This paper is available online at www.jbc.org

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 283, NO. 23, pp. 15515–15519, June 6, 2008

Nitro-fatty Acid Formation and Signaling*[S]

Published, JBC Papers in Press, February 19, 2008, DOI 10.1074/jbc.R800004200

Bruce A. Freeman[‡1], Paul R. S. Baker[‡], Francisco J. Schopfer[‡], Steven R. Woodcock[‡], Alessandra Napolitano[§], and Marco d'Ischia[§2]

From the [‡]Department of Pharmacology & Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213 and the [§]Department of Organic Chemistry and Biochemistry, University of Naples, I-80126 Naples, Italy

Enzymatic and non-enzymatic oxygenations of unsaturated fatty acids yield a broad family of autocrine and paracrine cell signaling mediators. Soon after nitric oxide (•NO) was described as a mediator of vascular relaxation, it was appreciated that this lipophilic free radical species strongly influences fatty acid oxygenation at multiple levels. For example, •NO terminates peroxyl radical-induced chain propagation reactions of lipid peroxidation at rate constants $>10^3$ faster than tocopherols (1, 2). Also, the gene expression and catalytic activity of enzymes responsible for prostaglandin, thromboxane, and leukotriene biosynthesis are regulated by •NO (3–5). Another convergence of lipid and •NO signaling is manifested in the form of nitrated unsaturated fatty acids. Fatty acid nitration is induced by •NO-derived species reacting via multiple mechanisms that share a proclivity for the homolytic addition of nitrogen dioxide ($^{\bullet}NO_2$) to the double bond, yielding an array of regio- and stereoisomers (6). NO_2-FAs[3] display both cGMP-independent and receptor-dependent signaling actions as well as robust electrophilic reactivity, facilitating reversible adduction by nucleophilic targets (*e.g.* protein Cys and His residues) (7, 8). This reactivity in turn supports the post-translational modification of protein distribution and function. Both NO_2-FAs and their protein or GSH adduction products are detected clinically in healthy individuals, become elevated postprandially, and are formed by oxidative inflammatory reactions (8–12). Current data indicate that NO_2-FAs signal via predominantly •NO-independent mechanisms, acting via electrophilic and receptor-mediated reactions to exert adaptive and anti-inflammatory cell responses.

Mechanisms of Fatty Acid Nitration

The biochemistry of fatty acid nitration stems from the reactions of •NO, •NO-derived oxides of nitrogen (*e.g.* nitrogen dioxide ($^{\bullet}NO_2$) and peroxynitrite ($ONOO^-$)), and oxygen-derived inflammatory mediators (*e.g.* superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and lipid peroxyl radicals ($LOO^{\bullet}$)). Multiple mechanisms induce biomolecule nitration, with this redundancy supporting the concept that nitration reactions transduce •NO signaling and tissue inflammatory responses. The formation of secondary •NO-derived species and the subsequent reactions that mediate biomolecule nitration are dictated by •NO concentration; oxygen tension; site of generation; local concentrations of targets, catalysts, and scavengers; and partitioning between hydrophobic and hydrophilic compartments (13). These factors also reflect cell metabolic and inflammatory status and ultimately govern relative extents of target molecule oxidation, nitrosation, and nitration.

Peroxynitrite-mediated—Appreciation of the •NO- and $O_2^{\bullet-}$-dependent formation of the oxidizing and nitrating species $ONOO^-$ shed new light on the myriad of downstream effects this reaction has on redox signaling (14, 15). In addition to reaction with thiols, $ONOO^-$ reaction with carbon dioxide predominates in tissues, yielding $ONOOCO_2^-$. In an inflammatory milieu, both protonated $ONOO^-$ ($ONOOH$) and $ONOOCO_2^-$ yield $^{\bullet}NO_2$ along with •OH and $^{\bullet}CO_3^-$ as products that can support biomolecule nitration reactions (16).

Peroxidase-catalyzed and Fenton-induced Oxidation of NO_2^- to $^{\bullet}NO_2$—Peroxidases catalyze the reduction of H_2O_2 and organic peroxides and in turn catalyze the oxidation of organic compounds, inorganic anions, and halides. Upon oxidation by hydroperoxides, the oxidation of NO_2^- to $^{\bullet}NO_2$ is mediated by peroxidases, microperoxidases, hemeproteins, and transition metals (*e.g.* Fe(II) acting via iron-catalyzed Fenton chemistry) (17–19).

Protonation of NO_2^-—Nitrite (NO_2^-), the principal physiologic metabolite of •NO, can be protonated to nitrous acid (HNO_2, pK_a 3.4) in acidic tissue environments such as the gastric compartment, phagolysosomes. and endosomes. The further reactions of HNO_2 in an aqueous milieu then yield a spectrum of nitrating and nitrosating species that induce biomolecule nitration via either free radical or ionic addition reactions.

