INDUCTION OF DETOXIFYING ENZYMES IN RODENT WHITE ADIPOSE TISSUE BY ARYL HYDROCARBON RECEPTOR AGONISTS AND ANTIOXIDANTS

Kouichi Yoshinari, Nao Okino, Takeshi Sato, Junko Sugatani, and Masao Miwa

Department of Pharmaco-Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Received September 13, 2005; accepted March 27, 2006

ABSTRACT:
The liver is the main organ of drug metabolism, but the expression and induction by xenobiots of drug-metabolizing enzymes is also often observed in extrahepatic tissues. Recently, we reported that lipophilic cytochrome P450 inducers, β-naphthoflavone (BNF), phenobarbital, and dexamethasone, induced CYP1, CYP2B, and CYP3A enzymes, respectively, in rat epididymal white adipose tissue (WAT) at both mRNA and protein levels. To further confirm the xenobiotic-induced expression of drug-metabolizing enzymes in adipose tissue, we studied the induction of CYP1A1 and other detoxifying enzymes by aryl hydrocarbon receptor (AhR) agonists and antioxidants. BNF increased CYP1A1 mRNA levels in several visceral WATs (epididymal, perirenal, and mesenteric) to a greater degree than in subcutaneous WAT in rats. Using C57BL/6 and DBA/2 mice with different responsiveness to aryl hydrocarbons and detecting cytoplasmic levels of AhR proteins, we have demonstrated that AhR mediates this CYP1A1 induction by BNF in WAT. Moreover, the NF-E2-related factor 2 (Nrf2)/antioxidant responsive element pathway is also functional in WAT, since BNF, which is known to activate both AhR and Nrf2, and antioxidants including tert-butylhydroquinone, 1-chloro-2,4-dinitrobenzene, and menadione induced the expression of Nrf2-target genes (NAD(P)H:quinone oxidoreductase, glutathione S-transferase A subunits, and heme oxygenase-1) in rats and mice. These results suggest that both AhR and Nrf2 pathways are active in WAT and that lipophilic compounds accumulated in WAT can activate these transcription factors to increase detoxification capability in the tissue.

Upon xenobiotic exposure, the transcription of genes encoding drug-metabolizing enzymes such as cytochromes P450 (P450s) is activated, increasing the metabolic capacity of organisms. The mechanisms of this chemical-induced expression of P450 genes have been revealed in detail, in which two classes of transcriptional factors, zinc finger-containing nuclear receptors and helix-loop-helix aryl hydrocarbon receptor (AhR), play critical roles (Whitlock, 1999; Willson and Kliwer, 2002; Denison and Nagy, 2003). The constitutive androstane receptor and pregnane X receptor, members of the nuclear receptor superfamily, regulate the expression of CYP2B, CYP2C, and CYP3A subfamily genes (Wilson and Kliwer, 2002), whereas AhR transactivates CYP1 family and CYP2S1 genes (Whitlock, 1999; Willson and Kliwer, 2002; Denison and Nagy, 2003; Saarikoski et al., 2005). A typical CYP1A1 inducer, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), binds to AhR, which resides in the cytoplasm as a complex with 90-kDa heat shock protein (Hsp90) and an immunophilin-like protein under unstimulated conditions. The ligand-bound AhR translocates into the nucleus and forms a heterodimer with the AhR nuclear translocator (Arnt) to bind to the xenobiotic response element (XRE), a promoter sequence found in aryl hydrocarbon-inducible genes such as CYP1A1, to activate transcription.

In addition to P450s, genes encoding other detoxifying enzymes, such as NAD(P)H:quinone oxidoreductase (NQO1), glutathione S-transferase (GST), UDP-glucuronosyltransferase (UGT), and sulfotransferase, are also transcriptionally activated by a variety of stimuli including xenobiotic exposure and oxidative stress. Among the transcription factors involved, NF-E2-related factor 2 (Nrf2) plays a pivotal role in the expression of NQO1 and GST genes by antioxidants such as tert-butylhydroquinone (BHQ) (Jaiswal, 2004; Motohashi and Yamamoto, 2004). Similar to AhR, Nrf2 is located in the cytoplasm and translocates into the nucleus upon stimuli, where it dimerizes with a small Maf protein and binds to the antioxidant responsive element (ARE), a cis-acting sequence in the target genes (Jaiswal, 2004; Motohashi and Yamamoto, 2004). In addition to Nrf2 activation, NQO1 is also induced by AhR ligands including TCDD and β-naphthoflavone (BNF) (Prochaska and Talalay, 1988; Jaiswal, 1994; Nebert et al., 2000). This induction seems to be mediated through both AhR-XRE and Nrf2-ARE pathways (Favreau and Pickett, 1991; Radjendirane and Jaiswal, 1999; Noda et al., 2003; Ma et al., 2004).

