Hypoxia and CYP1A1 induction-dependent regulation of proteins involved in glucose utilization in Caco-2 cells

VÉRONIQUE CARRIÈRE,1 ANNE RODOLOSSE,1 MICHEL LACASA,1
DANIELLE CAMELIER,1 ALAIN ZWEIBAUM,1 AND MONIQUE ROUSSET1

1Unité de Recherches sur la Différenciation Cellulaire Intestinale, Institut National de la Santé et de la Recherche Médicale U178, 94807 Villeguy Cedex; and 2Université Pierre et Marie Curie, 75252 Paris Cedex 05, France

Carrière, Véronique, Annie Rodolosse, Michel Lacasa, Danielle Cambier, Alain Zweibaum, and Monique Rousset. Hypoxia and CYP1A1 induction-dependent regulation of proteins involved in glucose utilization in Caco-2 cells. Am. J. Physiol. 274 (Gastrointest. Liver Physiol. 37): G1101–G1108, 1998.—Although induction of cytochrome P-450 1A1 (CYP1A1) in the Caco-2 clone TC7 alters glucose utilization and modifies the expression of sucrose-isomaltase (SI) and hexose transporters, nothing is known of the events that control these effects. In this study, we analyzed the effects of β-naphthoflavone (β-NF) and hypoxia on these parameters and expression of key enzymes of glucose metabolism. Both β-NF and hypoxia induce similar changes: 1) induction of CYP1A1 mRNA; 2) increased glucose consumption and lactic acid production and lower glycogen content; 3) downregulation of SI and upregulation of GLUT1 mRNAs; 4) downregulation of fructose-1,6-bisphosphatase and pyruvate kinase mRNAs and upregulation of phosphoenolpyruvate carboxykinase, pyruvate dehydrogenase, lactate dehydrogenase, and phosphofructokinase mRNAs; and 5) upregulation of c-fos and c-jun mRNAs. Although addition of inhibitors of CYP1A1 catalytic activity to β-NF-treated cells totally inhibits the enzyme activity, it does not modify CYP1A1 mRNA response and associated effects, thus excluding a direct role for the enzyme per se. These results point to a possible physiological implication of the signal-transduction pathway responsible for CYP1A1 induction.

β-naphthoflavone; cobalt chloride; sucrose-isomaltase; GLUT1; glucose metabolism

Expression of cytochrome P-450 1A1 (CYP1A1), a monoxygenase involved in the metabolism of xenobiotics, is highly inducible by polycyclic aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin, β-naphthoflavone (β-NF), or imidazole derivatives. Although most studies on the induction of CYP1A1 have concerned the liver or animal models, recent studies have shown that CYP1A1 is also inducible in the human small intestine and in the enterocytic human cell line Caco-2.

Among clonal populations isolated in our laboratory from the Caco-2 cell line (7), one clone, TC7, has been shown to be even closer to the small intestinal enterocytes than the parental population as to the level of expression of differentiation-associated proteins (7, 23). We have previously reported that the treatment of TC7 cells with CYP1A1 inducers results in increases of glucose consumption and lactic acid production and in marked modifications, at the mRNA and protein levels, of the expression of membrane proteins involved in the uptake and transport of hexoses (6). These modifications include a downregulation of the brush-border hydrolase sucrose-isomaltase (SI) and of hexose transporters GLUT2, GLUT5, and SGLT1 and an upregulation of hexose transporters GLUT1 and GLUT3 (6). These results raised the question of whether CYP1A1 per se or the signal-transduction system that controls its induction could be involved in these regulations.