Acidic nitration can be a confounding event in NO_2-FA analysis and quantification because of the low pH and adventitious NO_2^- that can be experienced upon sample extraction, hydrolysis, derivatization, and chromatographic separations.

Aerobic Reactions of •NO—Because of a small molecular radius and uncharged nature, •NO is lipophilic and can concentrate up to 20-fold and more readily react with the molecular O_2 that also preferentially partitions into a hydrophobic milieu (20). This molecular "lens" effect induced by •NO and O_2 concentration in hydrophobic compartments can accelerate •NO oxidation by 2–3 orders of magnitude. This lens effect thus promotes the formation of secondary •NO-derived species from O_2 ($k = 2 \times 10^6\ M^{-2}\ s^{-1}$), including $^{\bullet}NO_2$ (Fig. 1) (21).

The chemistry of unsaturated fatty acid nitration and detection of reaction products *in vivo* is dictated not only by the intrinsic reactivity of the double bonds, but also by the biological microenvironment of target molecules that includes factors such as (*a*) relative fluxes of instigating oxidizing and nitrating

* This work was supported, in whole or in part, by National Institutes of Health Grants R01 HL58115 and R01 HL64937. This work was also supported by the American Diabetes Association, the American Heart Association, and the Ministry of Health (Rome). This is the second article of seven in the Oxidized Lipids Minireview Series. This minireview will be reprinted in the 2008 Minireview Compendium, which will be available in January, 2009.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3, Tables 1–7, and additional references.

[1] To whom correspondence may be addressed. E-mail: freerad@pitt.edu.

[2] To whom correspondence may be addressed. E-mail: dischia@unina.it.

[3] The abbreviations used are: NO_2-FA, nitro-fatty acid; MS, mass spectrometry; HPLC, high pressure liquid chromatography; RSNO, *S*-nitrosothiol; LNO_2, 9,10,12- and 13-nitro-9,12-*cis*-octadecadienoic acid (nitrated linoleic acid); PMN, polymorphonuclear leukocyte; VCAM-1, vascular cell adhesion molecule-1; heme oxygenase-1, HO-1; PPAR, peroxisome proliferator-activated receptor; TZD, thiazolidinedione.

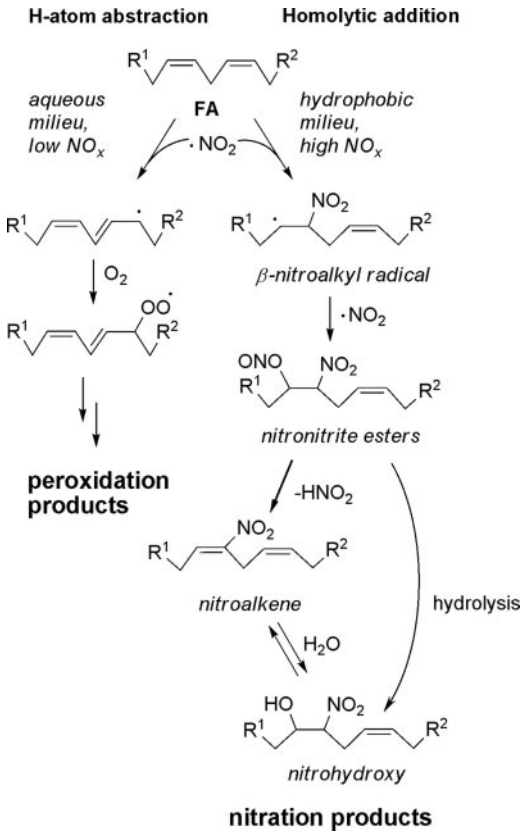

FIGURE 1. **Mechanisms of fatty acid nitration and oxidation by nitric oxide-derived species.**

species; (*b*) adventitious catalysts, antioxidants, and nucleophiles; and (*c*) local rates of fatty acid esterification, de-esterification, and β-oxidation reactions. Unsaturated fatty acids such as linoleic acid, oleic acid, and their congeners represent important targets because of both their abundance and an availability of double bonds susceptible to $^{\cdot}NO_2$ attack via both homolytic and heterolytic (ionic) reactions. In addition, the bisallylic methylene centers represent another potential reactive site of polyunsaturated fatty acids because of their susceptibility to hydrogen atom abstraction.