These drug-metabolizing enzymes play important roles in detoxification.
fying and eliminating a variety of xenobiotics from the body. Even with this sophisticated drug-excreting system, however, a number of highly lipophilic environmental contaminants or pharmaceutical drugs, such as TCDD and its related compounds (i.e., dioxins), poly-chlorinated biphenyls, 1,1-dichloro-2,2-bis-(p-chlorophenyl)ethylene, and amiodarone, are eventually accumulated at high levels in white adipose tissue (WAT). These compounds demonstrate long half-lives in the body (tissue) on the order of months to years (Bickel, 1984; Latini et al., 1984; Muhlebach et al., 1985; Flesch-Jansys et al., 1996; Michalek et al., 1996). Because some of these compounds are considered endocrine disruptors, their effects on development and reproduction as well as cellular and metabolic toxicity have been studied intensively (Birnbaum, 1995; Longnecker et al., 1997). In contrast, their direct effects on adipose tissue remain unclear despite WAT being considered as not only a lipid store, as an energy source, but an important endocrine organ secreting a large number of cytokines and hormones (i.e., adipokines) including adiponectin, resistin, leptin, and tumor necrosis factor α, and expressing an array of genes involved in lipid and glucose metabolism (Ahima and Flier, 2000; Large et al., 2004).

Although the liver is the main organ of drug metabolism, the expression and induction of drug-metabolizing enzymes by xenobiotics is also often observed in extrahepatic tissues. Recently, we reported in rats that typical P450 inducers, namely, phenobarbital, dexamethasone, and BNF, increase both mRNA and protein levels of CYP2, CYP3, and CYP1 family members, respectively, in WAT as well as in the liver (Yoshinari et al., 2004). Here, we show that AhR agonists increase the expression of several detoxifying enzymes such as NQO1, GST, and aldehyde dehydrogenase (ALDH) in addition to CYP1A1 in rodent WAT, and that this induction is likely to be mediated by AhR and Nr2f. Moreover, we observed the induction of NQO1 in WAT by antioxidant BHQ. Our results suggest that lipophilic compounds accumulated in WAT can activate AhR and Nr2f pathways, increasing the tissue’s detoxifying capability, and raising the possibility that these compounds dysregulate the gene expression profile in WAT, causing unexpected effects on the tissue.

Materials and Methods

Materials. BNF, BHQ, menadione, 2,6-dichlorophenoleindophenol (DCPIP) sodium salt hydrate, FAD, Tween 20, and collagenase (type II) were obtained from Sigma (St. Louis, MO). 1-Chloro-2,4-dinitrobenzene (CDNB) and NADH were obtained from Wako Pure Chemical Industries (Osaka, Japan). Polyvinylidene fluoride (PVDF) membranes were obtained from Millipore (Billerica, MA). Oligonucleotides were synthesized by Greiner Bio-one (Tokyo, Japan). Anti-AhR, anti-Hsp90, and anti-NQO1 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-histone H1 antibody was purchased from Stressgen (Victoria, Canada). Oligonucleotides and antisense primers were designed by Greiner Bio-one (Tokyo, Japan) and their sequences are shown in Table 1.

Animal Treatment. Six- to 7-week-old male Sprague-Dawley rats, C57BL/6, and DBA/2 mice (Japan SLC, Hamamatsu, Japan) were acclimated for at least a week before the experiments. The animals were housed under a constant dark/light cycle (12 h each) and given standard chow and water ad libitum. They were given intraperitoneal injections of BNF (0.05–80 mg/kg), 3MC (40 mg/kg), BHQ (100 mg/kg), CDNB (40 mg/kg), menadione (40 mg/kg), or vehicle (corn oil) for the times indicated in the figure legends; then, they were killed, and the liver and WAT were excised for further analyses.

Adipocyte Separation. Separation of adipocytes and stromal-vascular (SV) cells from WAT was carried out according to the methods reported by Rodbell (1964) and Bornstein et al. (2000) with minor modifications. Briefly, rat epididymal WAT was minced and digested with collagenase at 37°C for 1 h. The digested tissue was filtrated with nylon mesh and centrifuged at 400g for 1 min, and floating adipocytes were washed three times and collected. Sedimented cells were suspended in 154 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA to lyse erythrocytes, and the remaining cells were washed and collected as SV cells.

