4-Hydroxynonenal (4-HNE) is one of the major end products of lipid peroxidation. It has been widely accepted that 4-HNE can induce oxidative stress, implicating into extensive stress-related diseases. In the present study, however, 4-HNE was found to exert adaptive cytoprotective effect at low concentrations, which was primarily through induction of thioredoxin reductase 1 (TR1) via transcriptional activation of NF-E2-related factor 2 (Nrf2). Pretreatment with 4-HNE at sublethal concentrations significantly protected PC12 cells against the subsequent oxidative cell death induced by H$_2$O$_2$ and 6-hydroxydopamine. The cellular antioxidant glutathione system did not show any considerable changes, whereas the TR1 activity as well as the mRNA level was significantly elevated by the 4-HNE treatment. Cells treated with TR1 small interfering RNA exhibited less resistance to oxidative stress, and the adaptive response was completely abolished. The Nrf2 was transcriptionally activated by 4-HNE. Cells treated with Nrf2-small interfering RNA exerted lower constitutive levels of TR1 and exhibited less resistance to oxidative stress, and the 4-HNE-induced TR1 expression and subsequent adaptive response were again abolished in such cells. Treatment with 4-HNE at the adaptive concentration induced transient activation of extracellular signal-regulated protein kinase 1/2 and Akt/protein kinase B. Pharmacological inhibition of both these kinase pathways effectively attenuated 4-HNE-induced TR1 expression and subsequent adaptive protection. The above findings, taken together, suggest that stimulation with 4-HNE at sublethal concentrations induces adaptive response and enhances cell tolerance, primarily through induction of TR1 via transcriptional activation of Nrf2 signaling pathway, thereby protecting cells against the forthcoming oxidative stress.

"Oxidative stress" was defined as a disturbance in the prooxidant-antioxidant balance in favor of the prooxidant (1). However, even if the level of stress is less than that of antioxidant capacity, stress functions as a signal to which the body responds irrespective of its level. When the stress level exceeds defense capacity, it may induce oxidative damage, whereas the low level stress may stimulate defense network and acts as a good stress, "eustress."

4-Hydroxynonenal (4-HNE) is one of the major end products of lipid peroxidation and has been found to induce oxidative stress, as it is involved in the pathogenesis of a number of degenerative diseases such as Alzheimer disease (2), atherosclerosis (3), cataract (4), and cancer (5). However, increasing evidence has suggested that 4-HNE at low concentrations takes an important role in cell signal transduction and gene expression (6–11). 4-HNE can react in Michael addition across its carbon-carbon double bond with a wide variety of cellular components, including DNA and proteins (12). Thus, it has been suggested that 4-HNE could act as a potential activator of NF-E2-related factor 2 (Nrf2) and induce the expression of phase II detoxification enzymes (13–15).

The transcription factor Nrf2 has been implicated as the central protein that interacts with the antioxidant response element (ARE) to activate gene transcription constitutively or in response to an oxidative stress signal (16). Under homeostatic conditions, Kelch-like ECH-associated protein 1 (Keap1) binds to Nrf2 and facilitates the degradation of Nrf2 via the proteasome system (17). Upon stimulation, Nrf2 dissociates from its cytoplasmic inhibitor Keap1, translocates to the nucleus, and by heterodimerizing with a small Maf protein, transactivates the expression of ARE-dependent genes (18–20).

Thioredoxin reductase (TR), thioredoxin (Trx), and NADPH comprise a highly conserved, ubiquitous system (21) that plays an important role in the redox regulation of multiple intracellular processes, including DNA synthesis, transcriptional regulation, cell growth, and resistance to cytotoxic agents that induce oxidative stress and apoptosis (22, 23). TR can catalyze the NADPH-dependent reduction of the redox protein Trx as well as of other endogenous and exogenous compounds, including lipoic acid (24), the cytotoxic peptide (25), lipid hydroperoxides (26) and the tumor suppressor protein p53 (27). Recent study (28) has also suggested that the induction of TR might serve as an adaptive response to the oxidative damage induced by acrolein. To date, three TR isoforms have been identified, predominantly cytosolic TR1, mitochondrial TR2, and the recently cloned TR3 (29). Among these three isoforms of TR, TR1 is widely expressed and up-regulated by various stimuli and thought to take a prominent role in the cell defense against oxidative stress (30). The detailed mechanisms by which TR1 is regulated have not been clearly clarified, and Nrf2 is considered to be one of the potential candidates (31).