Nitrated unsaturated fatty acids identified *in vivo* to date belong to two main groups, nitroalkene and nitrohydroxy (nitroalcohol) derivatives, with preliminary observations also suggesting fully saturated and allylic NO_2-FA derivatives. Chemically, *E*-nitroalkene derivatives of unsaturated fatty acids and their esters are formed *in vitro* under a variety of conditions. They represent relatively abundant and less polar components of mixtures obtained by reaction of fatty acids or esters with (i) $^{\cdot}NO$ in air-equilibrated cyclohexane (22, 23) or (ii) acidic NO_2^- in a biphasic cyclohexane system (24, 25). Fatty acid nitroalkene formation is invariably accompanied by other nitration products, including, besides the *Z*-isomers, allylic nitro, nitronitrate, and dinitro derivatives as well as hydroxynitro compounds (see supplemental material for NMR, IR, and MS characteristics of many of these species) (24, 26). Oxygen is essential for fatty acid reactions with $^{\cdot}NO$, consistent with the chemical inertness of $^{\cdot}NO$ toward alkenes. In aerobic $^{\cdot}NO$-promoted reactions, the chemical course and product composition change considerably with $^{\cdot}NO$ concentration and solvent polarity: nitrated species are the main products in cyclohexane, whereas peroxidation products predominate in neutral aqueous phosphate buffer (22, 23).

In the first step of fatty acid nitration, $^{\cdot}NO_2$ adds to alkene double bonds via a fast and reversible process to initially form β-nitroalkyl radicals. In hydrophobic environments, where the solubility of $^{\cdot}NO$ and related nitrogen oxides is relatively high, these radicals can evolve to give nitrated derivatives. However, in an aqueous milieu, where the solubility of $^{\cdot}NO$ and related species is about an order of magnitude lower, trapping of the β-nitroalkyl radical is less efficient. The radical intermediates then revert, and the slow but irreversible allylic hydrogen atom abstraction by $^{\cdot}NO_2$ (for $^{\cdot}NO_2$, $k_{add}/k_{abs} \sim 10^3$–$10^6$) eventually prevails, initiating chain peroxidation reactions (27). Under conditions in which O_2 tension is low (*e.g.* ischemia and anoxia), the balance between peroxyl radical formation and coupling with $^{\cdot}NO_2$ shifts toward nitration reactions. With regard to stereochemistry of the products, the nitro group preferentially constrains the double bond of nitroalkene derivatives in the *cis* (*E*)-configuration, with only a minor proportion present as *trans*-isomers. This implies that the original double bond configuration of the parent fatty acid is retained in nitrated derivatives. The two primary reaction pathways of polyunsaturated fatty acids with the proximal nitrating species $^{\cdot}NO_2$ are outlined in Fig. 1. Chemical evidence indicates that nitration reactions can also occur in the course of lipid peroxidation, modifying conjugated diene intermediates that are generated by enzymatic and non-enzymatic peroxidation of linoleic acid. Carbon chain breakdown and rearrangements induced by HNO_2 and $ONOO^-$, respectively, are also possible events leading to secondary fatty acid nitration products (28).

Chemical Reactivities of Nitro-fatty Acids

Analysis of synthetic nitroalkene derivatives of oleic, linoleic, and arachidonic acid reveals that these species possess unique chemical reactivities that support pluripotent cell signaling events. NO_2-FAs act via both receptor-dependent and -independent mechanisms that are subject to regulation by the relative distribution of NO_2-FAs in hydrophobic and aqueous microenvironments (Fig. 2). At present, there is not strong support for a mitigating role by NO_2-FA-derived $^{\cdot}NO$ production.

Electrophilic Reaction with Water—The electrophilicity of fatty acid nitroalkene derivatives is central to their subsequent reactions, e.g. Michael conjugate addition with the low amounts of hydroxide anion always present in aqueous solution at physiologic pH. An equilibrium between nitroalkene derivatives and their corresponding vicinal nitrohydroxy fatty acids is indicated by nitrohydroxy fatty acid derivatives in cardiac tissue, blood, and urine (6, 12, 29). The presence of the highly polar hydroxyl group in nitrohydroxy fatty acid derivatives results in these products behaving more like saturated fatty acids.

Aqueous Decay and $^{\cdot}NO$ Release—A mechanism accounting for $^{\cdot}NO$ release by nitroalkenes comes from a reaction of organic nitro derivatives, the Nef reaction. Support for this reaction comes from HPLC-MS detection of expected Nef reaction products, a requirement for an aqueous milieu, the pH dependence of the reaction, and the direct detection of $^{\cdot}NO$ release (30). Under neutral aqueous conditions, nitroalkene equilibrium with vicinal