Reverse Transcription-Polymerase Chain Reaction. Total RNAs were prepared by the acid guanidine-phenol-chloroform method using TRIzol reagent (Invitrogen, Carlsbad, CA), and first-strand cDNAs were synthesized with SuperScript III (Invitrogen) according to the manufacturer’s protocols. Conventional and quantitative real-time polymerase chain reaction experiments were carried out as described previously (Yoshinari et al., 2004) using rTaq polymerase (Takara Bio, Ohtsu, Japan) and SYBR Premix Ex Taq (Takara Bio).

Detection of AhR Protein. Cytoplasmic fractions were prepared as described previously (Yoshinari et al., 2003) from pooled rat livers or epididymal fat (n = 3 or 4), and 100 μg (liver) or 200 μg (WAT) of proteins were separated by SDS-polyacrylamide gel electrophoresis (9% gel) and immunostained with anti-AhR antibody or anti-Hsp90 antibody (as a loading control).

Enzymatic Assay and Detection of NQO1 Protein. Pooled mouse livers or epididymal fat (n = 3 or 4) were homogenized in 0.25 M sucrose/50 mM Tris-HCl (pH 7.5) and centrifuged at 105,000g for 60 min, and supernatants were collected as cytoplasmic fractions. The quinone reductase activity was determined as described previously (Shaw et al., 1991) with minor modifications. The final reaction mixture contained 25 mM Tris-HCl (pH 7.5), 0.18 mg/ml bovine serum albumin, 5 μM FAD, 0.01% Tween 20, 200 μM NADH, 5 to 20 μg of cytoplasmic protein, and 40 μM DCPIP (as a substrate). The reaction rate was determined by the decrease in absorbance at 600 nm.

Results

CYP1A1 Induction in Adipocytes. Previously, we demonstrated that BNF treatment of rats (40 mg/kg, 3 days) increased CYP1A1 mRNA, protein, and activities in epididymal WAT (Yoshinari et al., 2004). To examine whether this BNF induction of CYP1A1 is dependent on the anatomic site or type of adipose tissue, we treated rats with a single intraperitoneal injection of BNF (40 mg/kg) 24 h before killing and measured CYP1A1 mRNA levels by reverse transcription-polymerase chain reaction (RT-PCR) in different WATs, namely, epididymal, perirenal, mesenteric, and inguinal subcutaneous WAT, and interscapular brown adipose tissue (BAT), as well as in the liver. As shown in Fig. 1, A and C, significant induction of CYP1A1 protein levels, 150 μg of cytoplasmic proteins were separated by SDS-polyacrylamide gel electrophoresis with 12% gel and immunostained with anti-NQO1 or anti-Hsp90 antibodies.

All primers were designed to amplify both rat and mouse mRNAs. Sequences are shown in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense Primer</th>
<th>Antisense Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>CTTGCGGACTGTCCTTGTAGCACAGAC</td>
<td>CTTGCGGACTGTCCTTGTAGCACAGAC</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>CCAACCTTCTGACACGATTCTTT</td>
<td>CCAACCTTCTGACACGATTCTTT</td>
</tr>
<tr>
<td>AhR</td>
<td>TCTTCCTGCTGCAATTTGAGG</td>
<td>TCTTCCTGCTGCAATTTGAGG</td>
</tr>
<tr>
<td>Arnt</td>
<td>TGCCACCCAGATTGAGTTGG</td>
<td>TGCCACCCAGATTGAGTTGG</td>
</tr>
<tr>
<td>aP2</td>
<td>CATTCCGTGCAATGATACAT</td>
<td>CATTCCGTGCAATGATACAT</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>GCTGTGATGCCGACCACTGCC</td>
<td>GCTGTGATGCCGACCACTGCC</td>
</tr>
<tr>
<td>UCP1</td>
<td>CTGGCACTGCACGATCTTC</td>
<td>CTGGCACTGCACGATCTTC</td>
</tr>
<tr>
<td>RPS9</td>
<td>TCGCCGAAACCACAGTTGAGG</td>
<td>TCGCCGAAACCACAGTTGAGG</td>
</tr>
<tr>
<td>NQO1</td>
<td>GACAGTACACAGCATCTCTCTG</td>
<td>GACAGTACACAGCATCTCTCTG</td>
</tr>
<tr>
<td>GSTA1/2</td>
<td>GAGGATCTGATGGACAGAGTCAGG</td>
<td>GAGGATCTGATGGACAGAGTCAGG</td>
</tr>
<tr>
<td>ALDH3A1</td>
<td>AAATGATCCCCATGCGAACCCG</td>
<td>AAATGATCCCCATGCGAACCCG</td>
</tr>
<tr>
<td>HO-1</td>
<td>TAAAGCGCCTCCACCCAGGCAGG</td>
<td>TAAAGCGCCTCCACCCAGGCAGG</td>
</tr>
<tr>
<td>Nr2f</td>
<td>CTGCCAATGTCCTCCACCTATT</td>
<td>CTGCCAATGTCCTCCACCTATT</td>
</tr>
</tbody>
</table>