Although 4-HNE at low concentrations has been suggested to take an

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1 The abbreviations used are: 4-HNE, 4-hydroxynonenal; ARE, antioxidant response element; HO-1, heme oxygenase-1; Keap1, kelch-like ECH-associated protein 1; Nrf2, NF-E2-related factor 2; 6-OHDA, 6-hydroxydopamine; ROS, reactive oxygen species; siRNA, small interfering RNA; Trx, thioredoxin; TR, thioredoxin reductase; MTT, dimethylthiazol-2-yI-2,5-diphenyl tetrazolium bromide; RT, real time; PBS, phosphate-buffered saline.

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important role in cell signal transduction, the detailed molecular mechanisms are not fully understood. In the present study we attempted to explore the possible adaptive response of 4-HNE in neuronal PC12 cells. We found that stimulation of PC12 cells with sublethal concentrations of 4-HNE induced adaptive response and protected cells against the subsequent oxidative stress, which was critically mediated through induction of TR1 via transcriptional activation of the Nrf2 signaling pathway.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s modified Eagle’s medium/F-12, horse serum, and fetal bovine serum were obtained from Invitrogen. NADPH was the product of Nacalai, Kyoto, Japan. Zinc protoporphyrin was purchased from Wako, Osaka, Japan. 4-HNE was from Calbiochem. 6-Hydroxydopamine (6-OHDA), LY294002, and U0126 were obtained from Sigma. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was from Dojindo, Kumamoto, Japan. Nrf2, phosphorylated extracellular signal-regulated protein kinase (p-ERK), and actin antibodies were obtained from Santa Cruz Biotechnology, Santa Cruz, CA. Heme oxygenase-1 (HO-1) antibody was purchased from Stressgen Biotechnologies. The antibody of phosphorylated Akt was product of Cell Signaling Technology. All other chemicals were of the highest quality commercially available.

Cell Culture—PC12 cells were routinely cultured in Dulbecco’s modified Eagle’s medium/F-12 medium with 1-glutamine, sodium bicarbonate, and pyridoxine hydrochloride supplemented with 10% fetal bovine serum and 5% horse serum. Cell cultures were maintained at 37 °C in a humidified atmosphere of 5% CO2.

Determination of Cell Viability—For determination the cell viability, the conventional MTT reduction assay was used. The treated cells were incubated with 0.5 mg/ml MTT in fresh medium at 37 °C for 1 h. Isopropyl alcohol containing 0.04 N HCl was added to the culture medium (3:2 by volume) and mixed with a pipette until the formazan was completely dissolved. The optical density of formazan was measured at 570 nm using a Multiskan Ascent plate reader (Thermo Labsystems, Helsinki, Finland).

Preparation of the Cytosolic Fractions—The cytosolic fractions were prepared for the following TR1 enzyme assay, as described previously (32). After treatment cells were harvested, and the cell pellets were suspended into 50 mM Tris-HCl buffer at pH 7.4 containing 0.1 mM EDTA, 0.2 mM NADPH, 0.8 μM human recombinant Trx, and 80 μM insulin were used as described previously (32). The oxidation of NADPH was recorded at 340 nm. The TR1 activity was calculated by nmol of oxidized NADPH/min and expressed as relative activity.

Transfection of siRNA—Rat Nrf2- and TR1-siRNA were designed and manufactured by Invitrogen according to the current guidelines for effective knockdown by this method. The target sequences for Nrf2- and TR1-siRNA are 5'-UUU GAG UCU AAG GAG UUC AGC UGG C-3' (forward) and 5'-GCG AGC UGA ACU CCU UAG ACUCAA A-3' (reverse), and 5'-UUG UUG UAC ACA GGU UUC GAA GGA GCC GGC C-3' (forward) and 5'-GCG UCA CUG CGA AAG CUG UCA ACA A-3' (reverse), respectively. The siRNA was transfected into PC12 cells at a concentration of 20 pmol/105 cells by Lipofectamine (Invitrogen) 24 h before further experiments.

Real Time (RT)-PCR Analysis—Total RNA was isolated from cells using the RNeasy Mini Kit (Qiagen, Tokyo, Japan). cDNA synthesis was performed with a GeneAmp RNA PCR core kit (Applied Biosystems). Real time PCR was conducted using an ABI PRISM 7900 HT sequence detection system (Applied Biosystems), and the PCR amplification was then detected with the SYBR Green I nucleic acid gel stain (Cambrex Bio Science Rockland, Inc., Rockland, ME).