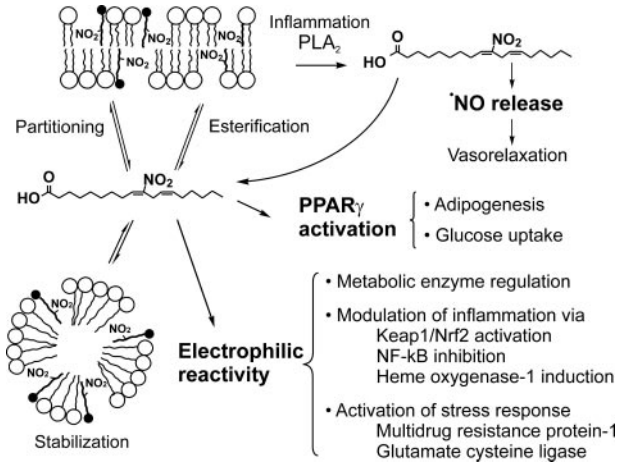

FIGURE 2. **Biological trafficking and actions of NO_2-FA derivatives.** Nitroalkenes are stable in free and/or esterified form in hydrophobic micellar, lipoprotein, or membrane environments and can serve as a reserve of NO_2-FAs in free and/or esterified form. Once access to an aqueous milieu is gained, nitroalkenes can decay or possibly be metabolized to release nitric oxide (•NO), bind and activate PPARs, or elicit multiple biological responses as a consequence of electrophilic reactivity.

nitrohydroxy derivatives facilitates formation of the nitronate anion (R_2-$C^{(-)}$-NO_2). Following concerted protonation and deprotonation events, the resulting nitroso intermediate provides a pathway that yields •NO via a reaction facilitated by reductants such as ascorbate (29). This nitroso intermediate has a weak C–N bond that yields •NO and a carbon-centered radical product stabilized by conjugation with both the alkene and the OH group. The carbon-centered radical can be reduced by various cellular reductive mechanisms to yield the conjugated ketone derivative detected as the final product by HPLC-MS (30). An alternative mechanism for NO release from nitroalkenes has been proposed, involving nitroalkene isomerization to a nitrite ester followed by N–O bond homolysis and/or one-electron reduction to form the corresponding functional enol group and nitric oxide (29, 31). The direct release of •NO from synthetic vicinal nitrohydroxylinoleic and nitrohydroxyarachidonic acid precursors, analogous to those detected in human plasma and urine and bovine cardiac lipid extracts, has also been proposed based on the observation that this species induces vasorelaxation of rat aortic rings (12, 29). Present results indicate that synthetic hydroxy derivatives of fatty acid nitroalkenes are not direct precursors to •NO release, but rather require equilibrium between nitrohydroxy and nitroalkene derivatives. It is important to note that the hydrophobic stabilization of fatty acid nitroalkenes can render pathways leading to •NO release of little or no biological significance.

Electrophilic Adduction of Protein Thiol and Histidine Residues—The post-translational modification of proteins by electrophilic oxidative reaction products is typically viewed as an index of toxicity. Electrophilic adduction reactions are now also viewed to instigate adaptive responses that are mediated by the nitroalkylation of critical thiols and other nucleophilic moieties of transcription factors, ion channels, and cell signaling proteins (32). Nitroalkenes react with the thiolate anion of GSH and Cys via Michael addition at robust bimolecular rate constants significantly exceeding most fatty acid-derived electrophiles, displaying second order rate constants of up to 355 M^{-1} s^{-1} at pH 7.4 and 37 °C (7). These are orders of magnitude greater than for GSH reaction with H_2O_2 and electrophilic lipids such as 8-isoprostaglandin A_2 and 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. The electrophilic adduction of proteins by NO_2-FAs gains additional significance in the context of cell signaling, as this reaction is reversible, a necessary trait for signaling mediators. Protein adduction by NO_2-FAs is detected clinically, thus representing a metabolic and redox-sensitive mechanism for regulating protein distribution and function.

Hydrophobic Stabilization—Fatty acid nitroalkene derivatives are highly stable in polyethylene glycols, alcohols, and other less polar organic solvents. When partitioned in non-ionic detergent micelles or phosphatidylcholine/cholesterol liposomes, the parent nitroalkene is stabilized, thus inhibiting (*a*) decomposition via the Nef reaction and •NO release and (*b*) electrophilic reaction with thiols (7, 30). This suggests that upon partitioning or esterification to complex lipids in cell membranes and lipoproteins, NO_2-FAs exist in a stabilized form that can be mobilized for downstream cell signaling actions upon activation of esterases and A_2-type phospholipases.

Signaling Actions of Nitro-fatty Acids

cGMP-dependent Vessel Relaxation—Diverse •NO-derived and •NO-generating molecules regulate vascular tone. Current evidence reveals endogenously produced NO_2^- and RSNO derivatives as metabolic "reserves" for •NO- and downstream cGMP-dependent signaling (33). NO_2-FAs also induce •NO-dependent vasorelaxation with an IC_{50} of 0.1–1 μM when LNO_2 is added to preconstricted rat aortic vessel rings (29, 34). Nitrohydroxy derivatives of arachidonic acid have also been suggested to induce vasorelaxation (12). In an aqueous milieu, nitrohydroxy derivatives of fatty acids will be in equilibrium with their dehydration product, the nitroalkene, so the immediate precursor of •NO is uncertain, and the equilibrium constants for nitrohydroxy fatty acid dehydration remain to be defined (30). Although NO_2-FAs release •NO in aqueous media following Nef-like acid-base chemistry (12, 29, 30), this reaction is inhibited by intercalation in micelles and liposomes (30). This apparent contradiction with vessel relaxation studies, where •NO intermediacy is implicated by guanylate cyclase inhibitors abolishing vessel responses to NO_2-FAs (34), has not been resolved. Possibly, •NO generation by NO_2-FAs is enzymatically catalyzed or the result of NO_2-FA activation of a signaling pathway dependent on •NO. In summary, nitroalkene-dependent •NO release and vessel relaxation may be an anomaly, as the signaling actions described below are not the consequence of •NO-dependent, cGMP-mediated reactions.

