1994; Hubatsch *et al.*, 1998; Ketterer, 1998) and, possibly, the formation of conjugates between GSH and end products of HNE metabolism, such as the GSH–dyhydroxynonene conjugate (Siems *et al.*, 1998). Although the relative generation of GSH–HNE conjugate, 4-hydroxynonenoic acid, and 1,4-dihydroxynonene may vary in different cultured or isolated cells or experimental models, they are by far the major metabolites found during metabolism of exogenous HNE. Interestingly, several groups have detected the presence in the urine of mercapturic acid conjugates of degraded HNE. 1,4-Dihydrox-

TABLE 5. HNE AND HNE–PROTEIN ADDUCTS IN ISCHEMIA-REPERFUSION CONDITIONS

| *Experimental model* | *Detection time* | *References* |
|---|---|---|
| Myocardium of normal and spontaneously hypertensive rats | Early reperfusion | Grune *et al.* (1993)<br>Blasig *et al.* (1995)<br>Grune *et al.* (1994b) |
| Rat brain | Post ischemic | Kondo *et al.* (1997)<br>Kunstmann *et al.* (1996)<br>Yoshino *et al.* (1997)<br>Urabe *et al.* (1998) |
| Rat kidney | During warm ischemia<br>After reperfusion | Eschwege *et al.* (1997)<br>Cristol *et al.* (1996) |
| Rat small intestine | Early reperfusion | Siems *et al.* (1995) |

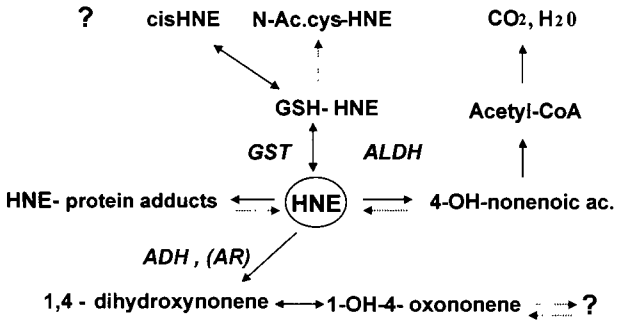


**FIG. 2. Cellular metabolism and fate of 4-hydroxynonenal.** Abbreviations used: HNE, 4-hydroxynonenal; GSH, glutathione; GST, glutathione-S-transferase isoforms; ALDH, aldehyde dehydrogenase isoforms; ADH, alcohol dehydrogenase isoforms; AR, aldose-reductase; N-Ac.cys, *N*-acetylated cysteine.

ynonene mercapturic acid is the major urinary metabolite found after administration of exogenous HNE, but it is also physiologically present in rat and human urine (Petras *et al.*, 1995; Alary *et al.*, 1995, 1998a). Detection of other polar urinary metabolites of HNE, such as 9-hydroxy-4-hydroxy-nonenoic acid, its mercapturic acid conjugate, and two diastereoisomers of the corresponding lactone, has led to the hypothesis that they may originate by omega oxidation of 4-hydroxy-2,3-nonenoic acid (Alary *et al.*, 1998b). Evidence has been reported also for omega oxidation of 1,4-dihydroxynonene with the formation of 9-hydroxy-1,4-dihydroxy- 2-nonene. Finally, it has been recently reported that an additional metabolism of HNE (and also HHE) may be operated by aldose reductase, an enzyme which is particularly present in retina (Ansari *et al.*, 1996; Spycher *et al.*, 1996, 1997; He *et al.*, 1998).

Interestingly, different cells may have relevant differences in the presence of the three main enzymes involved in HNE metabolism (GSTs, ADH, and ALDH), as well as in related relevant differences in overall HNE removal. Liver parenchymal cells (*i.e.*, hepatocytes) are extremely well equipped for this purpose (Esterbauer *et al.*, 1991; Canuto *et al.*, 1994; Hartley *et al.*, 1995; Leonarduzzi *et al.*, 1995; Siems *et al.*, 1997b). Other cells of mesenchymal origin have been reported to have less HNE-metabolizing enzymatic activities, such as in monocyte/macrophage cells (Leonarduzzi *et al.*, 1997), or to have negligible HNE metabolism, such as in human hepatic stellate cells (Parola *et al.*, 1998). If HNE is not rapidly removed by the above-mentioned pathways, the high chemical reactivity leads this molecule to react quickly with GSH, low molecular thiols, and cellular macromolecules. In particular, HNE is known to form adducts with proteins, and binding of HNE to cellular proteins has been described to give rise to multiple epitopes, including Schiff's bases, cross links, and Michael-type adducts by reacting with defined amino acid residues. Using Michael-type reactions HNE forms adducts by nucleophilic addition to the sulfur atom of cysteine, to the $\epsilon$-amino group of lysine, and to the imidazole ring nitrogen of histidine (Esterbauer *et al.*, 1991; Uchida and Stadtman, 1992, 1994; Friguet *et al.*, 1994; Nadkarni and Sayre, 1995; Waeg *et al.*, 1996). These reactions of HNE, particularly those with -SH groups, are probably the basis for most of the cytotoxic and mutagenic effects reported in the literature in addition to those effects that may depend on GSH depletion and related redox perturbation (Esterbauer *et al.*, 1991; Uchida and Stadtman, 1992; Uchida *et al.*, 1993, 1994; Friguet *et al.*, 1994; Nadkarni and Sayre, 1995; Waeg *et al.*, 1996). Formation of these adducts has been also suggested as a possible additional basis for the biological action of HNE and related HAKs of different chain

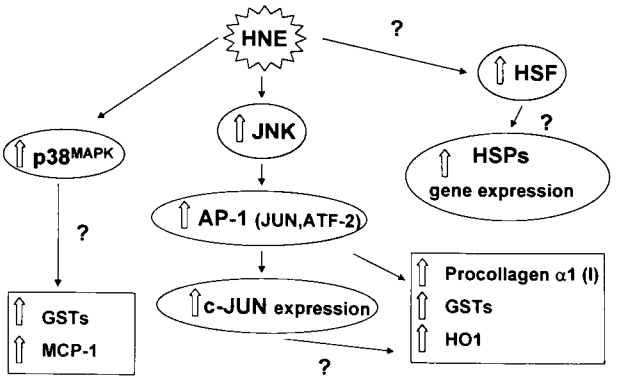


**FIG. 3. Signal transduction pathways known to be elicited by 4-hydroxynonenal in different cells with examples of related expression of defined genes.** Abbreviations used: HNE, 4-hydroxynonenal; JNK, c-Jun amino-terminal kinases; AP-1, transcription factor activator protein-1; HSF, heat shock transcription factor; HSPs, heat shock proteins; GSTs, glutathione-S-transferases; MCP-1, monocyte chemotactic protein 1; HO1, heme oxygenase 1.

lengths. However, although HNE interactions with proteins contribute unequivocally to the formation of adducts, only a few references (Blanc *et al.*, 1997; Parola *et al.*, 1998) provide direct evidence showing that formation of HNE adducts with specific proteins involved in signal transduction resulted in a well-defined biological effect. Several laboratories have developed polyclonal or monoclonal antibodies to recognize, *in vivo* or in cultured cells, the presence of these HNE-protein adducts (see Tables 1–5). The interested reader may find more chemical and biochemical details on HNE reactions elsewhere (Esterbauer *et al.*, 1991; Uchida and Stadtman, 1992, 1994; Friguet *et al.*, 1994; Nadkarni and Sayre, 1995; Waeg *et al.*, 1996).

## 4-HYDROXYNONENAL AS A BIOLOGICAL SIGNAL IN PATHOPHYSIOLOGY

The concept of HNE as a biological signal was first suggested in 1982 by a report describing chemotactic effects of this aldehyde (Curzio *et al.*, 1982). Within few years, many researchers realized that HNE and other HAKs were able to exert a number of biological actions when employed at very low (*i.e.*, nontoxic) concentrations in different experimental systems. Because the literature on this field is rapidly growing, we have decided tentatively to divide experimental and clinical evidence in a number of relatively homogenous aspects of pathophysiological conditions, rather than to simply review the available data.