CYP1A1 induction requires the recruitment of two members of the basic helix-loop-helix-PAS (bHLH-PAS) family of DNA-binding proteins: the aryl hydrocarbon receptor (AHr), associated in the cytoplasm with the heat-shock protein 90 (Hsp90), and the aryl receptor nuclear translocator (ARNT) proteins. The binding of the ligand to AHr results in the nuclear translocation of the complex followed by the release of Hsp90 and dimerization with the ARNT protein. Then the heterodimer AHr-ARNT interacts specifically with DNA on the xenobiotic-responsive element (XRE) and enhances the transcription of CYP1A1 and other specific genes (see Ref. 32 for review). To date no endogenous ligand has been found and the biological role of Ah and Arnt genes is under investigation. Recently, the establishment of Ahr null mice has indicated that AHR may play a role in the development of the liver and in the function of the immune system (see Ref. 32 for review). Concerning the physiological function of ARNT, a recent study has demonstrated that ARNT is identical to the β-subunit of the hypoxia-inducible factor 1 (HIF-1β) (39). Subsequently, several experiments have shown that ARNT/HIF-1β is able to dimerize with HIF-1α subunit and that this heterodimer binds to the hypoxia-responsive elements (HRE) and modulates the expression of oxygen-regulated genes (see Ref. 33 for review). Among them are the genes coding for erythropoietin (EPO) (34), the vascular endothelial growth factor VEGF (21), the hexose transporter GLUT1 (22), and several glycolytic enzymes such as phosphoglycerate kinase 1, lactate dehydrogenase A (LDH A), aldolase A, phosphofructokinase L (PFK L), and enolase 1 (13, 33), and pyruvate kinase M (PK M) (12). In addition, a recent report of targeted inactivation of the Arnt gene in mice indicates that Arnt, through its involvement in the cellular response to hypoxia, regulates angiogenesis and vasoforimation during mammalian embryonic development (24). Finally, the cellular response to hypoxia was also reported to be characterized by an increased glycolysis (22, 28) and by modifications of the expression of genes without any identified HRE such as the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (17) and the hexose transporters GLUT2 and GLUT3 (12).
Because of the striking similarities between some of these findings and our own data, and to decipher the mechanisms responsible for the changes observed when TC7 cells are exposed to CYP1A1 inducers, the purpose of the present work was to further investigate which physiological modifications are associated with the observed effects. Three approaches were used. First, we analyzed whether CYP1A1 inducers such as β-NF would modify the expression of key enzymes of glycogen synthesis or gluconeogenesis. Second, we compared the effects of β-NF with those of cellular hypoxia on 1) glucose utilization, 2) expression of some key enzymes of glucose metabolism, and 3) expression of the CYP1A1 gene compared with that of two membrane-associated proteins chosen for their inverse variations, i.e., SI and GLUT1 (6). Third, we analyzed whether the observed effects were dependent on CYP1A1 activity.

MATERIALS AND METHODS

Cell culture. TC7 cells (7) were seeded at 0.2 × 10^6 cells/25 cm² T flask (Corning Glassworks, Corning, NY) and cultured in DMEM (25 mM glucose) supplemented with 1% nonessential amino acids (GIBCO, Paisley, UK) and 20% heat-inactivated (30 min at 56°C) fetal bovine serum (Boehringer, Mannheim, Germany). Aliquots of concentrated solution (50 mM in DMSO) of β-NF (Sigma, Saint-Quentin-Fallavier, France) and α-NF (Sigma) were added to the culture medium at a final concentration of 20 or 200 µM (37). At these final concentrations, DMSO (0.04%) and chloroform (0.01% and 0.1%) have no effect on any parameter of this study. CoCl₂ (Sigma) was concentrated solution (200 mM in chloroform) of 8-methoxypsoralen (MOP) (Sigma) were added to the culture medium at a final concentration of 20 µM (6, 41). Aliquots of concentrated solution (200 mM in chloroform) of 8-methoxypsoralen (MOP) (Sigma) were added to the culture medium at a final concentration of 20 or 200 µM (37). At these final concentrations, DMSO (0.04%) and chloroform (0.01% and 0.1%) have no effect on any parameter of this study. CoCl₂ (Sigma) was added to the culture medium at a final concentration of 75 µM, a concentration currently used in various cellular systems (24, 39). TC7 cells were cultured under hypoxic conditions in an atmosphere of 1% O₂, 10% CO₂, and 89% N₂. I na l l

Glucose consumption, intracellular glycogen, cAMP concentrations, and protein assays. Glucose consumption was determined daily by measuring the amount of glucose remaining in the medium 16-18 h after the medium changes, unless otherwise indicated, with the use of a Beckman Glucose Analyzer 2. Glycogen content was measured with anthrone as previously reported (7). The concentration of intracellular cAMP was measured using a cAMP enzyme immunoassay system (Amersham). Protein content was measured with bichinchoninic acid protein assay reagent (Pierce, Rockford, IL).