Inhibition of Inflammatory Cell Function—NO_2-FA derivatives inhibit neutrophil (PMN) and platelet function via non-cGMP-dependent mechanisms (35, 36). In activated PMNs, LNO_2 inhibited O_2^- production, azurophilic degranulation, calcium mobilization, and CD11b expression via •NO-independent mechanisms. Exposure of both PMNs and platelets to LNO_2 increased cAMP levels (37). In platelets, LNO_2 inhibited thrombin-induced aggregation by increasing platelet cAMP levels, with inhibitors of adenylate cyclase restoring thrombin-induced aggregation to platelets pretreated with LNO_2 (36). Growing evidence supports that LNO_2 induces adenylate cyclase activity, which is in part mediated by the cAMP-de-

pendent phosphorylation of Ser^{159} in vasodilator-stimulated phosphoprotein, causing a decrease in cAMP hydrolysis in PMNs and macrophages. Activated macrophage nitric-oxide synthase-2 expression is down-regulated by nitroarachidonic acid, an event that also inhibits subsequent macrophage inflammatory responses (38). The transendothelial migration of monocytes is largely dependent on a class of adhesion molecules, including VCAM-1, E-selectin, and P-selectin. VCAM-1 is a central mediator of the selective recruitment of monocytes and lymphocytes to atherosclerotic lesions (39). In this regard, NO_2-FAs suppress the expression of VCAM-1, resulting in impaired monocyte rolling and adhesion and inhibition of monocyte infiltration (40). Expanding evidence supports that NO_2-FAs abrogate the activation of multiple inflammatory cell types via •NO-independent mechanisms and encourages further study of operative signaling mechanisms.

HO-1 Expression—HO-1 plays a central role in inflammatory signaling, mediating protective responses during inflammation. HO-1 catalyzes the degradation of heme to biliverdin, iron, and CO, the latter of which is isoelectronic with •NO and recently shown to mediate diverse anti-inflammatory, anti-apoptotic, and vasodilatory adaptive signaling actions (41). During inflammation, HO-1 gene expression is up-regulated, with induction typically occurring at the transcriptional level. HO-1 expression is potently induced by LNO_2 in human aortic endothelial cells compared with other established stimuli, including oxidized fatty acids and hemin. This induction is not mediated by •NO, NF-κB, or PPARγ (42). Current data support that up-regulation of human HO-1 expression by NO_2-FAs requires synergy between the cAMP-response element and AP-1 sequences in the −4.5-kb HO-1 promoter region (43).

Inhibition of NF-κB—The enzymatic and chemical oxidation of fatty acids plays a central role in regulating inflammation. Prostaglandins, thromboxanes, isoprostanes, and leukotrienes mediate the propagation and resolution of inflammation via G-protein-coupled and nuclear receptor activation and in some cases by post-translational protein modification (44, 45). In addition, electrophilic species contribute to the transcriptional regulation of inflammation via post-translational modification of NF-κB (46). NF-κB functions as a hetero- or homodimer of five different subunits (p50, p52, p65, p100, and p105). The dimer between p50 and p65 in particular promotes expression of genes involved with cell survival and the progression of inflammation. Biological electrophiles such as NO_2-FAs, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, and plant-derived sesquiterpene lactones suppress NF-κB transactivation, thus modulating inflammatory signaling. The most responsive sites of electrophile reaction appear to be the alkylation or inhibition of IκB kinase and inhibition of p65 DNA binding. Once activated, IκB kinase phosphorylates the inhibitory IκBα subunit, routing it to degradation. These events are followed by p50/p65 heterodimer nuclear translocation and gene transcription initiation. In particular, NO_2-FAs exert a potent inhibitory effect on inflammatory signaling by alkylating the p65 subunit and rendering it unable to bind to the promoter region of inflammatory-related genes. This in turn inhibits tumor necrosis factor-α and lipopolysaccharide-induced secretion of pro-inflammatory cytokines in macrophages and endothelial cells (40).