### *HNE, G protein-related signal trasduction systems, and calcium signaling*

The first study in this field was suggested by the evidence that the *in vivo* toxicity of haloalkanes (*i.e.*, well-known pro-oxidant agents) was accompanied by a significant increase in the hepatic level of cyclic nucleotides, particularly cyclic adenosine monophosphate (cAMP) (Paradisi and Dianzani, 1979; Paradisi *et al.*, 1984). Using highly purified rat liver plasma membranes, a peculiar biphasic, dose-dependent and time-dependent effect of HNE on adenylate cyclase activity was reported (Paradisi *et al.*, 1985). The stimulatory effect was detected at low micromolar concentrations and was rather specific because another enzymatic activity associated with plasma membranes, such as 5′-nucleotidase, was unaffected. Adenylate cyclase activation was later confirmed also in plasma membranes isolated from AH-130 ascites hepatoma cells (Canuto *et al.*, 1995). The original report on adenylate cyclase was followed by a series of studies showing an analogous effect of HNE and other HAKs on phosphatidylinositol-4,5-diphosphate hydrolase (phospholipase C, or PLC; Rossi *et al.*, 1988, 1990, 1991, 1994). Interestingly, both enzymes are regulated by specific membrane guanosine triphosphate (GTP)-binding proteins (G-proteins) and are stimulated by HNE at concentrations ranging from 0.1 to 1 $\mu M$. Enzyme stimulation was seen immediately after HNE addition to isolated membranes and lasted for about 10 min. Maximal stimulation of adenylate cyclase and PLC occurred with 1 $\mu M$ and 0.1 $\mu M$ HNE, respectively, whereas inhibition was detected when higher doses of HNE were employed. Data actually available concerning the adenylate cyclase system suggest that HNE is able to interact specifically with Gi (G-inhibitory protein of adenylate cyclase complex), and then the stimulatory effect of HNE should operate through the inactivation of Gi on the basis of a pertussis toxin-independent mechanism (Dianzani, 1998). Conversely, HNE did not affect the response of adenylate cyclase to glucagon (Paradisi *et al.*, 1985), cholera toxin, and forskolin, indicating that HNE does not interact with the glucagon receptor or with Gs (G-stimulatory protein) or the catalytic subunit (Dianzani, 1998). As a result of adenylate cyclase stimulation, HNE should be able to elicit an increase in intracellular levels of cAMP and this should, at least in theory, deeply affect the activity of cAMP-dependent protein kinases (PKAs). However, this aspect is still unexplored.

Recently, HNE has been shown to interact directly with a G protein in cultured rat cerebrocortical neurons (Blanc *et al.*, 1997). In these cells subtoxic doses of HNE have been shown to impair signal transduction elicited by carbachol, a muscarinic agonist, and by (RS)-3,5-dihydroxyphenyl glycine, a metabotropic gluta-

mate receptor agonist. HNE inhibited the biological effects usually induced by the agonists, including the increase in GTPase activity, inositol phosphate release, and intracellular calcium concentration. Authors suggested that in this experimental model HNE was able to disrupt coupling of the receptors to PLC-linked GTP-binding proteins and that the effect was likely to depend on the detected formation of adducts between HNE and the GTP-binding protein G alpha (q/11).

Concerning PLC activation, the effect of HNE was found to depend on the peculiar molecular structure and on the presence of the hydroxy group in position C4 (see the section on chemical reactivity), since corresponding saturated or 2,3-unsaturated aldehydes, such as nonanal and 2,3-nonenal, were almost completely uneffective (Rossi *et al.*, 1994; Dianzani, 1998). As we will see, this feature will be evident for other biological effects. HNE-induced activation of PLC, at least in theory, should result in an increased production of inositol triphosphate ($IP_3$) and diacylglycerol (DAG) within target cells exposed to the aldehyde. DAG is known to stimulate several protein kinase C (PKC) isoforms, and, indeed, exposure of isolated rat hepatocytes to low concentrations of HNE has been reported to lead to an increase in calcium- and phospholipid-dependent PKC detectable enzymatic activity (Pronzato *et al.*, 1990, 1993). In addition, very recently it has been shown that low doses of HNE may differentially modulate the expression of different PKC isoforms (Chiarpotto *et al.*, 1999). HNE increased specifically PKC$\beta$I and PKC$\beta$II activities, paralleled by a marked stimulation of PKC-dependent transport of lysosomal procathepsin D from the trans-Golgi network to the endosomal–lysosomal compartment and exocytosis of mature cathepsin D. Moreover, HNE specifically inhibited PKC$\delta$ activity.

PLC is not the only phospholipase affected by the action of HNE. In 1992, a study performed on cultured NIH-3T3 fibroblasts provided evidence for an inhibitory effect of HNE on the sphingosine-stimulated phospholipid hydrolysis that usually results in a rise in the cellular content of phosphatidic acid (Kiss *et al.*, 1992). This has been suggested to depend on a selective inhibition by HNE of sphingosine-stimulated phospholipase D (PLD). In other experiments performed on cultured vascular endothelial cells, an opposite effect was reported (Natarajan *et al.*, 1993) because HNE was found to activate PLD in a dose-dependent manner. The activation of PLD by HNE was independent on extracellular calcium and PKC. Moreover, PLD activation was obtained also by exposing cultured cells to equal amount of other HAKs such as 4-hydroxyoctenal and 4-hydroxyhexenal. Nonanal was uneffective, whereas a certain degree of stimulation by *trans*-2,3-nonenal and *trans*-2,3-*cis*-nonadienal was detected. Interestingly, the effect was detected when HAKs and related compounds were added to the medium of cultured viable cells, whereas the same stimulatory effect on PLD was not reported when the assay was performed on isolated plasma membranes (Natarajan *et al.*, 1993). The same authors recently proposed that HNE-mediated activation of PLD may depend on the HNE-mediated activation of tyrosine kinases (Natarajan *et al.*, 1997). Again they used cultured vascular endothelial cells and a panel of tyrosine kinase (TK) and protein tyrosine phosphatase (PTPase) inhibitors to modulate the action of HNE. The use of the TK inhibitors genistein, erbstatin, and herbimycin attenuated HNE-induced PLD activation, whereas the PTPase inhibitors vanadate, phenylarsine oxide, and diamide enhanced HNE-induced PLD activation. Moreover, effects of inhibitors were specific for HNE, because these compounds were not effective against TPA-induced activation of PLD. In addition, these authors reported that HNE induced tyrosine phosphorylation of unidentified proteins having molecular weights in the range 40–60 kDa, 70–90 kDa, and 110–130 kDa.

Interference with G-proteins and activation of PLC should affect calcium homeostasis. Actually, exposure of isolated rat hepatocytes to micromolar concentrations of HNE (effective range 0.1–1.0 $\mu M$) led to an early and transient increase in cytosolic $Ca^{2+}$ concentration, followed by a late, more pronounced, and progressive elevation of this parameter (Carini *et al.*, 1996). The late increase in intracellular $Ca^{2+}$ was prevented by $Ca^{2+}$ chelation by EGTA or by the addition of gadolinium chloride ($GdCl_3$),

an agent known to block the activity of store operated $Ca^{2+}$ channels in hepatocyte plasma membrane. In addition, both the early and the late increases in intracellular $Ca^{2+}$ were abolished by U73122, a PLC inhibitor. When HNE was added to the medium 5 min after thapsigargin, a compound able to empty intracellular $Ca^{2+}$ pools, the aldehyde was able to cause a further increase in $Ca^{2+}$ accumulation; once again this effect was prevented by $GdCl_3$. It was concluded that HNE was able to cause the influx of $Ca^{2+}$ into the hepatocytes across $GdCl_3$-sensitive $Ca^{2+}$ channels and that the mechanism responsible for such elevation was triggered by the emptying of intracellular $Ca^{2+}$ pools, likely depending on HNE-mediated stimulation of PLC. In addition, authors suggested that the aldehyde may also interfere with the channel protein(s) or with the mechanism regulating capacitative $Ca^{2+}$ inflow. A rise in intracellular $Ca^{2+}$ concentration elicited by HNE has been detected more recently also in human platelets (Fowler *et al.*, 1998) and in neuronal cells (Mark *et al.*, 1997). Other mechanisms potentially leading to an intracellular rise of $Ca^{2+}$ have been described, including inhibition of high-affinity $Ca^{2+}$-ATPase in plasma membranes (Parola *et al.*, 1990) and inhibition of $Ca^{2+}$ sequestration activity into liver microsomes (Benedetti *et al.*, 1984b), as well as increase in passive $Ca^{2+}$ permeability (Raess *et al.*, 1997). However, these effects were detected only in the presence of high (*i.e.*, toxic) concentrations of HNE (range 0.05–1.0 m*M* or more).