*This set of primers amplifies both GSTA1 and GSTA2 mRNAs.

Table 1: Sequences of primers used for RT-PCR analysis.
induction of CYP1A1 was also detected in BAT, and its mRNA levels were comparable to those in mesenteric WAT (Fig. 1, A and C). Another BNF target gene, CYP1B1, showed an expression pattern similar to that of CYP1A1, except for its extensively higher constitutive mRNA levels in adipose tissue than in the liver (Fig. 1, A and C). As a control, mRNAs for adipose fatty acid-binding protein aP2 (Bernlohr et al., 1995) and adiponectin (Scherer et al., 1995) as adipose-specific genes and for uncoupling protein 1 (UCP1) as a BAT-specific gene (Jacobsson et al., 1985) were also amplified and detected as expected (Fig. 1A).

Adipose tissue consists of fat-storing adipocytes and SV cells such as capillary, endothelial, and epithelial cells, and macrophages (Rodbell, 1964). We then investigated whether BNF induction of CYP1A1 occurred in adipocytes or other cells. To this end, RT-PCR analyses were performed with total RNAs prepared from adipocytes and SV cells separated from epididymal WAT of vehicle- or BNF-treated rats as well as RNAs from undigested WAT. Figure 1, B and D, shows that CYP1A1 and CYP1B1 induction was observed in both adipocytes and SV cells, and the degree of induction was higher in adipocytes than in SV cells. This difference in the mRNA level was also observed for Arnt, whereas AhR mRNA levels in SV cells were comparable to those in adipocytes (Fig. 1B). As expected, adipose-specific genes, aP2 and adiponectin, were exclusively detected in adipocytes but not in SV cells (Fig. 1B).

**Fig. 1.** Induction of CYP1A1 in various adipose tissues and adipocytes. Rats were treated with corn oil (lane C) or BNF (lane B; 40 mg/kg) 24 h before sacrifice, and livers and adipose tissue were excised for RT-PCR. A, RT-PCR analyses were performed with pooled total RNAs prepared individually from the liver (LV), epididymal WAT (EP), perirenal WAT (PR), mesenteric WAT (ME), and inguinal subcutaneous WAT (SC) \( n = 3 \), and RNAs from pooled interscapular BAT \( n = 3 \). Amplification was carried out for 24 (aP2), 26 (adiponectin, UCP1, and RPS9, 30 (Arnt), 32 (CYP1A1), or 36 (CYP1B1 and AhR) cycles. B, adipocytes (AD) and SV cells (SV) were separated from epididymal WAT. Total RNAs were prepared from the tissue and cells for RT-PCR. Amplification was carried out for 18 (aP2), 22 (adiponectin), 24 (RPS9), 28 (Arnt), 32 (CYP1A1 and CYP1B1), or 38 (AhR) cycles. Representative data of three independent experiments are presented. C and D, the same amount of RNA was subjected to quantitative RT-PCR, and CYP1A1 and CYP1B1 mRNA levels were determined with the corresponding standard DNAs. mRNA levels in the liver (C) or WAT (D) of corn oil-treated rats were set at 1. Data represent the means \( \pm \) S.D. of triplicate determinations. Open and closed bars indicated corn oil and BNF treatment, respectively. Numbers above bars indicate fold of induction. *\( p < 0.01. \)
whether CYP1A1 induction by BNF in WAT is mediated via an AhR-dependent pathway, we used aryl hydrocarbon-responsive C57BL/6 and -nonresponsive DBA/2 strains of mice (Poland et al., 1974, 1994). These mice were treated with BNF, 3MC, or vehicle 24 h before killing, and RT-PCR for CYP1A1 was performed with adipose and hepatic RNAs. When mice were treated with BNF (40 or 80 mg/kg), CYP1A1 induction was observed only in C57BL/6 strain but not in DBA/2 mice both in the liver and WAT (Fig. 3). Unexpectedly, 3MC treatment of DBA/2 mice increased CYP1A1 mRNA levels in WAT without affecting those in the liver (Fig. 3).