The housekeeping gene of rat GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as an endogenous control. The primers for amplification are: GAPDH, 5’-GGA CAC CAT GGC CTA CAT-3’ (forward) and 5’-GGT GGA CCT CAT GCC TGA TCC T-3’ (reverse); Nrf2, 5’-GCT AGT CTG ATG CAT CAT-3’ (forward) and 5’-GGA CCT GGA ATT GAG TCC CCA AAC-3’ (reverse); TR1, 5’-GCA GGA GCA GAT GGC GTC TTGA GCT-3’ (forward) and 5’-GGA CCT GGA ATT GAG TCC CCA AAC-3’ (reverse).

Western Blotting Analysis—Nuclear fractions or whole lysate of cells were prepared as described previously (33). The protein concentration was determined by using the BCA protein assay kit (Pierce). Protein samples were solubilized with SDS-polyacrylamide gel electrophoresis sample loading buffer and electrophoresed on a 12% SDS-polyacryl-
amide gel. Proteins were then transferred to a polyvinylidene difluoride membrane. The blots were blocked for 1 h at room temperature in fresh blocking buffer (0.1% Tween 20 in Tris-buffered saline, pH 7.4, containing 5% nonfat dry milk). Dilutions of primary antibodies were made in phosphate-buffered saline (PBS) with 3% nonfat dry milk. The blots were incubated overnight at 4 °C with the diluted primary antibody. After three washes with PBS and 0.1% Tween 20, the blots were incubated with the horseradish peroxidase-conjugated secondary antibody in PBS with 3% nonfat dry milk for 1 h at room temperature. The blots were again washed three times in PBS and 0.1% Tween 20 buffer, and the transferred proteins were incubated with ECL solution (Amersham Biosciences) for 1 min, in accordance with the manufacturer’s instructions, and visualized on radiographic film.

Statistics—All data of at least three independent experiments are expressed as the mean ± S.D. and analyzed by Student’s t test. Values of p < 0.05 were considered to be statistically significant.

RESULTS

4-HNE Induced Adaptive Response in PC12 Cells—As an initial approach toward determining the possible adaptive response by 4-HNE in PC12 cells, the conventional MTT assay was used for monitoring the 4-HNE-induced cytotoxicity. At concentrations higher than 20 μM, 4-HNE significantly decreased the cell viability in PC12 cells (Fig. 1A). Based on the above observations, 20 μM was regarded as a quasi-toxicological threshold at which 4-HNE caused no appreciable cytotoxicity in PC12 cells, and concentrations below that were then selected for further studies to examine the possible adaptive effect of 4-HNE. Pre-treatment with 15 μM 4-HNE for 24 h significantly protected PC12 cells against cell death induced by the subsequent treatment of H2O2 (Fig. 1B) or 6-OHDA (10 μM) for an additional 24 h. Cell viability were then determined by MTT assay. **, Significantly different from the corresponding value of control (p < 0.05). F, 4-HNE-induced adaptive response was abolished in cells treated with TR1-siRNA. PC12 cells were pretreated with TR1-siRNA for 24 h and followed by treatment with or without 4-HNE for 24 h. Cells were then challenged with 6-OHDA (30 μM) for an additional 24 h. Cell viability was determined by MTT assay. #, Significantly different from the control (p < 0.05). NS, non-significant difference.

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TR1 Was Responsible for the 4-HNE-induced Adaptive Response—We then attempted to determine the underlying molecular mechanisms responsible for such an adaptive response in PC12 cells. The cellular antioxidative glutathione system, including cellular glutathione peroxidase, glutathione S-transferase, glutathione reductase, and total glutathione contents, did not show any considerable changes in cells treated with 15 μM 4-HNE for 24 h (data not shown), whereas the TR1 activity was significantly elevated by the treatment with sublethal concentra-
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FIGURE 3. Central role of Nrf2 signaling pathway in 4-HNE-induced adaptive response. A, nuclear accumulations of Nrf2 by 4-HNE. PC12 cells were treated with 15 μM 4-HNE for indicated times, and nuclear fractions were extracted for Western blotting analysis. B, effect of Nrf2-siRNA on the constitutive expression of Nrf2 protein. PC12 cells were pretreated with Nrf2-RNA-interference or nonspecific RNA sample (C) for 24 h, and total protein lysate was subjected for Western blotting analysis. C and D, Nrf2-siRNA decreased the TR1 mRNA expression (C) and enzyme activity (D). Cells were treated with Nrf2-siRNA or nonspecific RNA sample (C) for 24 h, and total RNA or cytosolic fractions were extracted for RT-PCR analysis in 3 different mRNA transcript levels of TR1. E, Nrf2-siRNA sensitized PC12 cells to oxidative stress. Cells were treated with Nrf2-siRNA or control RNA sample (Control) for 24 h and followed by the treatment of 4-HNE for an additional 6 h. Total RNA was isolated and subjected for RT-PCR analysis. F, Nrf2-siRNA sensitized PC12 cells to oxidative stress. Cells were treated with Nrf2-siRNA or control RNA sample (Control) for 24 h and followed by the treatment of 4-HNE (15 μM) or 6-OHDA (10 μM) for an additional 24 h. Cell viability was then determined by MTT assay. **, Significantly different from the corresponding value of control (p < 0.05). G, 4-HNE-induced adaptive response was completely abolished in cells treated with Nrf2-siRNA. PC12 cells were pretreated with Nrf2-siRNA or control RNA sample for 24 h and followed by the treatment of 4-HNE (15 μM) or 6-OHDA (10 μM) for an additional 24 h. Cell viability was then determined by MTT assay. **, Significantly different from the control (p < 0.05). NS, non-significant difference.