### *4-Hydroxy-2,3-alkenals as chemotactic and pro-inflammatory stimuli*

The first report involving HAKs as bioactive molecules was published 17 years ago and showed that HAKs were able to exert chemotactic effects toward neutrophils (Curzio *et al.*, 1982). This original study was later confirmed and the pro-chemotactic effect was better characterized by several others studies published by the same group (Curzio *et al.*, 1982, 1983, 1985, 1986a, 1990; Rossi *et al.*, 1994) and by other researchers (Schaur *et al.*, 1994; Schaur and Curzio, 1995; Müller *et al.*, 1996). Curzio and co-workers, using the classic model of Boyden's chamber, showed that HAKs can exert chemotactic activity toward rat neutrophils and induce oriented migration and morphological polarization when used in a range of concentrations between $10^{-6}$–$10^{-11}$ *M*. The biological effect was independent of the chain length of the aldehyde. HNE was found to be active at concentrations around $10^{-6}$ *M*, whereas the most effective aldehyde in these experiments was found to be 4-hydroxyoctenal (HOE), still able to elicit chemotaxis at $10^{-11}$ *M*. Other HAKs have been described to elicit both oriented migration (chemotaxis) and random migration (chemokinesis) of rat neutrophils, including naturally occuring molecules such as 4-hydroxyhexenal (HHE) and 4-hydroxyundecenal (HUE), as well as the synthetic aldehydes 4-hydroxytetradecenal, 4-hydroxypentadecenal, and 4-hydroxyheptadecenal (Curzio *et al.*, 1982, 1983, 1985, 1986a). When the experiments were performed using human neutrophils, HNE was found to stimulate random migration in the range $10^{-6}$–$10^{-8}$ *M*, but a significant chemotactic effect exerted by HNE was observed only in some human neutrophil preparations (Curzio *et al.*, 1990).

Chemotaxis and chemokinesis were elicited only at concentrations lower than $10^{-6}$ *M*; when concentrations of HAKs higher than $10^{-5}$ *M* were used, a progressive, dose-dependent decrease of leukocyte motility was detected. Inhibition of cell motility was possibly the consequence of a dose-dependent increase in toxicity or the consequence of a block in cytoskeletal proteins (as shown in the past for tubulin) elicited by HAKs (Dianzani, 1982, 1998; Esterbauer *et al.*, 1991).

HNE-induced chemotaxis has been confirmed by Schaur and co-workers (Schaur *et al.*, 1994) using an acute and aseptic *in vivo* model of inflammation consisting of the subcutaneous injection of the polydextrane Sephadex G-200. HNE was detected in the exudate with a peak concentration concomitant with the highest turn-over rate of neutrophils and the highest rate of superoxide anion production, still preceeding the detection of the highest number of neutrophils. In addition, by adding synthetic HNE to the Sephadex gel, the number of neutrophils found at the inflammatory site was significantly enhanced. Finally, these authors

found also evidence suggesting that HNE could also be produced by self-destruction of neutrophils (Schaur *et al.*, 1994). In this connection, HNE generation may enter as a part of an autocatalytic cycle whereby neutrophils, which migrate into an inflammatory site, produce HNE that, in turn, stimulates the recruitment of new neutrophils. HNE may be generated as a result of NADPH oxidase activity in the phagosomes of human neutrophils (Quinn *et al.*, 1995).

Müller and co-workers (Müller *et al.*, 1996), have observed that HNE was able to exert a chemotactic effect toward human monocytes obtained from peripheral blood of healthy volunteers, confirming a previous report obtained with rabbit mononuclear cells (Moldovan *et al.*, 1994). Maximal chemotactic effect was reported at a concentration of 1.25–2.5 $\mu M$ that was not toxic versus monocyte–macrophages. This effect on human monocytes has been suggested to be relevant in conditions of atherosclerosis, because HNE has been found to be bound to oxidized low-density lipoprotein (LDL) (Esterbauer and Ramos, 1996).

Unfortunately, the pro-chemotactic mechanism of action of HNE and, more generally, of HAKs, is still unclear. From a chemical point of view, as for many other biological effects of these compounds, the presence of the hydroxyl group in position C4 seems necessary because alkanals and alkenals of corresponding chain length are usually devoid of any action. Interestingly, the CH=CH-CHO grouping that characterizes HAKs can be also found in a major aldehydic lipoxygenase metabolite, 12-oxododeca-5,8,10-trienoic acid, known to be formed from arachidonic acid in polymorphonuclear leukocytes (Glasgow *et al.*, 1986).

From a molecular point of view, it is well known that activation of PLC has been described as a common step in chemotaxis elicited by a series of well-characterized chemotactic peptides (f-met peptides), such as the model compound formyl-methionyl-leucyl-phenylalanine (fmlp). Actually, HNE has been found to stimulate, at very low concentrations, the activity of PLC in membrane preparations obtained from rat neutrophils and other cells (Rossi *et al.*, 1990, 1994). However, deactivation experiments clearly pointed out that HNE does not act on the receptors recognized by fmlp. Cells pretreated with fmlp were still able to respond to HNE and, similarly, neutrophils were still activated by fmlp after exposure to HNE. These results led to the search for a putative receptor for these compounds. By using [$^3$H]HNE no significant binding of this molecule was found at the level of the plasma membrane. However, Scatchard analysis revealed the possible existence of a yet undefined cytosolic receptor(s) that bind(s) HNE, obey the laws of agonist–receptor interactions, and behave as a medium-affinity receptor (Curzio *et al.*, 1994).

Other differences between the action of HNE and conventional chemoattractants have been described. fmlp, used as a model compound, is known to lead to an increased activity of NADPH oxidase and to the related increased generation of superoxide anion (*i.e.*, respiratory burst; Babior *et al.*, 1973, 1981). HNE is unable to evoke such a response in neutrophils but, curiously, is rather able to modulate in a dose-dependent way the respiratory burst elicited by fmlp (Di Mauro *et al.*, 1995; Dianzani, 1998). HNE concentration higher than $10^{-5}$ $M$ always resulted in inhibition of fmlp-induced superoxide anion generation in human neutrophils, whereas exposure of primed neutrophils to lower concentrations of the aldehyde resulted in a significant enhancement of this parameter (Dianzani *et al.*, 1996). HNE-dependent inhibition of NADPH oxidase-mediated superoxide anion formation in PMA-stimulated human neutrophils has been reported by others (Witz *et al.*, 1985; Siems *et al.*, 1997a). However, they described only an inhibitory effect with an $I_{50}$ value of 27 $\mu M$ (Witz *et al.*, 1985) and 19 $\mu M$ (Siems *et al.*, 1997b). Another major difference between HNE and other chemoattractants seems to exist: HNE has been reported to inactivate the synthesis of NO• from L-arginine (Di Mauro *et al.*, 1995), whereas chemoattractants usually lead to the generation of this reactive nitrogen intermediate, which is able to interact with superoxide anion to form peroxynitrite.

Whatever the mechanism elicited by HAKs, it is relevant to emphasize that HNE has been detected in inflammatory exudate (Curzio *et al.*, 1986b) and at the inflammatory site *in vivo*

(Schaur *et al.*, 1994; Schaur and Curzio, 1995). This suggests that these molecules, in the right place at the right time, may indeed play a role as mediators in the inflammatory process.

Recently, HNE has been suggested to stimulate the synthesis of monocyte chemotactic protein-1 (MCP-1) in hepatic stellate cells and then to promote the recruitment of circulating monocytes from peripheral blood to damaged hepatic parenchyma in the classic model of carbon tetrachloride ($CCl_4$)-induced liver injury (Marra *et al.*, 1999). Since HNE has been detected in several experimental and clinical conditions of chronic liver damage (see Poli and Parola, 1997), this finding may have relevant implications for the pathogenesis of liver fibrosis, which is known to depend on perpetuation of inflammatory reactions in hepatic parenchyma (Friedman, 1993; Pinzani, 1995) and, potentially, for any other chronic disease in which MCP-1 has been reported to be involved.