Upon ligand binding, cytoplasmic AhR translocates into the nucleus (Whitlock, 1999; Denison and Nagy, 2003). We thus prepared cytoplasmic fractions from rat epididymal WAT and livers after BNF treatment at several time points, and AhR protein levels were determined by Western blotting. In untreated rats, AhR protein levels in WAT were comparable to those in the liver (Fig. 4), consistent with its mRNA levels (Fig. 1). Four hours after BNF treatment (40 mg/kg), the AhR level in WAT had decreased to less than 10% of that of untreated rats, and this reduction continued at least 24 h after the treatment (Fig. 4A). On the other hand, AhR in the liver cytoplasm began to decrease as early as 1 h after treatment and returned to the control level after 24 h, although the amount of CYP1A1 mRNA remained at a high level (Fig. 4, A and B). BNF treatment did not change AhR mRNA levels significantly in either WAT or liver (Fig. 4C). The disappearance of AhR from the cytoplasm appears to differ in WAT as compared with the liver, and this could be a result of differences in either ligand-induced degradation or ligand-induced translocation of AhR. Taken together with the results of C57BL/6 and DBA/2 mice, these results strongly suggest that CYP1A1 induction by BNF in WAT is mediated by AhR as in the liver.

**Induction of NQO1 by BNF in WAT.** BNF is known as a bifunctional inducer, which enhances the expression of CYPI family members (CYP1A1, CYP1A2 and CYP1B1) through AhR and of other detoxifying enzymes (e.g., NQO1, GST, UGT) through Nrf2.
We thus investigated whether the expression of NQO1 in WAT was also induced by BNF. RT-PCR experiments demonstrated that BNF and 3MC effectively increased NQO1 mRNA levels in WAT as in the liver of C57BL/6 mice (Fig. 5A). Consistent with mRNA levels, NQO1 protein levels and quinone reductase activities were also elevated by BNF treatment (Fig. 5, B and C). In DBA/2 mice, no induction of NQO1 by BNF or 3MC was observed in mRNA, protein, and activity levels (data not shown).

**Induction of Phase II and Antioxidant Enzymes by Xenobiotics in WAT.** Nrf2 is involved in the expression of antioxidant enzymes such as heme oxygenase-1 (HO-1) in addition to NQO1 (Jaiswal, 2004; Motohashi and Yamamoto, 2004). Since BNF increased NQO1 expression in WAT (Fig. 5) and RT-PCR analyses demonstrated that Nrf2 mRNAs were abundantly expressed in the WAT of rats and mice (Fig. 6; data not shown), we also examined the expression of other Nrf2-target genes including ALDH3A1, GSTA1/2, and HO-1 in WAT. Both GSTA1/2 and ALDH3A1 mRNA levels in mouse WAT and livers were increased by treatment with BNF and 3MC, as was NQO1 mRNA (Fig. 5A). In contrast, HO-1 mRNA levels were unchanged by chemical treatment (Fig. 5A). When rats were used, similar results were obtained; in addition to NQO1, GSTA1/2 and ALDH3A1 mRNA levels were increased by BNF in various adipose tissues as well as in the liver, whereas no significant change was observed for HO-1 mRNA levels (Fig. 6). Interestingly, in rat visceral WAT, mRNA levels for NQO1, GSTA1/2, and ALDH3A1 as well as Nrf2 were comparable to or higher than those in the liver (Fig. 6).

Furthermore, we treated rats with another class of Nrf2 activators including BHQ, CDNB, and menadione (Ishii et al., 2000). NQO1 mRNA and protein levels, and quinone reductase activities in the liver were increased by treatment with these three compounds, although the magnitude of induction was much lower than that in BNF-treated rats (Fig. 7). In contrast, only BHQ and CDNB but not menadione increased NQO1 proteins and activities in WAT (Fig. 7, B and C), and neither BHQ, CDNB, nor menadione increased NQO1 mRNA levels (Fig. 7A). On the other hand, RT-PCR analyses demonstrated that CDNB and menadione increased HO-1 mRNA levels in WAT to greater degrees than in the liver, although there were large interindividual differences (Fig. 8; data not shown). In contrast, BHQ, CDNB, and menadione increased ALDH3A1 mRNAs in the liver more than they did in WAT (Fig. 8). No significant increases in GSTA mRNA in WAT were observed with these compounds (Fig. 8). These results indicate the inducer- and tissue-dependent regulation of these detoxifying enzymes.