PPAR Activation—The regulation of nuclear lipid receptor-dependent gene expression by fatty acid derivatives underscores the crucial signaling roles that lipids serve beyond their originally appreciated actions as energy-conserving molecules. For example, oxidized and nitrated fatty acids regulate metabolism and inflammation by activating PPARs (6, 47, 48). Concordant with this concept, elevated NO_2-FA levels occur in postprandial and hyperlipidemic human plasma, suggesting an involvement in metabolic homeostasis (10). Three different isoforms of PPAR (α, β, and γ) display unique tissue expression profiles and contribute to the regulation of fat metabolism, adipogenesis, glucose homeostasis, and inflammatory responses. Upon binding of lipophilic ligands, PPARγ forms a heterodimer with the retinoid X receptor, binds to PPAR-response elements, and regulates target gene transcription. PPARγ is broadly expressed by a variety of cells, including monocytes, macrophages, endothelial cells, vascular smooth muscle cells, and adipocytes. Luciferase-based transactivation analysis shows that NO_2-FAs are robust agonists of PPARγ and, to a lesser extent, PPARα and PPARδ. The affinity of NO_2-FAs as ligands for PPARγ rivals the TZD rosiglitazone, an antidiabetic drug (K_i for [^{3}H]rosiglitazone displacement = 133 nM for LNO_2 *versus* 53 nM for rosiglitazone) (6, 48). As for TZDs, NO_2-FAs induce increased adipocyte glucose uptake (6, 48). Despite transactivation assay potencies, receptor affinities, and effects on glucose uptake that are commensurate with TZDs, NO_2-FAs display a reduced capacity to induce adipogenesis compared with TZDs such as rosiglitazone. Rosiglitazone induces more extensive preadipocyte differentiation and adipocyte triglyceride accumulation compared with NO_2-FAs, suggesting unique properties of NO_2-FAs as PPAR ligands in the context of co-regulator protein interactions or other aspects of PPAR activation (6, 48).

Keap1/Nrf2 Activation—Covalent post-translational protein adduction by electrophilic fatty acid derivatives alters the structure, trafficking, and catalytic activity of proteins such as cathepsin B (49), insulin (50), glyceraldehyde-3-phosphate dehydrogenase (51, 52), and Keap1 (53). In particular, thiol adduction of Keap1 induces phase II gene expression, a mechanism that protects against pathogens, metabolic stress, and irreversible inflammatory injury. Phase II protein expression is regulated by the antioxidant-response element, a *cis*-acting DNA regulatory element also referred to as the electrophile-response element. The thiol oxidation-mediated mechanism that controls antioxidant-response element-dependent electrophile responses consists of the pivotal Nrf2 (nuclear factor erythroid 2-related factor 2) and the electrophile-reactive, cysteine-rich cytoplasmic suppressor protein Keap1 (Kelch-like ECH-associating protein 1). Keap1/Nrf2-mediated signaling is protective against toxicant exposure and acts through multiple kinase signaling pathways (54). The electrophilic reactivity of NO_2-FAs inhibits vascular smooth muscle cell proliferation via PPAR- and •NO-independent, Keap1/Nrf2-dependent signaling (55). Current data support that NO_2-FAs post-translationally modify critical thiols in Keap1 to ultimately facilitate Nrf2 translocation to the nucleus and transactivation of the expression of cytoprotective and anti-proliferative genes.

Summary

The endogenous generation of NO_2-FAs and other electrophilic lipids in turn activates networks of lipid receptors, transcription factors, and signaling pathways that sense changes in tissue levels of oxidative inflammatory mediators. These stimulus-response mechanisms confer the ability to sense and rapidly adapt to changes in metabolic, redox, and inflammatory status via an immediate impact on protein function and by altering patterns of gene expression. The formation and signaling actions of nitrated unsaturated fatty acids thus expand the array of molecular targets regulated by •NO and lipid signaling mediators and represent a redox-sensitive signaling mechanism that responds adaptively to multiple facets of cell metabolism.