Microsomal fraction preparation. TC7 cells were washed twice with Ca²⁺-Mg²⁺-free PBS, detached from their support by scraping, and immediately frozen and stored in liquid nitrogen. The cells were slowly thawed at 4°C in a 2 mM Tris-HCl (pH 7) buffer containing 50 mM mannitol and supplemented with protease inhibitors (100 µM phenylmethanesulfonyl fluoride and 25 µg/ml benzamidine). The cell suspension was sonicated at 4°C, and an aliquot of the homogenate was stored in liquid nitrogen for determination of the sucrose and dipetidyl-peptidase IV (DPP-IV) activities. The homogenate was centrifuged at 9,000 g for 20 min to remove nuclear and cell debris. The supernatant was then centrifuged at 100,000 g for 60 min. The pellet, corresponding to the microsomal fraction, was resuspended in a small volume of 100 mM NaPO₄ (pH 7.4) buffer containing 10 mM MgCl₂ and 20% glycerol, supplemented with protease inhibitors, and stored in liquid nitrogen until use.

Enzymatic activities. Surace and DPP-IV activities were measured in the cell homogenates as previously reported (7), using 1.5 mM L-glucyl-L-proline-4-nitroanilide as substrate for DPP-IV. Results are expressed as milliunits per milligram of protein; one unit is defined as the activity that hydrolyzes 1 micromole of substrate per minute at 37°C. The 7-ethoxyresorufin-O-deethylase activity was measured using the direct fluorimetric assay described by Burke and Mayer (5). Microsomal fractions (0.1–0.3 mg/ml of proteins) were incubated at 37°C in buffer containing 50 mM Tris-HCl (pH 7.5) and 25 mM MgCl₂ in the presence of 125 µM of reduced NADP (Boehringer Mannheim) and 2 µM of 7-ethoxyresorufin (Sigma). At the end of the assay, 10 pmol of resorufin (Sigma) were added to calibrate the amount of resorufin produced. Parameters for fluorescence detection were 522 nm for the excitation wavelength and 586 nm for the emission wavelength, determined with the use of a J obin Yvon spectrofluorimeter J YD3. Results are expressed as picomoles of resorufin produced per milligram of microsomal protein per minute.

RNA extraction and Northern blot analysis. Total RNA was extracted by the guanidinium isothiocyanate method (8). Twenty micrograms of total RNA denatured in 1 M glyoxal for 60 min at 50°C were applied to 1% agarose gel. After the run in 10 mM sodium phosphate buffer, the RNA was transferred to nylon (Hybond N; Amersham) with 20× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0). Prehybridization was performed overnight at 42°C in the presence of 50% formamide, and hybridization was performed at 42°C in the presence of 40% formamide and 10% dextran sulfate. The probes were 32P labeled using a megaprime DNA labeling kit (Amersham). After hybridization, the blots were washed twice in 2× SSC-0.1% SDS for 10 min at room temperature, once in 0.1× SSC-0.1% SDS for 15 min at 50°C, and once in 0.1× SSC-0.1% SDS for 15 min at 65°C. For control of RNA loading, filters were dehybridized and stained with methylene blue (25). Quantitation was achieved with a densitometric scanner (Mark I II CS; Joyce, Loebi, Gatehead, UK).