![Fig. 4. BNF-induced AhR activation in WAT. Livers and epididymal WAT were excised from untreated rats (lane 0) or rats treated with BNF (40 mg/kg) at indicated time points. A, cytoplasmic fractions were prepared from pooled WAT and livers (n = 3 or 4), and a portion of the cytosol (WAT, 200 μg/lane; liver, 100 μg/lane) was subjected to Western blotting with anti-AhR and anti-Hsp90 (loading control) antibodies. Band intensities were measured with NIH Image software, and relative AhR protein levels were normalized with Hsp90 levels. Results are presented as a percentage of the AhR levels for untreated rats. Experiments were repeated twice, and representative data are shown. B, quantitative RT-PCR analyses were carried out with total RNAs prepared from pooled WAT and livers (n = 3 or 4). Relative CYP1A1 mRNA levels were calculated by raising 2 to the power of differences in Ct (2(ΔΔCt)) and were normalized with RPS9. mRNA levels of untreated rats were set at 1. Data are the means of duplicate determinations for each time point. C, RT-PCR analyses were carried out with total RNAs prepared from pooled WAT and livers (n = 3 or 4).](https://journals.asm.org/doi/10.1128/microbiolspec.ADS1003-0111.2020)
Lipophilic xenobiotics are usually metabolized into more hydrophilic forms by drug-metabolizing enzymes, mainly in the liver, and excreted from the body. However, several highly lipophilic compounds are known to be accumulated and reside in adipose tissue for a long time, from months to years in some cases. Because some of these compounds activate nuclear receptors, AhR or Nrf2, to increase the gene expression of drug-metabolizing enzymes, at least in the liver, this has raised the possibility that these transcription factors can also be activated in adipose tissue when a sufficient amount of chemicals is accumulated in the tissue. In this study, we demonstrated that BNF, an activator of both AhR and Nrf2, increased the expression of CYP1A1, CYP1B1, NQO1, and phase II enzymes in WAT, as in the liver. Furthermore, antioxidants including BHQ, CDNB, and menadione induced the expression of Nrf2 target genes, NQO1 and HO-1, in WAT. Previously, we demonstrated that phenobarbital and dexamethasone induced CYP2B and CYP3A expression, respectively, in rat WAT (Yoshinari et al., 2004). Taken together, these results suggest that adipose tissue has functional AhR, Nrf2, and nuclear receptor systems to activate the expression of a variety of detoxifying enzymes.

TCDD is one of the best known compounds that accumulate in adipose tissue. It is resistant to metabolism and its half-life is estimated as between 7 and 10 years in humans (Flesch-Janys et al., 1996; Michalek et al., 1996). Most, if not all, systemic and cellular effects of TCDD, including CYP1A1 induction, are mediated through the
activation of AhR (Fernandez-Salguero et al., 1996; Shimizu et al., 2000). Consistent with the high expression of AhR in WAT, a lipophilic AhR ligand, BNF, induced CYP1A1 and CYP1B1 in WAT (Yoshinari et al., 2004; this study). Furthermore, BNF treatment of rats resulted in loss of AhR from WAT cytoplasm as well as from liver cytoplasm (Fig. 4A), consistent with a functional AhR system in WAT. DBA/2 mice showed a significantly lower response to aryl hydrocarbons than C57BL/6 mice (Poland et al., 1974, 1994), further supporting a role for the AhR in CYP1A1 induction by BNF in WAT (Fig. 3.). Unexpectedly, 3MC treatment of DBA/2 mice induced CYP1A1 in WAT but not in the liver (Fig. 3). Although the exact reason remains to be elucidated in future studies, we speculate that, since DBA/2 mice have defects in the induction of hepatic CYP1A1 and other drug-metabolizing enzymes, which metabolize 3MC into more hydrophilic forms, 3MC was accumulated in the adipose tissue of DBA/2 mice at an extremely high level so that it was able to activate DBA/2 AhR despite its lower affinity for BNF compared with that of C57BL/6. Nonetheless, the results obtained in this study demonstrate that lipophilic AhR agonists activate AhR in adipose tissue, increasing the tissue’s metabolic capability. This suggests xenobiotic metabolism in adipose tissue and its importance in determining the elimination of lipophilic compounds from the tissue (and also from the body). This hypothesis is consistent with metabolism-resistant TCDD having an extremely long half-life (Bickel, 1984; Flesch-Janys et al., 1996; Michalek et al., 1996).