### *4-Hydroxynonenal and adaptative/defensive response of target cells to oxidative stress*

Exposure of bacteria and mammalian cells to oxidative stress and oxidative stress-related molecules is known to result in the induction of heat shock proteins (hsps) as well as of a number of enzymes able to defend the cells against oxidative stress itself. In 1988, evidence for oxidative stress-dependent induction of a subset of hsps in isolated rat hepatocytes and in rat hepatoma $MH_1C_1$ cells was reported (Cajone and Bernelli-Zazzera, 1988). Oxidative stress was induced by incubating cells with the pro-oxidant stimuli ADP-iron or, interestingly, mimicked by adding increasing concentrations of HNE to the medium. Results were compared with those obtained using a classic "heat shock" (1 hr of preincubation at 42°C). ADP-iron induced the synthesis of only three hsps in hepatocytes (having apparent molecular weights of 95, 80, and 31 kDa) and of only two hsps in $MH_1C_1$ cells (hsps of 100 and 85 kDa). When $MH_1C_1$ cells were exposed to HNE, only one hsp protein of 31 kDa, among those induced by heat shock, was found to be increased significantly. This increase in hsp 31, not found in $MH_1C_1$ cells exposed to ADP-iron, was dose-dependent and the effects of HNE and heat shock were found to be additive. Using human hepatoma cells (HepG2 cell line), in which ADP-iron was uneffective on hsps synthesis, HNE elicited a specific induction of hsp 70 gene expression and protein synthesis (Cajone and Bernelli-Zazzera, 1989). This paper was the first to show that HNE can stimulate the expression of a defined gene by inducing an increased transcription of its specific mRNA. In other experiments authors used electrophoretic gel mobility shift assay (EMSA) to prove that HNE was able to mimick heat shock in HeLa cells: HNE induced the appearance in cell extracts of a transcription factor (heat shock factor or HSF) able to bind the DNA sequence specific for the induction of heat shock genes, known as HSE or heat shock element (Cajone *et al.*, 1989). The activation of HSF by micromolar concentrations of HNE was later confirmed also in *in vitro* conditions (Cajone and Crescente, 1992) and found to be apparently independent on the action of HNE on sulphydryl groups. The same authors also showed that all the tested (E)-HAKs of different chain lengths were able to activate HSF and heat shock genes in HeLa cells. Once again this suggested that the (E)-2 double bond and the hydroxy group in position C4 were essential for activation (Allevi *et al.*, 1995). The major metabolite of HNE (E)-4-hydroxy-nonenoic acid as well as (e)-2-nonen-1,4-diol were uneffective whereas the glutathione-HNE adduct was able to elicit activation. Authors concluded, but did not prove, that an irreversible binding of HNE and related HAKs to proteins could be the first step of the mechanism by which these compound exerted biological effects.

HNE-induced activation of hsps has been confirmed *in vivo* by Hamilton and co-workers, who showed that HNE was responsible, at least in part, for the response of human lung cells to ozone. Human volunteers were exposed to defined levels of ozone in the air and then submitted to bronchoalveolar lavage to obtain lung cells; these cells, mostly alveolar macrophages, were found to contain increased amount of 32-kDa HNE-protein adducts and of 72-kDa HSP (Hamilton *et al.*, 1998). Interestingly, the same results were obtained by exposing alveolar macrophages directly to HNE.

Apart from hsps, exposure of cultured cells to micromolar concentrations of HNE has been shown to result in the induction of defined enzymatic activities that are known to recognize HNE, HAKs, and, more generally, α,β-unsaturated aldehydes as substrates. As mentioned earlier, HNE is an excellent substrate for GSTs (Alin *et al.*, 1985; Ishikawa *et al.*, 1986), and GSTs have been shown to exert a major role in the intracellular metabolism of α,β-unsaturated aldehydes (Esterbauer *et al.*, 1991; Canuto *et al.*, 1989, 1994; Singhal *et al.*, 1994, 1995; Hartley *et al.*, 1995; Hubatsch *et al.*, 1998). Addition of 50 μ*M* HNE and of 50 μ*M* HHE to the medium of rat liver epithelial cells (RL34 cell line) resulted in an increase of detectable GST enzymatic activity (Fukuda *et al.*, 1997). Induction was time-dependent and reached a plateau after 16 hr. GST-P homodimer (π class of GSTs) was identified as the major GST isoform induced by HNE in RL34 cells. HNE induced a significant increase in GST-P mRNA after 1 hr, with maximal transcription observed at 3 and 6 hr. A significant increase in the correspondent protein was evident at 16 and 24 hr. It is relevant to note that GST-P gene expression is dominantly regulated by an enhancer (GPE I) located approximatively 2.5 kb upstream from the transcriptional initiation site of GST-P gene, which contains the TPA-responsive element (TRE)-like sequence (Sakai *et al.*, 1987). Moreover, TRE is known to be a specific binding site for the transcription factors of the AP-1 family (Sakai *et al.*, 1987; Diccianni *et al.*, 1992). In this connection, it has been shown in different cells that HNE, even at lower concentrations (1–10 μ*M*), is able to activate AP-1 transcription factor very rapidly in a rather specific manner, being uneffective on DNA binding of the other redox sensor nuclear factor kappa B (NF-κB) (Camandola *et al.*, 1997; Parola *et al.*, 1998).

GST's induction by HNE has been recently confirmed using rat clone 9 hepatoma cells (Tjalkens *et al.*, 1998). To understand further the mechanism of induction, these cells were transfected with a luciferase reporter construct containing the 5'-flanking antioxidant response element (ARE) from rGST A1. HNE was able to induce an increased transcription for the α-class GST genes GST A1 and GST A4 mRNAs, as well as to induce an increase in the correspondent proteins (particularly the dimer GST A4-4) and enzymatic activities, likely by acting as activators of the ARE sequence. Once again, it is relevant to note that induction of AP-1 has been shown in the past to be a necessary step mediating the activation of ARE-dependent GST gene expression by several chemical agents (see Bergelson *et al.*, 1994 and references therein).

Another enzyme that is supposed to be involved in HNE metabolism is aldose reductase (Srivastava *et al.*, 1995; Vander Jagt *et al.*, 1995). This α-keto reductase is particularly abundant in the ocular lens (Das *et al.*, 1988), and its role as HNE metabolizing enzyme has been emphasized by the discovery that HNE added to ocular lens is able to induce *in vitro* cataract (Ansari *et al.*, 1996). Spycher and co-workers, using cultured rat vascular smooth muscle cells (A7r5 cell line) have shown a dose-dependent (range 1–10 μ*M*) and time-dependent induction of aldose reductase mRNA by HNE (Spycher *et al.*, 1996, 1997). Induction was confirmed at the protein level by immunoblotting and by assaying enzymatic activity; in addition, a very similar effect of induction was also reported for hydrogen peroxide (Spycher *et al.*, 1997). When A7r5 cells were exposed to toxic levels of HNE, the concomitant use of Sorbinil, an aldose reductase inhibitor, resulted in a marked increase of HNE-mediated cell death, suggesting that the induction of aldose reductase by HNE and hydrogen peroxide during oxidative stress may represent a general and relevant antioxidant defense mechanism.

Another interesting example of induction of a defensive enzyme has been provided by Liu and co-workers (Liu *et al.*, 1998). Using rat lung epithelial cells (L2 cell line), they have shown that HNE (range 5–20 μ*M*) was able to increase the transcription rates and the stability of mRNA for both the catalytic and regulatory subunits of γ-glutamyl-cysteine synthetase (GCS). GCS is a well known rate-limiting enzyme for *de novo* synthesis of GSH, the major water-soluble antioxidant which is known to form rapidly GSH-HNE adducts in the presence of HNE (Esterbauer *et al.*, 1991). Although HNE elicited the induction of both GCS subunits, the use of emetine, a protein synthesis inhibitor, resulted in the block of the increase in GCS regulatory subunit mRNA only (Liu *et al.*,

1998). *De novo* synthesis of GSH (after initial depletion) was also observed after treatment with rather high doses of HNE (250 $\mu M$) in the yeast *Saccharomyces cerevisiae* (Wonisch *et al.*, 1997).