Probes. The probes used for the detection of CYP1A1, SI, DPP-IV, and GLUT1 mRNAs were the same as previously reported (6). AHR was detected with phuARNT (11) and ARNT with phuARNT (36) obtained from C. Bradford (Northwestern University Medical School, Chicago, IL). Hexokinase II (HK II) was detected with a rat cDNA probe (38) obtained from J. E. Wilson (Michigan State University, East Lansing, MI). The c-fos probe was generated by reverse transcription-PCR from mouse mRNA (as described in Ref. 2), and the c-jun probe (3) was obtained from B. Binetruy (Institut Fédératif sur le Cancer, Villejuif, France). The other cDNA probes for human glucose metabolic enzymes were obtained by reverse transcription-PCR of total RNA isolated from TC7 postconfluent cells. Reverse transcription was performed at 42°C for 2 h, using a first-strand cDNA synthesis system kit (Amersham) with 6 µg of total RNA, 20 U of RNasin, 1 mM each of deoxynucleotide triphosphates (dNTPs), 1.6 µg of oligo(dT) primers, and 40 U of RT in 20 µl of final reactional volume. PCR was performed on one-fifth of the reverse-transcription PCR in 25 µl of Taq polymerase buffer (Tebu, Houdan, France) containing 0.5 µM of oligonucleotide primers, 125 µM each of dNTPs, and 25 U of Tfl polymerase. The sequences of the primers chosen for their inverse variations, i.e., SI and GLUT1 (6). Third, we analyzed whether the observed effects were dependent on CYP1A1 activity.

MATERIALS AND METHODS

Cell culture. TC7 cells (7) were seeded at 0.2 × 10⁶ cells/25 cm² T flask (Corning Glassworks, Corning, NY) and cultured in DMEM (25 mM glucose) supplemented with 1% nonessential amino acids (GIBCO, Paisley, UK) and 20% heat-inactivated (30 min at 56°C) fetal bovine serum (Boehringer, Mannheim, Germany). Aliquots of concentrated solution (50 mM in DMSO) of β-NF (Sigma, Saint-Quentin-Fallavier, France) and α-NF (Sigma) were added to the culture medium at a final concentration of 20 or 200 µM (37). At these final concentrations, DMSO (0.04%) and chloroform (0.01% and 0.1%) have no effect on any parameter of this study. CoCl₂ (Sigma) was added to the culture medium at a final concentration of 75 µM, a concentration currently used in various cellular systems (24, 39). TC7 cells were cultured under hypoxic conditions in an atmosphere of 1% O₂, 10% CO₂, and 89% N₂. I na l l
RESULTS

Permanent exposure of TC7 cells to β-NF results in modifications of the parameters of glucose utilization and the pattern of expression of glucose metabolism enzymes. Permanent exposure of TC7 cells to β-NF results, as previously reported (6), in a high level of CYP1A1 and, compared with untreated cells, a permanently higher level of GLUT1 and a much lower expression of SI in postconfluent differentiated cells (Fig. 1A). These changes were associated with increased rates of glucose consumption and lactic acid production (Table 1). Interestingly, the ratio between the amount of glucose consumed and the amount of lactic acid produced indicates that in β-NF-treated cells >90% of the glucose consumed is metabolized into lactic acid, vs. 55% in control cells. Although the intracellular cAMP concentration was unchanged in both treated and untreated cells, the intracellular glycogen content was much lower in β-NF-treated than in control cells (Table 1), indicating that the variations observed are not related to CAMP-dependent glycogenolysis.

To determine whether these effects of β-NF occur via perturbations of glucose metabolism, we have analyzed the mRNA levels of key enzymes of the glycolytic and gluconeogenic pathways during the time course of cell proliferation and differentiation, i.e., from seeding to late postconfluence. In control cells, as shown in Fig. 1B, PK, PyC, Fru(1,6)P2-ase, and PEPCK increase, whereas HK I, PDH, and G-6-PDH decrease during the time course of the culture. The same pattern was observed in β-NF-treated cells, but quantitative changes are characterized by a marked decrease of Fru(1,6)P2-ase compared with control cells (Fig. 1B). No changes were observed for PyC, HK I, and G-6-PDH. No HK II mRNA could be detected in either control or β-NF-treated cells.