The nuclear Nrf2 level was rapidly accumulated by the treatment of 15 μM 4-HNE (Fig. 3A), suggesting a possible involvement of the Nrf2 signaling pathway in the adaptive response. Cells treated with Nrf2-siRNA showed distinctly lower levels of constitutive Nrf2 protein (Fig. 3B) and lower TR1 mRNA transcript (~80% that of control, Fig. 3C) as well as decreased TR1 activity (~80% of control, Fig. 3D). Moreover, the 4-HNE-induced TR1 expression was completely abolished in cells treated with Nrf2-siRNA (Fig. 3E). In such cells 4-HNE at low concentrations (5, 10, or 15 μM) did not induce any considerable changes on the TR1 mRNA levels. All these findings strongly suggested a pivotal role of the Nrf2 transcription pathway in regulation of TR1 in PC12 cells. Similar to the effect of TR1-siRNA, treatment with Nrf2-siRNA also sensitized PC12 cells to the oxidative stress induced by 4-HNE or 6-OHDA, which both again caused higher cytotoxicity than they did in control cells (Fig. 3F). The adaptive response was abolished in such cells as well without any considerable protective effect by 4-HNE at low concentrations (Fig. 3G).

Lesser Predominant Role of HO-1 in the Adaptive Response—The induction of TR1 may be regulated by several transcription factors, including Nrf2 (31). Therefore, we investigated the possible involvement of Nrf2 transcription pathway in the expression of TR1. The nuclear Nrf2 level was rapidly accumulated by the treatment of 15 μM 4-HNE (Fig. 3A), suggesting a possible involvement of the Nrf2 signaling pathway in the adaptive response. Cells treated with Nrf2-siRNA showed distinctly lower levels of constitutive Nrf2 protein (Fig. 3B) and lower TR1 mRNA transcript (~80% that of control, Fig. 3C) as well as decreased TR1 activity (~80% of control, Fig. 3D). Moreover, the 4-HNE-induced TR1 expression was completely abolished in cells treated with Nrf2-siRNA (Fig. 3E). In such cells 4-HNE at low concentrations (5, 10, or 15 μM) did not induce any considerable changes on the TR1 mRNA levels. All these findings strongly suggested a pivotal role of the Nrf2 transcription pathway in regulation of TR1 in PC12 cells. Similar to the effect of TR1-siRNA, treatment with Nrf2-siRNA also sensitized PC12 cells to the oxidative stress induced by 4-HNE or 6-OHDA, which both again caused higher cytotoxicity than they did in control cells (Fig. 3F). The adaptive response was abolished in such cells as well without any considerable protective effect by 4-HNE at low concentrations (Fig. 3G).
ifying enzymes, including HO-1, which is able to be induced by a wide variety of stimuli and protect cells against oxidative damage (36–40).

Therefore, we explored the possible role of HO-1 in the 4-HNE-induced adaptive response. Treatment with 4-HNE at 15 μM for 24 h significantly induced the expression of HO-1 (Fig. 4A). However, the constitutive level of HO-1 only significantly declined in such cells treated with Nrf2-siRNA but did not show any considerable variations in cells treated with TR1-siRNA (Fig. 4B), in which the 4-HNE-induced adaptive response was completely abolished (Fig. 2F). In addition, zinc protoporphyrin, a pharmacological HO-1 inhibitor widely used in a variety of biosystems (41–43) including the PC12 cell line (44), only slightly but not significantly attenuated the 4-HNE-induced adaptive response (Fig. 4C). All these findings strongly suggested that, although HO-1 was induced by 4-HNE, it appeared to take a less predominant role in the 4-HNE-induced adaptive response.