Finally, HNE (1–30 $\mu M$) has been reported to activate human heme oxygenase 1 gene (HO-1) in human skin fibroblasts, one of the two known isoenzymes of HO system, the rate-limiting enzyme in heme catabolism (Basu-Modak *et al.*, 1996). HO-1 is a peculiar enzyme, considered as a redox sensor because it can be readily induced by oxidants in human skin fibroblasts and other cultured mammalian cell types (Keyse *et al.*, 1990; Applegate *et al.*, 1991). The report by Basu-Modak and co-workers establishes for the first time the existence of a direct correlation between exposure of cells to ultraviolet A (UVA) radiation, induction of lipid peroxidation in cell membranes, and a strong induction of the classic target gene for pro-oxidants induced by HNE. In this connection, it is well known that exposure of mammalian cells to UV light results in a gene induction response called the "UV response" (Holbrook and Fornace, 1991). This response includes the immediate early genes, particularly c-*jun*, and transcription factors AP-1 and NF-$\kappa$B (Büscher *et al.*, 1988; Stein *et al.*, 1989; Devary *et al.*, 1991). Increased transcription of c-*jun* and c-*fos* induced by UV light is known to be mediated through phosphorylation of transcription factors of the AP-1 family, such as c-Jun and ATF-2, by members of the so called c-Jun $NH_2$-terminal kinases (JNKs), also known as stress-activated protein kinases (SAPKs; Hibi *et al.*, 1993; Derijard *et al.*, 1994; Kyriakis *et al.*, 1994). If one excludes the lack of activation of NF-$\kappa$B, as we will see in more details in the next paragraph, HNE in the range 1–10 $\mu M$, is able to elicit exactly such a signaling pathway: activation of JNK isoforms, increased activation of AP-1 binding activity, and increased transcription of c-*jun* (Parola *et al.*, 1998; Uchida *et al.*, 1999). Actually, this pathway can lead to increased expression of several genes (Fig. 3).

### *HNE as a pro-fibrogenic molecule in chronic liver diseases*

*In vivo* detection of HNE and of HAKs is a common finding in several experimental and clinical conditions of chronic liver injury associated with active fibrogenesis. Clinical evidence for HNE and HAKs generation (see Table 2) has been provided for patients affected by chronic hepatitis C (Paradis *et al.*, 1997a), by alcoholic liver disease (Paradis *et al.*, 1997b; Aleynik *et al.*, 1998; Ohira *et al.*, 1998), by Wilson's disease, genetic hemochromatosis, and primary biliary cirrhosis (Paradis *et al.*, 1997b). Homologous experimental evidence has been obtained during liver fibrosis associated with chronic administration of the toxic pro-oxidant model compound $CCl_4$ (Parola *et al.*, 1992a; Bedossa *et al.*, 1994), extrahepatic cholestasis (Parola *et al.*, 1996a), iron overload (Houglum *et al.*, 1990), chronic ethanol consumption alone (Kamimura *et al.*, 1992; Niemela *et al.*, 1995, 1998; Li *et al.*, 1997), or chronic ethanol consumption associated with iron supplementation (Tsukamoto *et al.*, 1995).

Several lines of research suggest the existence of a close relationship between the generation of HNE (and HAKs) and excess deposition of extracellular matrix (ECM) components in the liver. This relationship was first suggested by the protective role exerted by vitamin E pretreatment (a procedure that is known to prevent the spreading of lipid peroxidation and then the generation of aldehydic end-products) in an experimental model of liver fibrosis (Parola *et al.*, 1992ab). Moreover, an evident association between HNE generation, infiltration of monocyte/macrophage cell populations, and collagen deposition has been detected in different experimental models of liver fibrosis (Parola *et al.*, 1992a, 1996a), suggesting that HNE (and related HAKs) may act as a profibrogenic signal. Because a crucial feature of chronic liver diseases is represented by chronic active hepatitis, HNE and HAKs may act simply as direct chemotactic agents as well as, indirectly, by eliciting recruitment of monocytes by sustaining the synthesis and the release of MCP-1 (Marra *et al.*, 1999), as previously mentioned in this review.

However, vitamin E pretreatment was able not only to reduce the extent of inflammatory reaction and of collagen deposition but also the hepatic gene expression for transforming growth factor-$\beta$1 (TGF-$\beta$1), a key profibrogenic

cytokine in liver fibrosis (Friedman, 1993) and for procollagen type I.

TGF-$\beta$1 is produced in the liver mainly by activated mononuclear cells or, in an autocrine loop, by activated hepatic stellate cells (HSC). HSC are known to be the cells responsible for excess deposition of ECM in fibrotic liver and the main cellular target for the profibrogenic action of TGF-$\beta$1 (Friedman, 1993; Pinzani, 1995). A recent study has provided *in vitro* evidence for an HNE-dependent up-regulation of TGF-$\beta$1 gene expression in cultured macrophage cell lines, suggesting that this event as a possible link between oxidative injury and fibrosclerosis (Leonarduzzi *et al.*, 1997). HNE (active range 1–10 $\mu M$) has been shown to elicit increased transcription of mRNA for TGF-$\beta$1 in either J774 murine macrophages and U937 human promonocytic cells as well as in rat Kupffer cells isolated from the liver of rat undergoing a model of cirrhosis obtained by chronic treatment with thioacetamide. The synthesis of the cytokine was not stimulated by nonanal and 2-nonenal in J774 and U937 cells.

A third direct mechanism by which HNE and other HAKs may elicit a profibrogenic effect has been outlined using cultured human hepatic stellate cells (hHSC) as experimental model. hHSC (also known as Ito's cells, liver fat-storing cells, or liver specific pericytes) are now recognized as the major source of ECM components in human fibrotic liver as well as cells able to contribute to hepatic fibrogenesis by producing growth factors, cytokines, and proinflammatory mediators (Friedman, 1993; Pinzani, 1995; Pinzani *et al.*, 1998). Cultured hHSC are also recognized as an excellent experimental model to study fibrogenesis, because these cells undergo in culture the same process of activation seen during chronic liver injury—transdifferentiation into an activated myofibroblast-like phenotype characterized by marked proliferation and secretion of ECM components. Addition of 1.0 $\mu M$ HNE to hHSC cultured for 24–48 hr in a serum- and insulin-free medium, to have quiescent cells and to avoid unspecific binding of HNE to serum proteins, resulted in an early and very significant increase in mRNA transcription for procollagen $\alpha$1(I) gene and synthesis of the protein (Parola *et al.*, 1993). mRNA was already up-regulated 1 hr after HNE addition and the increased transcription lasted up to 6 hr. The effect was rather specific because other ECM components, such as procollagen type III or fibronectin, were not apparently affected. This datum is conceptually relevant since it is known that during the development of liver fibrosis the physiological equilibrium between collagen type I and III disappears, and an uncontrolled rise in collagen type I synthesis and secretion occurs (Friedman, 1993). HNE-dependent stimulation of procollagen type I synthesis was confirmed either by exposing hHSC to the pro-oxidant stimulus ascorbate–iron (Parola *et al.*, 1993) or by co-culturing hHSC in the presence of fmlp-activated human neutrophils, a procedure that elicited a superoxide anion-mediated increase in lipid peroxidation (Casini *et al.*, 1997). In both cases the increase in procollagen type I gene expression was almost completely prevented with antioxidants, particularly with vitamin E, suggesting that the anti-fibrogenic effect of vitamin E described *in vivo* (Parola *et al.*, 1992a,b) may also depend on prevention of HNE and HAKs generation. Once again, all of the tested HAKs having chain lengths of 6, 8, and 11 carbon atoms (HHE, HOE, and HUE) were equally able to elicit procollagen type I gene expression in cultured hHSC. Nonanal and 2,3-nonenal were uneffective (Parola *et al.*, 1996b).