The inhibitory effect of β-NF on SI expression does not require CYP1A1 enzymatic activity. To analyze whether the effect of β-NF on the expression of SI, chosen as a

![Fig. 1. Analysis of mRNA levels of cytochrome P-450 1A1 (CYP1A1), sucrase-isomaltase (SI), and GLUT1 (A) and some enzymes of glucose metabolism (B) during the time course of cell proliferation and differentiation, i.e., from seeding to late postconfluence. In control cells, as shown in Fig. 1B, PK, PyC, Fru(1,6)P2-ase, and PEPCK increase, whereas HK I, PDH, and G-6-PDH decrease during the time course of the culture. The same pattern was observed in β-NF-treated cells, but quantitative changes are characterized by a marked decrease of Fru(1,6)P2-ase compared with control cells (Fig. 1B). No changes were observed for PyC, HK I, and G-6-PDH. No HK II mRNA could be detected in either control or β-NF-treated cells. The inhibitory effect of β-NF on SI expression does not require CYP1A1 enzymatic activity. To analyze whether the effect of β-NF on the expression of SI, chosen as a
marker of the effect, is dependent on CYP1A1 catalytic activity per se, TC7 cells were exposed between days 7 and 9 of culture to enzymatic inhibitors of CYP1A1 activity in the absence or presence of β-naphthoflavone (β-NF) (20 µM) and analyzed at late confluence (day 15), 20 h after the medium change. UT, untreated cells.

Exposure of TC7 cells to CoCl2 results in the same alterations of glucose utilization as observed with β-NF. In contrast to β-NF, which has no effect on cell growth and viability (6), exposure of the cells to CoCl2 [which mimics cellular hypoxia (20)] from the beginning of culture, i.e., during the phase of cell proliferation, resulted in a high rate of mortality, thus excluding a protocol of permanent exposure. We turned therefore to the same short-term treatment of 48 h as reported above, starting on the day of confluence, i.e., day 7 under our conditions of seeding. We first established that, at this time of culture, CoCl2 has no deleterious effect on cell viability. As shown in Table 2, both β-NF and CoCl2 induced similar alterations of glucose utilization, namely, a smaller decrease of the rates of glucose consumption and lactic acid production associated with a stabilization of the ratio of lactic acid production to glucose consumption and a smaller increase of glycogen concentration.

Similar effects of β-NF and CoCl2 on the level of expression of SI, GLUT1, and glucose metabolism enzyme mRNAs. Northern blot analysis of total RNA isolated from cells exposed for 48 h, between days 7 and 9, to β-NF or CoCl2 is shown in Fig. 3. Analysis of these results shows that 1) short-term treatment with β-NF and permanent exposure to the drug have the same effect on the genes studied here (see Fig. 1), and 2) CoCl2 induces the same changes as β-NF, i.e., a smaller increase of the level of SI and increased expression of GLUT1, as well as a smaller increase of F(1,6)P2ase and PK and an increase of PEPC, PFK, and LDH. The decrease of SI gene expression after β-NF or CoCl2 treatment was confirmed by enzyme activity assays (Fig. 4). In contrast, DPP-IV, analyzed here as a control of differentiation not linked to glucose utilization, was found to be unchanged at both mRNA (Fig. 3) and activity (Fig. 4) levels.

To analyze whether CoCl2 would modify the effects of β-NF on the genes studied, CoCl2 was added for 48 h between days 7 and 9 to cells permanently exposed to β-NF from the beginning of the culture. No additional effect was observed for most of the genes studied.

Table 1. Glucose utilization parameters in postconfluent TC7 cells

<table>
<thead>
<tr>
<th></th>
<th>UT</th>
<th>β-NF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose consumption</td>
<td>8.5 ± 0.5</td>
<td>51.5 ± 7</td>
</tr>
<tr>
<td>rate, µg·mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>protein⁻¹·h⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>4.7 ± 0.05</td>
<td>48 ± 5.4</td>
</tr>
<tr>
<td>production rate, µg·mg</td>
<td>0.55</td>
<td>0.94</td>
</tr>
<tr>
<td>protein⁻¹·h⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycogen content, µg/mg protein</td>
<td>350 ± 55</td>
<td>159 ± 25</td>
</tr>
<tr>
<td>cAMP, pmol/mg protein</td>
<td>19 ± 2</td>
<td>21 ± 3</td>
</tr>
</tbody>
</table>

Data are means ± SD of 3 different cultures. TC7 cells were grown, from the first day of culture on, in the presence or absence of β-naphthoflavone (β-NF) (20 µM) and analyzed at late confluence (day 15), 20 h after the medium change. UT, untreated cells.