Role of Extracellular Signal-regulated Protein Kinase 1/2 and Akt/Protein Kinase B Signaling Pathways in the 4-HNE-induced Adaptive Response—The transcriptional activation of Nrf2 may be due to the direct Michael addition reaction with 4-HNE, which modified the thiol that resides in Keap1 and, consequently, liberated Nrf2 (13, 14). Alternatively, it could also be regulated by several upstream protein kinase pathways, including mitogen-activated protein kinases, protein kinase C, and phosphatidylinositol 3-kinase, which modified either Keap1 or Nrf2 through phosphorylation and disassociated Nrf2 from Keap1 (15, 37, 39, 44–46). We then investigated the possible involvement of these kinases in the adaptive response. Cells treated with 15 μM 4-HNE transiently activated the extracellular signal-regulated protein kinase 1/2 and Akt via phosphorylation, which both peaked at ~60 min and slightly decreased thereafter (Fig. 5A). The 4-HNE-induced nuclear Nrf2 accumulation, TR1 mRNA expression, and adaptive protection were effectively attenuated by U0126 (25 μM) and LY294002 (10 μM) (Fig. 5, B–D), the pharmacologic inhibitor of MEK (mitogen-activated protein kinase/extracellular signal-regulated kinase) and phosphatidylinositol 3-kinase, which are upstream of extracellular signal-regulated protein kinase 1/2 and Akt.

DISCUSSION

It has been widely accepted that low levels of H$_2$O$_2$ and the related reactive oxygen species (ROS) play essential roles in the cell signal transduction (47) and can induce adaptive response (48–52). Such roles of some other physiological oxidative stimuli or oxidation products in modulating and participating in signal transduction have received increased attention. For instance, cyclopentenone prostaglandins, which were derived from arachidonic acid via enzymatic conversion by cyclooxygenase and have been widely known to have proapoptotic effect in a variety of cell types (53, 54), now have been reported to show a protective effect at low concentrations via different mechanisms (55–58). Likewise, 4-HNE, an α,β-unsaturated aldehyde that is formed by the reaction of reactive oxygen or nitrogen species with arachidonic acid in cellular membranes during oxidative stress, has now also been recognized as a signaling molecule at low concentrations and considered to bridge the fields of oxidative stress and redox signaling (6–11), although the detailed molecular mechanisms by which 4-HNE involves in the signal transduction and induces adaptive response are not fully understood. In the present study we clearly demonstrated that 4-HNE at low concentrations could induce adaptive response, protecting PC12 cells against the subsequent oxidative stress, which was critically mediated through induction of TR1 via transcriptional activation of Nrf2.

The mammalian thioredoxin system consists of the two antioxidant oxidoreductase enzymes Trx and TR. The latter catalyzes the reduction of the active disulfide site in Trx as well as of other endogenous and exogenous compounds, including lipoic acid, the cytotoxic peptide, lipid hydroperoxides, and the tumor-suppressor protein p53, using NADPH (21–27). Even though mammalian TR exhibits a wide substrate specificity, it should be emphasized that the physiological effects of cellular TR activity are closely linked to the different cellular functions of Trx, although TR may also carry functions not related to those of Trx (21). In general, Trx modulates the signal transduction properties of ROS by striving toward reduction of the intracellular disulfides induced by ROS and by lowering the levels of ROS directly (59, 60). Because TR is upstream of and regulates Trx and Trx may also be regulated by Nrf2, it would seem logical that TR may also be involved in the 4-HNE-induced adaptive response. In addition and similar to TR, the chemical or genetic inhibition of Trx resulted in a cell cycle arrest and cytotoxicity that was enhanced when combined with several anticancer agents (61). Although this work did not specifically examine Trx, the role of Trx in the 4-HNE-induced adaptive response was obviously important.

The inhibition of TR should be profound in view of the multiple functions of the thioredoxin system in DNA synthesis and inflammatory processes as well as in the general defense against oxidative damage. Pharmacologic inhibition of TR sensitized tumor cells to oxidative
stress, which made TR a potential molecular target for anticancer agents that induced oxidative stress (62). Likewise, inhibition of TR activity might account for the cancer preventive property of some phytochemicals such as curcumin (63). In agreement with the above results, it has also been found in our study that genetic inhibition of TR1 by siRNA abolished the adaptive response and sensitized PC12 cells to oxidative stress, indicating a pivotal role of TR1 in the adaptive response as well as in the cell defense tolerance against oxidative stress. It has to be pointed out that, in PC12 cells, treatment of TR1-siRNA at higher concentrations or for longer times would significantly decrease the cell viability (data not shown), which is not surprising because TR1 and Trx have been shown to play an important role in regulating cell growth and apoptosis (21).