REFERENCES

1. Rubbo, H., Radi, R., Anselmi, D., Kirk, M., Barnes, S., Butler, J., Eiserich, J. P., and Freeman, B. A. (2000) *J. Biol. Chem.* **275,** 10812–10818
2. O'Donnell, V. B., Chumley, P. H., Hogg, N., Bloodsworth, A., Darley-Usmar, V. M., and Freeman, B. A. (1997) *Biochemistry* **36,** 15216–15223
3. Mollace, V., Muscoli, C., Masini, E., Cuzzocrea, S., and Salvemini, D. (2005) *Pharmacol. Rev.* **57,** 217–252
4. O'Donnell, V. B., Coles, B., Lewis, M. J., Crews, B. C., Marnett, L. J., and Freeman, B. A. (2000) *J. Biol. Chem.* **275,** 38239–38244
5. O'Donnell, V. B., Taylor, K. B., Parthasarathy, S., Kuhn, H., Koesling, D., Friebe, A., Bloodsworth, A., Darley-Usmar, V. M., and Freeman, B. A. (1999) *J. Biol. Chem.* **274,** 20083–20091
6. Baker, P. R. S., Lin, Y., Schopfer, F. J., Woodcock, S. R., Groeger, A. L., Batthyany, C., Sweeney, S., Long, M. H., Iles, K. E., Baker, L. M. S., Branchaud, B. P., Chen, Y. E., and Freeman, B. A. (2005) *J. Biol. Chem.* **280,** 42464–42475
7. Baker, L. M. S, Baker, P. R. S., Golin-Bisello, F., Schopfer, F. J., Fink, M., Woodcock, S. R., Branchaud, B. P., Radi, R., and Freeman, B. A. (2007) *J. Biol. Chem.* **282,** 31085–31093
8. Batthyany, C., Schopfer, F. J., Baker, P. R. S., Duran, R., Baker, L. M. S., Huang, Y., Cervenansky, C., Branchaud, B. P., and Freeman, B. A. (2006) *J. Biol. Chem.* **281,** 20450–20463
9. Lima, E. S., Di Mascio, P., Rubbo, H., and Abdalla, D. S. (2002) *Biochemistry* **41,** 10717–10722
10. Rubbo, H., Radi, R., Trujillo, M., Telleri, R., Kalyanaraman, B., Barnes, S., Kirk, M., and Freeman, B. A. (1994) *J. Biol. Chem.* **269,** 26066–26075
11. Alexander, R. L., Bates, D. J., Wright, M. W., King, S. B., and Morrow, C. S. (2006) *Biochemistry* **45,** 7889–7896
12. Balazy, M., Iesaki, T., Park, J. L., Jiang, H., Kaminski, P. M., and Wolin, M. S. (2001) *J. Pharmacol. Exp. Ther.* **299,** 611–619
13. Schopfer, F. J., Baker, P. R. S, and Freeman, B. A. (2003) *Trends Biochem. Sci.* **28,** 646–654
14. Beckman, J. S., Beckman, T. W., Chen, J., Marshall, P. A., and Freeman, B. A. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 1620–1624
15. Radi, R., Beckman, J. S., Bush, K. M., and Freeman, B. A. (1991) *J. Biol. Chem.* **266,** 4244–4250
16. Radi, R., Denicola, A., and Freeman, B. A. (1999) *Methods Enzymol.* **301,** 353–367
17. Brennan, M. L., Wu, W., Fu, X., Shen, Z., Song, W., Frost, H., Vadseth, C., Narine, L., Lenkiewicz, E., Borchers, M. T., Lusis, A. J., Lee, J. J., Lee, N. A., Abu-Soud, H. M., Ischiropoulos, H., and Hazen, S. L. (2002) *J. Biol. Chem.* **277,** 17415–17427
18. Eiserich, J. P., Hristova, M., Cross, C. E., Jones, A. D., Freeman, B. A., Halliwell, B., and Van der Vliet, A. (1998) *Nature* **391,** 393–397
19. Castro, L., Eiserich, J. P., Sweeney, S., Radi, R., and Freeman, B. A. (2004) *Arch. Biochem. Biophys.* **421,** 99–107
20. Moller, M., Botti, H., Batthyany, C., Rubbo, H., Radi, R., and Denicola, A. (2005) *J. Biol. Chem.* **280,** 8850–8854
21. Lancaster, J. R., Jr. (2006) *Chem. Res. Toxicol.* **19,** 1160–1174
22. d'Ischia, M., Rega, N., and Barone, V. (1999) *Tetrahedron* **55,** 9297–9308
23. d'Ischia, M. (1996) *Tetrahedron Lett.* **37,** 5773–5774
24. Napolitano, A., Camera, E., Picardo, M., and d'Ischia, M. (2000) *J. Org. Chem.* **65,** 4853–4860
25. Napolitano, A., Crescenzi, O., Camera, E., Giudicianni, I., Picardo, M., and d'Ischia, M. (2004) *Tetrahedron* **58,** 5061–5067
26. Napolitano, A., Panzella, L., Savarese, M., Sacchi, R., Giudicianni, I., Paolillo, L., and d'Ischia, M. (2004) *Chem. Res. Toxicol.* **17,** 1329–1337
27. Pryor, W. A., Lightsey, J. W., and Church, D. F. (1982) *J. Am. Chem. Soc.* **104,** 6685–6692
28. Napolitano, A., and d'Ischia, M. (2002) *J. Org. Chem.* **67,** 803–810