Because the biological effects of HNE have been suggested to depend also on its ability to form adducts with proteins by interacting with either sulphydryl groups of cysteine or amino groups of lysine and histidine (Esterbauer *et al.*, 1991; Uchida *et al.*, 1993, 1994; Uchida and Stadtman, 1994), monoclonal antibodies specific for HNE–histidine adducts (Waeg *et al.*, 1996) have been employed in the hHSC model to analyze morphologically and in terms of molecular biology the effects of HNE. This strategy was also suggested by the knowledge that collagen type I synthesis in HSC is not apparently affected by experimental manipulations of intracellular GSH levels (Maher and Neuschwander-Tetri, 1997). HNE (1–10 $\mu M$), led to an early generation of HNE–protein adducts that, by means of immunofluorescence combined with confocal laser microscopy, were detected in the nuclei of hHSC as soon as 5 min

after HNE addition. Nuclear fluorescence for immunoreactive HNE–protein adducts reached the highest level after 30 min (Parola *et al*, 1996c, 1998). Nuclear HNE–protein adducts of 46, 54, and 66 kDa were detected and p46 and p54 isoforms of JNKs were identified as HNE targets (Parola *et al.*, 1998). HNE not only led to nuclear translocation of JNK isoforms but also to their activation, particularly of p54, as a consequence of direct interaction of HNE to critical histidine residues in JNKs. This interpretation was suggested by the fact that upstream kinases in the JNK cascade were not involved and JNK isoforms translocated into the nuclei of hHSC were not phosphorylated (Parola *et al.*, 1998). In addition, NF-κB binding activity, a classic redox sensor, was not increased by HNE in hHSC, confirming previous results (Camandola *et al.*, 1997). This is relevant because NF-κB activation is known to require the activation of IκBα kinase complex, which in turn depends on the activation of the upstream kinase MEKK1 of the JNK cascade (Lee *et al.*, 1997). Moreover, JNK activation by HNE was not abolished by pretreatment of nuclear extracts with a specific low-molecular-weight protein tyrosine phosphatase, a procedure that affected interleukin-1α (IL-1α)-dependent conventional activation of the JNK cascade in the same cells (Parola *et al.*, 1998). JNK activation was followed by an early, impressive, and biphasic increase in AP-1 binding activity and by an increased transcription of c-*jun* proto-oncogene mRNA. The early increase in AP-1 was mainly represented by recruitment of Jun–Jun homodimers. The effect on JNKs was specific, since extracellularly regulated kinases 1 and 2 (ERK1/2) were not activated by HNE and no effect on cell proliferation was detected in hHSC (Parola *et al.*, 1998).

The prefential JNK/AP-1/c-*jun* pathway elicited by HNE and the lack of significant effects on ERK cascade have been confirmed by Uchida and co-workers in cultured rat liver epithelial RL34 cells (Uchida *et al.*, 1999). However, these authors suggested a mechanism involving HNE dependent intracellular generation of peroxides, mainly hydrogen peroxide, to explain the reported effects of HNE. It should be noted, however, that in these experiments HNE was used at a relatively high dose (25 μ*M*). In addition, they also described the HNE-dependent activation of p38 mitogen-activated protein kinase ($p38^{MAPK}$), another kinase belonging to the group of SAPKs.

Whatever the mechanism, it should be emphasized that activation of the JNK/AP-1/c-*jun* signaling pathway has been shown to be a relevant and necessary step for increased transcription of human procollagen type I gene expression in HSC (Armendariz-Borunda *et al.*, 1994; Chen and Davis, 1998, 1999). Furthermore, hHSC were found to be extremely sensitive to HNE for their substantial lack in HNE metabolizing activities (Parola *et al.*, 1998).

These data suggest that HNE generation in chronic liver diseases may have a role in sustaining chronic inflammation and excess deposition of ECM components in liver parenchyma.

### *HNE and development and progression of the atherosclerotic disease*

Experimental and clinical evidence suggests that oxidative modifications occurring in LDLs may play a crucial role in the development and progression of atherosclerotic disease (Witztum and Steinberg, 1991; Ross, 1993). Oxidized LDL (oxLDL) are known to activate endothelial cells to express adhesion molecules and to promote the recruitment of circulating monocytes in the subendothelial space and their transformation in resident macrophages. Moreover, oxLDL are avidly taken up by macrophages to form foam cells, are cytotoxic (at high concentrations) for endothelial cells, and can perturb endothelium-dependent vasomotion (Fogelman *et al.*, 1980; Berliner *et al.*, 1990; Rajavashisth *et al.*, 1990; Galle *et al.*, 1994; Escargueil-Blanc *et al.*, 1997). Considerable amounts of HNE are formed in LDL during peroxidation of polyunsaturated fatty acids (PUFA) and derivatize lysine, histidine, and cysteine residues in apoprotein B100 (Esterbauer *et al.*, 1992). Oxidized LDL have been found *in vivo* within the atherosclerotic plaques of different degrees of maturation, and immunostaining of these lesions with anti-HNE-lysine or anti-HNE-histidine monoclonal and polyclonal antibodies has revealed a strong positivity (Palinski *et al.*, 1990; Jurgens *et al.*,

1993; Napoli *et al.*, 1997). A colocalization of this material with apoprotein B100 has also been frequently found (Jurgens *et al.*, 1993; Napoli *et al.*, 1997), Although it could be extrapolated from these data that at least some of the biological features of oxLDL could be ascribed to HNE and its derivatization products, only scattered investigations to validate this hypothesis, however, have been performed so far.

In a recent study, Napoli *et al.* (1997) reported that HNE and oxidized LDL are present in the subendothelial space of human fetal aortas and precede the appearance of monocyte/macrophages and foam cells. The possibility that HNE could participate in recruiting circulating monocytes was experimentally validated, as already mentioned, by Müller *et al.* (1996), who found that HNE was a rather potent chemotactic stimulus (approximately 80% of the maximal chemotactic stimulation exerted by fMLP). Interestingly, this effect was achieved at 2.5 $\mu M$ HNE, a concentration well below that found in oxidized LDL and of the same order of magnitude of that employed in most investigations performed *in vitro* using oxidized LDL (2–5 $\mu M$, Müller *et al.*, 1996).

HNE-modified LDL is rapidly and efficiently taken up by macrophages. Extensive derivatization of $\epsilon$-amino groups on lysine residues of apo B100 resulted in LDL aggregation and marked increase in macrophage uptake and degradation of the lipoprotein aggregates (Hoff *et al.*, 1989). The uptake occurred by phagocytosis and was inhibited by cytochalasin D. It did not involve the operation of either LDL receptor or the classical scavenger receptor because neither native LDL nor acetyl-LDL failed to inhibit uptake and degradation competitively. These findings were confirmed by ultrastructural studies (Hoff and Cole, 1991), which revealed a close association of HNE-modified LDL aggregates with clathrin-coated pits on the cell surface, frequently surrounded by pseudopodia. A time-dependent increase was also found in the amount of HNE-LDL within vacuoles, some of which were secondary lysosomes. HNE-derivatized LDL were also shown to be more resistant than native LDL or MDA-derivatized LDL to proteolysis by lysosomal enzymes (Jessup *et al.*, 1992) and to accumulate within macrophages as a high-molecular-weight fraction.

Smooth muscle cell proliferation, differentiation, and migration are all rather typical features of atherosclerotic lesions (Ross, 1993). Ruef *et al.* (1998) have investigated the effects of HNE on rat aortic smooth muscle cell growth. They found that concentrations below 2.5 $\mu M$ significantly stimulated cell growth as measured by cell count, [$^3$H]thymidine uptake, and incorporation of bromodeoxyuridine. This was related to extracellular signal-regulated protein kinases ERK1 and ERK2, induction of c-*fos* and c-*jun*, and increase in transcription factor AP-1–DNA binding activity. In addition, HNE induced the expression of platelet-derived growth factor (PDGF)–AA and a monoclonal anti-PDGF–AA antibody markedly prevented HNE-induced proliferation effects.