Table 2. Glucose utilization parameters in confluent TC7 cells treated with β-NF and CoCl2 or left untreated

<table>
<thead>
<tr>
<th></th>
<th>UT</th>
<th>β-NF(s)</th>
<th>CoCl2(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose consumption</td>
<td>38.6 ± 5.9</td>
<td>81.7 ± 16.6</td>
<td>53 ± 5.8</td>
</tr>
<tr>
<td>rate, µg·mg·protein⁻¹·h⁻¹</td>
<td>36.7 ± 15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactic acid production rate, µg·mg·protein⁻¹·h⁻¹</td>
<td>60.8 ± 6.8</td>
<td>21.4 ± 2.3</td>
<td>39 ± 5.8</td>
</tr>
<tr>
<td>Glycogen content, µg/mg protein</td>
<td>132 ± 12</td>
<td>228 ± 27</td>
<td>208 ± 28</td>
</tr>
<tr>
<td>Lactic acid production/glucose consumption</td>
<td>0.71</td>
<td>0.55</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Data are means ± SD of 3 different cultures. TC7 cells were grown, from the first day of culture on, in the presence or absence of β-naphthoflavone (β-NF) (20 µM) or CoCl2 (75 µM) from the day of confluence (day 7) for 48 h (short treatment(s)). The cells were analyzed on day 9, 24 h after the medium change, *P < 0.05 and †P < 0.01. β-NF- and CoCl2-treated compared with control untreated cells (UT day 9).

Fig. 2. Effect of inhibitors of CYP1A1 activity on expression of SI and CYP1A1. TC7 were analyzed on day 9 of culture after a 48-h treatment with α-NF (20 µM) or β-methoxyresorafon (MOP; 20 µM and 200 µM) in presence or absence of β-NF (20 µM). A: Northern blot analysis of CYP1A1 and SI mRNAs. Same amount of total RNA (20 µg) was loaded in each lane. After hybridization with indicated probes, filters were stained with methylene blue. B: CYP1A1 activity toward 7-ethoxyresorufin. nd, Not detectable.
CoCl2 upregulates CYP1A1 at the mRNA level but inhibits its activity. The expression of CYP1A1 was analyzed at both mRNA and enzyme activity levels in cells treated with CoCl2, β-NF, or a combination of the two drugs. As shown in Fig. 6A, a 48-h exposure of TC7 cells to CoCl2 between days 7 and 9 resulted in a threefold increase of CYP1A1 mRNA, an increase that is, however, lower than the 80-fold increase observed with β-NF during the same period. Interestingly, CoCl2, when added for 48 h to cells permanently treated with β-NF, potentiates the effect of β-NF on the increase of CYP1A1 mRNA levels (Fig. 6B). These effects on CYP1A1 occur without modifications of AHR and ARNT mRNA levels (Fig. 6A). Contrasting with the high activity of CYP1A1 observed in cells exposed to a short or a permanent treatment with β-NF, and despite the increase of CYP1A1 mRNA, not only was CYP1A1 activity undetectable in CoCl2-treated cells but the drug completely inhibited the activity of CYP1A1 when added to β-NF-treated cells (Fig. 6C).

β-NF and CoCl2 upregulate c-jun and c-fos. The short treatment with CoCl2 also induced an increase of c-jun and c-fos mRNA levels, an increase that, in cells treated permanently with β-NF, occurred only after confluence, i.e., on day 9 (Fig. 7). It must be noted that a 48-h
exposure to CoCl₂ of cells treated permanently with β-NF resulted in a marked increase in c-jun but not of c-fos mRNA compared with treatment with β-NF alone (Fig. 7).