BNF, 3MC, and TCDD are known as bifunctional inducers (Prochaska and Talalay, 1988; Jaiswal, 1994; Nebert et al., 2000), and induce phase I and phase II enzymes through the activation of both AhR-XRE and Nrf2-ARE pathways (Xie et al., 1995; Radijendirane and Jaiswal, 1999; Whitlock, 1999; Denison and Nagy, 2003; Noda et al., 2003; Ma et al., 2004). Like AhR, Nrf2 was expressed in visceral WAT (Fig. 6; data not shown). Moreover, we have demonstrated that
NQO1, a typical Nrf2 target gene, increased its expression with BNF treatment in both mouse and rat adipose tissue (Figs. 5–7). These results suggest the existence of an active Nrf2-ARE pathway in adipose tissue. Nrf2 is activated by oxidative stresses produced by a variety of stimuli such as oxidative metabolism, UV light, radiation, heavy metal, inflammation, and viral infection (Jaiswal, 2004; Motohashi and Yamamoto, 2004). We thus treated rats with antioxidant or chemopreventive agents including BHQ, CDNB, and menadione, which seem to generate reactive oxygen species during their metabolism. These chemicals increased the expression of NQO1 and/or HO-1 in WAT (Figs. 7 and 8), although the compounds showed different selectivity toward target genes in WAT; BHF and CDNB for NQO1, and CDNB and menadione for HO-1. The reason for this is unknown at present. Nevertheless, these results corroborate the existence of an active Nrf2-ARE pathway in adipose tissue.

NQO1, which catalyzes the two-electron reduction of quinones, is expressed ubiquitously in animal tissues (Benson et al., 1980; Martin et al., 1987; Jaiswal, 2000). In this study, we have detected high levels of NQO1 protein, mRNA, and activity in epididymal WAT comparable to those in the liver (Figs. 5–7), consistent with earlier reports (Benson et al., 1980; Martin et al., 1987; Gaikwad et al., 2001). On the other hand, there is a discrepancy in the inducibility of adipose NQO1. We have demonstrated that BNF or BHQ treatment increased NQO1 levels in WAT as well as in the liver (Figs. 5–7), whereas in mice fed a diet containing 0.75% 2-(3-tert-butyl-4-hydroxyanisole for 2 weeks, quinone reductase activities were increased in several tissues including the liver, kidney, lung, stomach, and intestines but not in adipose tissue (Benson et al., 1980). This inconsistency might be due to differences in inducing agents, doses of inducers, and routes of administration. Moreover, the magnitude of NQO1 induction by BHF or CDNB was considerably lower than that by BNF, and menadione increased the expression of NQO1 in the liver but not in WAT (Fig. 7). This is due, at least in part, to differences in lipophilicity between these compounds, with BNF being the most lipophilic; logP values of BNF, BHF, CDNB, and menadione calculated with ChemDraw Ultra software (CambridgeSoft, Cambridge, MA) were 4.65, 2.53, 2.19, and 2.45, respectively. In addition, BHF and CDNB increased both NQO1 mRNA and protein levels in the liver, whereas they increased NQO1 proteins but not mRNAs in WAT (Fig. 7). Because we performed experiments using tissues 24 h after the last injection of inducers, the concentrations of these compounds in WAT at this time point could be too low to increase NQO1 mRNA levels. Further experiments for the time- or dose-dependent induction of NQO1 by these compounds are necessary to confirm this possibility. Interestingly, menadione enhanced the expression of HO-1 but not NQO1 in WAT (Fig. 8), suggesting the inducer- and tissue-specific induction of NQO1 and HO-1. These results support the idea that Nrf2 can activate NQO1 gene expression in adipose tissue as well as in the liver, although the reason for the high-level expression of NQO1 in WAT is unknown at present. In this regard, Gaikwad et al. (2001) have described an intriguing role of NQO1 in the glucose and fatty acid metabolism associated with the accumulation of abdominal adipose tissue.