Cytotoxic activity of HNE may also account for direct and indirect destruction of both lipid-loaded macrophages and endothelial cells (Müller *et al.*, 1996; Karlhuber *et al.*, 1997). However, HNE may affect endothelium-dependent vasomotion at lower and nontoxic concentrations. Martinez *et al.* (1994) and Romero *et al.* (1997) have demonstrated that HNE promotes relaxation of both human cerebral and mesenteric arterial rings in a dose-dependent manner. Removal of endothelium or treatment with NG-nitro-L-arginine methyl ester hydrochloride partially prevented HNE-induced relaxation, thus suggesting the intervention of nitric oxide from endothelial origin in the vascular effects of oxidized LDL-derived HNE.

As reported above, various steps of the atherogenic process seem to be critically related to oxLDL, and few investigations indicate that HNE may exert comparable effects. It must be stressed that clear-cut evidence in favor of the direct involvement of HNE in atherosclerosis is still lacking. Moreover, the possibility that all the biological properties of oxLDL could be linked to HNE derivatization is highly unlikely. Nonetheless, the available evidence allows the tempting assumption that HNE might be one of the various signal molecules involved in the complex biology of the atherosclerotic lesions.

*HNE, cell proliferation, and gene expression*

Lipid peroxidation is usually low or negligible in rapidly proliferating tissues such as testis, bone marrow, intestinal epithelium, and regenerating liver, as well as in highly proliferating neoplastic cells (Dianzani, 1993). Moreover, a direct correlation between the degree of tumor cell differentiation and the susceptibility to undergo lipid peroxidation has been shown to exist (Dianzani, 1989). In this connection, HNE levels are lower in poorly differentiated Yoshida AH-130 hepatoma cells than in highly differentiated $MH_1C_1$ hepatoma cells (Hammer *et al.*, 1997). Similarly, lipid peroxidation does not occur in highly undifferentiated leukemic cells, such as K562 and HL-60 cells, even after prolonged exposure to prooxidant agents (Barrera *et al.*, 1991c; Fazio *et al.*, 1992). These data suggest an inverse relationship between lipid peroxidation and cell growth.

HNE acts as an inhibitor of cell proliferation, as demonstrated unequivocally in several cell lines: Ehrlich ascites tumor cells (Hauptlorenz *et al.*, 1985), human leukemic K562 cells (Barrera *et al.*, 1987; Fazio *et al.*, 1992), amniotic fluid fibroblast-like cells and human diploid skin-derived cells (Poot *et al.*, 1988), human diploid skin fibroblasts (Poot *et al.*, 1988), HL-60 human leukemic cells (Barrera *et al.*, 1991c), *Saccaromyces cerevisiae* (Wonisch *et al.*, 1995), murine melanoma B16-F10 cells (Zarkovic *et al.*, 1995), and human peripheral blood lymphocytes challenged with PHA (Cambiaggi *et al.*, 1997). Only two reports seem to contradict this generalized inhibitory effect of HNE. Physiological doses of HNE (Zarkovic *et al.*, 1993) were able to induce a modest increase of proliferation in HeLa cells after a transient inhibitory effect. More recently, as mentioned before, HNE has been described (Ruef *et al.*, 1998) to induce rat aortic smooth muscle cell growth.

Results obtained from different groups should be analyzed carefully by considering that, if compared with other cytostatic substances, HNE is highly reactive and disappears from the culture medium in a few minutes. HNE easily reacts with sulphydryl and amino groups of serum proteins, and then in the presence of serum HNE may be not completely available for the cells (Barrera *et al.*, 1991a, 1996a). In addition, as already mentioned, quantitative and qualitative aldehyde-metabolizing enzyme patterns vary in cells of different origin, also depending on the degree of differentiation (Canuto *et al.*, 1993a,b). As an example, HNE consumption rate was 130–230 nmol/min per $10^6$ cells in normal rat hepatocytes (Ferro *et al.*, 1988), 14 nmol/min per $10^6$ cells in MH1C1 hepatoma cells (Ferro *et al.*, 1988), 9 nmol/min per mg in Ehrlich mouse ascites cells (Grune *et al.*, 1994a), and only 2 nmol/min per $10^6$ cells in K562 cells, in the first 5 min after addition (Barrera *et al.*, 1991b).

The mechanisms by which HNE may control cell proliferation have been investigated mainly using cultured cells as experimental models. HNE, at concentrations compatible with those observed in normal, nondividing cells (1–10 $\mu M$), was found to inhibit ornithine decarboxylase activity in a dose-dependent way (Barrera *et al.*, 1991a) and to affect the expression of crucial oncogenes involved in the control of cell proliferation in K562 and HL-60 cells (Fazio *et al.*, 1992, 1993; Barrera *et al.*, 1994, 1996b). HNE was found to down-modulate the expression of c-*myc* and c-*myb* from 1 to 6 hr after the treatment. Run-on transcription analysis demonstrated that early and rapid decline of c-*myc* gene expression was likely to depend on a trascriptional block of the third exon, the subsequent decrease of the steady state level of c-*myc* mRNA being dependent on a post-trascriptional mechanism (Fazio *et al.*, 1992). Both duration and intensity of the HNE-mediated inhibitory effect on c-*myc* and c-*myb* expression were dose-dependent in K562 and HL-60 cells. However, the expression of N-*ras* and c-*fos* oncogenes was unchanged, suggesting that HNE may act preferentially on defined genes. All of these experiments were performed by exposing cultured cells to a single dose of HNE.

To investigate whether an increase of the time of exposure of cultured cells to HNE could amplify its biological effects, cells were repeatedly treated with single doses of HNE (1 $\mu M$) at intervals of 45 min for several hours (from 8 to 12 treatments) (Barrera *et al.*, 1991c).

Using this experimental procedure, a higher degree of inhibition of cell growth as well as of c-*myc* and c-*myb* gene expression has been observed. Interestingly, the repeated treatment with 1 $\mu M$ HNE resulted in a stronger inhibition of c-*myc* and c-*myb* expression in HL-60 cells than that observed with a single treatment with 10 $\mu M$ HNE. Moreover, the inihibitory effect lasted for a longer period of time (Barrera *et al.*, 1994, 1996b).

*HNE, cell differentiation, and cell cycle progression*

It is well known that several biologically active molecules, such as dimethylsulfoxide (DMSO) and retinoids, are able to inhibit cell proliferation but also to induce differentiation in leukemic cells when these cells are maintained for some hours in the presence of the inducer. When K562 cells were treated with a single dose of HNE (1 or 10 $\mu M$), a marked, but transient, increase of gamma globin gene expression was detected, suggesting that leukemic cells were entering the differentiation pathway (Fazio *et al.*, 1992). To study the effects of HNE on differentiation, Barrera and co-workers (1991c) used HL-60 cells and the procedure of repeated single treatments of HNE. HL-60 cells can be induced to differentiate along the granulocytic or the monocytic–macrophagic lineage depending on the inducer used. DMSO is a common inducer of the granulocytic lineage (Collins *et al.*, 1978), whereas TPA induces a monocytic-macrophagic differentiation pattern (Rovera *et al.*, 1979). After HNE treatments, HL-60 cell differentiation was evaluated by assaying the phagocytic activity, generation of chemiluminescence, and expression of the differentiation-associated surface antigens CD11b, CD67, and CD36. Data were compared with those obtained by exposing cells to DMSO for 7.5 hr (same overall time of aldehyde treatment) or for the whole length of the experiments (5 days) (Barrera *et al.*, 1991c, 1996c). In HNE-treated cells, the number of phagocytic cells gradually increased from day 2 up to a maximum of 35% at day 5. In DMSO-treated cultures, the increase of phagocytic cells was negligible after the short (7.5 hr) treatment, whereas on continuous exposure the fraction of phagocytic cells progressively increased from day 2 up to a maximum of 63% at day 5. Expression of CD11b (a leukocyte integrin subunit that occurs on the surface of both human monocyte and monocyte–macrophages) and CD67 (a granulocyte-specific antigen) increased in cells treated with HNE or continuously exposed to DMSO, whereas CD36 (monocytic-specific antigen) was always expressed at low levels. Antigen expression studies and morphological analysis indicated that HNE, like DMSO, induced preferentially a pattern of granulocytic differentiation in HL-60 human leukemic cells.

Additional indirect evidence that HNE is involved in the induction of leukemic cell differentiation was provided by Rinaldi *et al.* (1998), who showed that treatment of K562 cells for 12 hr with micromolar HNE concentrations strongly reduced susceptibility of K562 cells to NK cells. This event occurs with other well-known inducers of differentiation.