29. Lima, E. S., Bonini, M. G., Augusto, O., Barbeiro, H. V., Souza, H. P., and Abdalla, D. S. (2005) *Free Radic. Biol. Med.* **39,** 532–539
30. Schopfer, F. J., Baker, P. R. S., Giles, G., Chumley, P. H., Batthyany, C., Crawford, J., Patel, R. P., Hogg, N., Branchaud, B. P., Lancaster, J. R., Jr., and Freeman, B. A. (2005) *J. Biol. Chem.* **280,** 19289–19297
31. Gorczynski, M. J., Huang, J., and King, S. B. (2006) *Org. Lett.* **8,** 2305–2308
32. Kobayashi, A., Kang, M. I., Watai, Y., Tong, K. I., Shibata, T., Uchida, K., and Yamamoto, M. (2006) *Mol. Cell. Biol.* **26,** 221–229
33. Gladwin, M. T., Schechter, A. N., Kim-Shapiro, D. B., Patel, R. P., Hogg, N., Shiva, S., Cannon, R. O., III, Kelm, M., Wink, D. A., Espey, M. G., Oldfield, E. H., Pluta, R. M., Freeman, B. A., Lancaster, J. R., Jr., Feelisch, M., and Lundberg, J. O. (2005) *Nat. Chem. Biol.* **1,** 308–314
34. Lim, D. G., Sweeney, S., Bloodsworth, A., White, C. R., Chumley, P. H., Krishna, N. R., Schopfer, F., O'Donnell, V. B., Eiserich, J. P., and Freeman, B. A. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 15941–15946
35. Coles, B., Bloodsworth, A., Clark, S. R., Lewis, M. J., Cross, A. R., Freeman, B. A., and O'Donnell, V. B. (2002) *Circ. Res.* **91,** 375–381
36. Coles, B., Bloodsworth, A., Eiserich, J. P., Coffey, M. J., McLoughlin, R. M., Giddings, J. C., Lewis, M. J., Haslam, R. J., Freeman, B. A., and O'Donnell, V. B. (2002) *J. Biol. Chem.* **277,** 5832–5840
37. Wanikiat, P., Woodward, D. F., and Armstrong, R. A. (1997) *Br. J. Pharmacol.* **122,** 1135–1145
38. Trostchansky, A., Souza, J. M., Ferreira, A., Ferrari, M., Blanco, F., Trujillo, M., Castro, D., Cerecetto, H., Baker, P. R. S., O'Donnell, V. B., and Rubbo, H. (2007) *Biochemistry* **46,** 4645–4653
39. Ley, K. (1996) *Cardiovasc. Res.* **32,** 733–742
40. Cui, T., Schopfer, F. J., Zhang, J., Chen, K., Ichikawa, T., Baker, P. R. S., Batthyany, C., Chacko, B. K., Feng, X., Patel, R. P., Agarwal, A., Freeman, B. A., and Chen, Y. E. (2006) *J. Biol. Chem.* **281,** 35686–35698
41. Dulak, J., Deshane, J., Jozkowicz, A., and Agarwal, A. (2008) *Circulation* **117,** 231–241
42. Wright, M. M., Schopfer, F. J., Baker, P. R. S., Vidyasagar, V., Powell, P., Chumley, P., Iles, K. E., Freeman, B. A., and Agarwal, A. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103,** 4299–4304
43. Wright, M. W., Hock, T. D., Freeman, B. A., and Agarwal, A. (2007) *Free Radic. Biol. Med.* **43,** S161
44. Serhan, C. N., and Oliw, E. (2001) *J. Clin. Investig.* **107,** 1481–1489
45. Smith, W. L., and Langenbach, R. (2001) *J. Clin. Investig.* **107,** 1491–1495
46. Vermeulen, L., De Wilde, G., Notebaert, S., Vanden Berghe, W., and Haegeman, G. (2002) *Biochem. Pharmacol.* **64,** 963–970
47. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) *Cell* **83,** 803–812
48. Schopfer, F. J., Lin, Y., Baker, P. R., Cui, T., Garcia-Barrio, M., Zhang, J., Chen, K., Chen, Y. E., and Freeman, B. A. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102,** 2340–2345
49. Crabb, J. W., O'Neil, J., Miyagi, M., West, K., and Hoff, H. F. (2002) *Protein Sci.* **11,** 831–840
50. Uchida, K., and Stadtman, E. R. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 4544–4548
51. Ishii, T., Tatsuda, E., Kumazawa, S., Nakayama, T., and Uchida, K. (2003) *Biochemistry* **42,** 3474–3480
52. Uchida, K., and Stadtman, E. R. (1993) *J. Biol. Chem.* **268,** 6388–6393
53. Levonen, A. L., Landar, A., Ramachandran, A., Ceaser, E. K., Dickinson, D. A., Zanoni, G., Morrow, J. D., and Darley-Usmar, V. M. (2004) *Biochem. J.* **378,** 373–382
54. Kensler, T. W., Wakabayashi, N., and Biswal, S. (2007) *Annu. Rev. Pharmacol. Toxicol.* **47,** 89–116
55. Villacorta, L., Zhang, J., Garcia-Barrio, M. T., Chen, X. L., Freeman, B. A., Chen, Y. E., and Cui, T. (2007) *Am. J. Physiol.* **293,** H770–H776