The detailed mechanisms by which TR1 is regulated have not been clearly clarified. Recently, Sakurai et al. (31) have reported that Nrf2 might play an important role in the expression of TR1 by cadmium in vascular endothelial cells. Hintze et al. (64, 65) have reported that a TR/luciferase reporter gene construct is transcriptionally regulated by broccoli-derived sulforaphane through an ARE sequence, which has led to the hypothesis that the regulation of TR, similar to many phase II enzymes, responds to transcriptional activation through the ARE. In the present study we also clearly demonstrated the critical involvement of Nrf2 transcription pathway in the adaptive response and in the induction of TR1. Nrf2 was rapidly accumulated in the nucleus by the treatment of 4-HNE, which bound to the ARE response, thereby up-regulating the expression of detoxifying enzymes, including HO-1 and TR1. The treatment of PC12 cells with Nrf2-siRNA decreased the constitutive TR1 mRNA level as well as the TR1 activity and abolished the 4-HNE-induced TR1 mRNA expression, suggesting a pivotal role of Nrf2 in the regulation of TR1 in PC12 cells.

It might be noteworthy that the induction of HO-1 in the present study appeared to take a less predominant role in the 4-HNE-induced adaptive protection (Fig. 4), although it is widely accepted that HO-1 is able to protect various types of cells against oxidative stress (37–41). In PC12 cells, HO-1 has also been found as the major mediator of the cytoprotective effect induced by nerve growth factor (66) and resveratrol (44). One plausible explanation for such different conclusions is that, whereas HO-1 could function as a potential cellular antioxidative defense, TR1 might be a much more powerful eudemon for cells against oxidative damage. Therefore, in cells highly expressed with TR1, HO-1 may take a lesser prominent role.

The results shown in Fig. 5 demonstrated that the activation of Nrf2 by 4-HNE was, at least partly, mediated by the extracellular signal-regulated protein kinase and Akt signaling pathways. Multiple protein kinase pathways, such as mitogen-activated protein kinases, protein kinase C, and phosphatidylinositol 3-kinase, have been proposed to play a role in Nrf2 activation (37, 39, 44–46), although the role of each pathway in the regulation of Nrf2 and its molecular targets is contro-
versial and probably specific to a given gene and cell type. These findings could not rule out the possibility that 4-HNE might directly modify the thiou group in Keap1. As an α,β-unsaturated aldehyde, 4-HNE is able to react through Michael addition reaction across its carbon-carbon double bond with cellular DNA and proteins, including Keap1, thereby liberating and activating Nrf2 (13–15).

Under physiological conditions, the cellular concentration of 4-HNE ranges from 0.1 to 3 μM (12, 68). However, under conditions of oxidative stress, 4-HNE can accumulate until much higher concentrations of 10 μM to 5 mM (12, 67, 68). Thus, based on the present study and other previous reports, it would be rational to assume that, at initial times of oxidative stress, the accumulation of 4-HNE and other lipid peroxidation products like prostaglandins serves as a feedback that transmits an SOS signal and warns cells of the oxidative status, thereby triggering the early response enzymes or proteins to cope with the forthcoming oxidative disaster.

In conclusion, the present study clearly demonstrates that 4-HNE at sublethal concentrations induces adaptive protection in PC12 cells. Treatment of PC12 cells with 4-HNE leads to a rapid nuclear translocation of Nrf2, which turns on to regulate the TR1, thereby enhancing the cell tolerance against the forthcoming oxidative stress. These results represent an initial effort to demonstrate the 4-HNE-induced adaptive protection at sublethal concentrations and to identify TR1 as a potential target to the oxidative stimuli that induce adaptive response. Moreover, this study in conjunction with previous reports clearly shows some instructive information for, taking TR1 as the target, enhancing the TR1 to the oxidative stimuli that induce adaptive response. Moreover, the accumulation of 4-HNE and other lipid peroxidation products like prostaglandins serves as a feedback that transmits an SOS signal and warns cells of the oxidative status, thereby triggering the early response enzymes or proteins to cope with the forthcoming oxidative disaster.

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