Finally, HNE was tested for its effects on cell cycle progression. When HL-60 cells were analyzed after 10 repeated treatments with 1 $\mu M$ HNE (Barrera *et al.*, 1996c), a marked increase in the proportion of $G_0/G_1$ cells (up to 80% of total cell number) was observed. A similar increase in the number of $G_0/G_1$ cells after treatment with HNE was also reported in *Saccharomyces cerevisiae* by Wonisch and co-workers (1998). In this connection, very recently Pizzimenti and co-workers (1999) demonstrated that HNE (single treatment with 1 and 10 $\mu M$ concentrations and repeated treatments with 1 $\mu$M concentration) down-regulated both mRNA and protein contents of cyclins D1, D2, and A until 24 hr after the treatment. These three cyclins are involved in $G_1$ phase progression (D1 and D2 cyclins) and in the $G_1/S$ transition (cyclin A) (Dulic *et al.*, 1992). On the contrary, cyclin E, which is known to be involved in the S-phase progression, and the mitotic cyclin B were not affected by the aldehyde (Pines and Hunter, 1990; Walker and Maller, 1991; Pagano et al., 1992; Scherr, 1993). These results are in agreement with the previously reported observations indicating an accumulation of HL-60 cells in the $G_0/G_1$ phase accompanied by a reduction of cells in S phase at 24 and 48 hr after HNE treatment.

## CONCLUSIONS

We have reviewed current literature that suggests a role for HNE and related HAKs as biological signals potentially able to modulate the response of cells exposed to low, nontoxic, concentrations of these compounds (range 0.1–1 $\mu M$) that can be reached in normal conditions as well as in conditions of mild to moderate oxidative stress. An overall survey of the literature in this field (*i.e.*, biological effects of HNE and HAKs) suggests that these compounds, when present at relatively high concentrations (usually over 10 $\mu M$), should be considered mainly as toxic and mutagenic mediators of oxidative stress-dependent injury, particularly under conditions of acute oxidative stress, leading to necrosis or apoptosis. Although the two main effects (toxic/mutagenic and as a signal) may be both present in the same disease, as probably may happen in degenerative diseases of central nervous system (Markesbery, 1997; Keller and Mattson, 1998), it is tempting to speculate that the reported action of HAKs as biological signals should be mainly involved in the regulation of physiological events and in the pathogenesis of some chronic diseases in which HAKs have been reported to occur (see Tables 1–5). In particular, HAKs, as a consequence of their reported pro-chemotactic, pro-inflammatory, and pro-fibrogenic action, may contribute to the progression of those human diseases characterized by chronic tissue injury, chronic active inflammation and excess deposition of extracellular matrix.

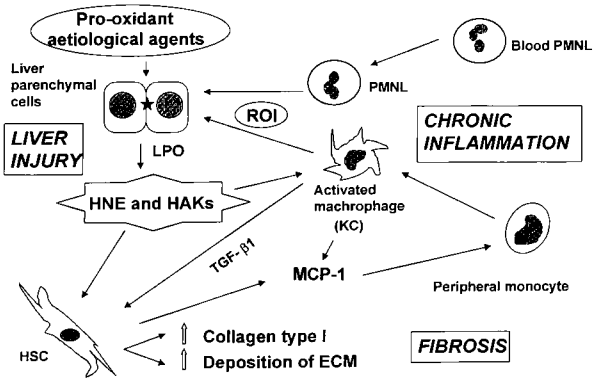


**FIG. 4. Proposed role for 4-hydroxynonenal in the development of chronic liver diseases.** Abbreviations used: HNE, 4-hydroxynonenal; HAKs, 4-hydroxyalkenals; LPO, lipid peroxidation; ROI, reactive oxygen intermediates; PMNL, polymorphonuclear leukocytes; KC, kupffer cells; HSC, hepatic stellate cells; TGF-$\beta$1, transforming growth factor-$\beta$1; MCP-1, monocyte chemotactic protein 1.

The authors' experience is mainly in the field of liver fibrosis, and available data indeed suggest that the contribution of HNE and HAKs may rely in the perpetuation of chronic inflammation by recruiting either polymorphonuclear leukocytes and monocytes in the injured liver parenchyma. HAKs may also contribute to fibrosis by stimulating macrophage populations (essentially Kupffer cells) to synthetize TGF-$\beta$1 and to stimulate hepatic stellate cells (*i.e.*, the cell responsible for excess deposition of ECM in liver fibrosis) to produce increased amount of fibrillar collagen (see Figure 4), particularly when these cells are in the activated state that is typical in chronic active hepatitis. This may apply also to other human chronic diseases. Moreover, it should be mentioned that HNE has been detected as a physiological component of human plasma at levels of 0.2–0.6 nmol/ml (Esterbauer *et al.*, 1991; Strohmaier *et al.*, 1995). HNE may then act in normal conditions as a factor able to contribute potentially to the regulation of the equilibrium between proliferation and differentiation. In this connection, HNE may favor differentiation, as suggested by experiments in which cultured cells were repeatedly exposed to nontoxic, low levels of HNE (1 $\mu M$), and by the notion that HNE and lipid peroxidation are usually extremely low or even absent in rapidly dividing cells.

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## ABBREVIATIONS:

ADH, Alcohol dehydrogenase; ADP, adenosine diphosphate; cAMP, cyclic adenosine monophosphate; ALDH, aldehyde dehydroge-

nase; AP-1, activator protein-1; ARE, antioxidant responsive element; ATPase, adenosine triphosphatase; ATF-2, activator transcription factor-2; $CCl_4$, carbon tetrachloride; DAG, diacylglycerol; DMSO, dimethylsulfoxide; ECM, extracellular matrix; EGTA, ethylene glycol-bis($\beta$-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; EMSA, electrophoretic mobility shift assay; ERK-1, extracellular regulated kinase 1; ERK-2, extracellular regulated kinase 2; FCS, fetal calf serum; fmlp, *N*-formyl-met-leu peptide; G-proteins, GTP-binding proteins; GCS, $\gamma$-glutamyl-cysteine synthetase; $GdCl_3$, gadolinium chloride; Gi, G-inibitory protein; GPE I, $\pi$ class of glutathione-S-transferase enhancer I; Gs, G-stimulatory protein; GSH, glutathione; GST, glutathione-S-transferase; GST A1, glutathione-S-transferase A1; GST A4, glutathione-S-transferase A4; GST-P, $\pi$ class of glutathione-S-transferase; GTP, guanosine triphosphate; GTPase, guanosine triphosphatase; HAKs, 4-hydroxy-2,3-alkenals; HHE, 4-hydroxy-2,3-hexenal; HO, heme oxygenase; HO-1, heme oxygenase 1 gene; HOE, 4-hydroxy-2,3-octenal; HNE, 4-hydroxy-2,3-nonenal; HSC, hepatic stellate cells; hHSC, human hepatic stellate cells; HSE, heat shock element; HSF, heat shock factor; HSP, heat shock protein; HUE, 4-hydroxy-2,3-undecenal; $IP_3$, inositol-3-phosphate; JNK, c-*jun* $NH_2$-terminal kinase; LDL, low-density lipoprotein; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotactic peptide 1; MDA, malonyldialdehyde; MEKK-1, mitogen-activated protein kinase/ERK kinase kinase-1; NADPH, nicotinamide adenine dinucleotide phosphate reduced form; NF-$\kappa$B, nuclear factor Kappa B; NO•, nitric oxide; ox, oxidized; PDGF-AA, platelet derived growth factor AA; PDGF-BB, platelet derived growth factor BB; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PLD, phospholipase D; PMA, phorbol myristate acetate; $P^{38MAPK}$, $P^{38}$ mitogen-activated protein kinase; PTPase, phosphotyrosine phosphatase; PUFA, polyunsaturated fatty acids; rGST A1, rat glutathione-S-transferase A1; ROI, reactive oxygen intermediates.; SAPK, stress activated protein kinase; TGF-$\beta$1, transforming growth factor-$\beta$1; TK, tyrosine kinase; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; TRE, TPA-responsive element; UV, ultraviolet; UVA, ultraviolet A.

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