In this study, we have examined whether the BNF induction of drug-metabolizing enzymes differs with the anatomic site or type of adipose tissue and found that visceral (epididymal, perirenal, and mesenteric) WAT shows significant induction of CYP1A1, CYP1B1, and NQO1 compared with inguinal subcutaneous WAT (Figs. 1 and 6). The main, if not only, reason for this is probably that the inducers were administrated intraperitoneally in this study. Because lipophilic compounds are able to enter adipocytes by passive diffusion, it is possible that BNF concentration in visceral WAT was higher than that in subcutaneous WAT after intraperitoneal injection. Moreover, mRNA levels of AhR, Arnt, and Nrf2 were much higher in visceral WAT than in subcutaneous WAT (Figs. 1 and 6). Thus, BNF might be able to activate gene transcription effectively in visceral WAT compared with subcutaneous WAT, although we cannot rule out the possibility that a visceral WAT-specific factor(s) plays an essential role in BNF induction in the tissue.

In conclusion, we have demonstrated that rodent WAT contains functional AhR and Nrf2 and that lipophilic xenobiotics accumulated in WAT activate these transcription factors to enhance the transcription of an array of genes encoding detoxifying enzymes. Given that AhR and Nrf2, as well as nuclear receptors, are suggested to play important roles in diverse cellular functions in addition to detoxification, and that adipose tissue is an important organ controlling lipid and carbohydrate homeostasis in the body, lipophilic xenobiotics accumulated in adipose tissue may exhibit as yet unknown effects in the tissue through the activation of these transcription factors.

Acknowledgments. We are grateful to Dr. Yasushi Yamazoe (Tohoku University, Sendai, Japan) for the generous gift of 3MC.

References
hydrocarbon receptor-deficient mice are resistant to 2,3,7,8-tetrachlorodibenzo-p-dioxin- 
Elimination of polychlorinated dibenzodioxins and dibenzofurans in occupationally ex-
oxidoreductase 1 (NQO1) in the regulation of intracellular redox state and accumulation of 
Transcription factor Nr2f2 coordinately regulates a group of oxidative stress-inducible genes in 
Jaiswal AK (2000) Regulation of genes encoding NAD(P)H:quinone oxidoreductases. Free 
Lau D, Tripathi RC, Pirkle JL, Caudill SP, Michalek JE, Pirkle JL, Caudill SP, Tripathi RC, 
Patterson DG Jr, and Needham LL (1996) Elimination of polychlorinated dibenzo-p-dioxins and dibenzo- 
Longnecker MP, Rogan WJ, and Lucier G (1997) The human health effects of DDT (dichloro-
diphenyltrichloroethane) and PCBs (polychlorinated biphenyls) and an overview of organo-
Motohashi H and Yamamoto M (2004) Nrf2-Keap1 defines a physiologically important stress 
Muhlebach S, Wyss PA, and Bickel MH (1985) Comparative adipose tissue kinetics of chilenol-
Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y, and Dalton TP (2000) Role of the aromatic 
hydrocarbon receptor and AhR gene battery in the oxidative stress response, cell cycle control 
expression of detoxifying enzymes in AhR and Nr2f2 compound null mutant mouse. Biochem 
Biophys Res Commun 303:105–111.
Poland AP, Glover E, Robinson JR, and Nebert DW (1974) Genetic expression of aryl hydro-
carbon hydroxylase activity. Induction of monoxygenase activities and cytochrome P450 
formation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice genetically “nonresponsive” to other 
Poland A, Palen D, and Glover E (1994) Analysis of the four alleles of the murine aryl 
Prochaska HJ and Talalay P (1988) Regulatory mechanisms of monofunctional and bifunctional 
Radljevindare V and Jaiswal AK (1999) Antioxidant response element-mediated 2,3,7,8-
tetrachlorodibenzo-p-dioxin (TCDD)-induction of human NAD(P)H:quinone oxidoreductase 1 
Rodbell M (1964) Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism 
Saarikoski ST, Rivera SP, Hankinson O, and Husafvel-Pursiainen K (2005) CYP2S1: a short 
dioxin-inducible NAD(P)H:quinone oxidoreductase cDNA-encoded protein expressed in 
Shimizu Y, Nakaturo Y, Ichinose M, Takahashi Y, Kume H, Mimura J, Fujii-Kuriyama Y, and 
Ishikawa T (2000) Benz[a]pyrene carcino genesis is lost in mice lacking the aryl hydrocarbon 
103–125.

Address correspondence to: Dr. Masao Miwa, School of Pharmaceutical 
Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka-
ken 422-8526 Japan. E-mail: miwa@u-shizuoka-ken.ac.jp