Hypoxia induces the same changes as β-NF or CoCl₂ treatment on the rate of glucose consumption and the expression of CYP1A1 and of proteins involved in glucose utilization. The culture of TC7 cells under hypoxic conditions for 48 h between days 7 and 9 results in an increase in the rate of glucose consumption (53 ± 6 µg·mg⁻¹·h⁻¹ protein compared with 40 ± 3 µg·mg⁻¹·h⁻¹ protein in control cells), an upregulation of CYP1A1 and GLUT1 mRNAs, and decreased levels of SI and Fru(1,6)P₂ase mRNAs (Fig. 8). Exposure of the cells to both β-NF and hypoxic conditions enhances the inhibitory effects of β-NF on SI and Fru(1,6)P₂ase expression (Fig. 8), while totally inhibiting CYP1A1 activity (data not shown).

**DISCUSSION**

The present results show that treatment of TC7 cells with CoCl₂ results in the same modifications as those previously reported with inducers of CYP1A1 (6), namely, increased glucose utilization (i.e., increased

---

**Fig. 6.** Effects of β-NF and CoCl₂ on mRNA levels of CYP1A1, aryl hydrocarbon receptor (AHR), and aryl receptor nuclear translocator (ARNT) and on CYP1A1 activity. A: Northern blot analysis of CYP1A1, AHR, and ARNT mRNAs in TC7 cells analyzed on days 7 and 9 of culture under same treatment conditions as in Fig. 3. B: quantification of CYP1A1 mRNA as deduced from a scanning of a Northern blot of TC7 cells analyzed on day 9 of culture, with same treatment conditions as in Fig. 5. C: activity of CYP1A1 toward hydroxylation of 7-ethoxyresorufin in β-NF and CoCl₂-treated TC7 cells. CYP1A1 activity was measured in control TC7 cells (UT), after 48-h exposure to β-NF (β-NF(s)), or after permanent exposure to β-NF (β-NF(p)) with or without a short treatment with CoCl₂ during the last 48 h. All cells were analyzed on day 9. Results are means ± SD of 3 different cultures.

---

**Fig. 7.** Effect of β-NF and CoCl₂ on levels of c-jun and c-fos mRNAs. Control TC7 cells and cells permanently exposed to β-NF and either not treated or treated with CoCl₂ for 48 h, starting on day 7 of the culture, were harvested on indicated days. Same amount of total RNA (20 µg) was loaded in each lane. After hybridization the filter was stained with methylene blue.

---

**Fig. 8.** Effect of β-NF and hypoxia on CYP1A1, SI, GLUT1, and fructose-1,6-bisphosphatase mRNA levels. TC7 cells were analyzed on day 9, after 48 h of culture under indicated conditions. Same amount of total RNA (20 µg) was loaded in each lane. Filter was hybridized with indicated probes and then dehybridized and stained with methylene blue.
rutes of glucose consumption and lactic acid production and lower glycogen content) and alterations of the expression of membrane proteins involved in glucose uptake and transport, i.e., a downregulation of SI and an upregulation of GLUT1. These results also show that both CoCl₂ and β-NF, utilized here as an inducer of CYP1A1, produce marked changes in the mRNA levels of enzymes involved in glucose metabolism, namely, a downregulation of Fru(1,6)P₂ase and PK and an upregulation of PEPCK and, to a lesser extent, LDH, PFK, and PDH. CoCl₂ also induces an increase in CYP1A1 mRNA, similar to β-NF but to a lesser extent. Culture of TC7 cells in hypoxic conditions results in an induction of CYP1A1 mRNA, increased glucose consumption, and the same modifications of expression of SI, Fru(1,6)P₂ase, and GLUT1 as observed when the cells were exposed to β-NF and CoCl₂. The unexpected finding that CoCl₂ and hypoxia induce an increased level of CYP1A1 mRNA in TC7 cells can be explained by the induction of c-jun and c-fos, as previously reported in other cell systems (40). Indeed, c-jun and c-fos could be responsible for the increase in CYP1A1 mRNA levels via the AP-1 site located in the promoter region of the CYP1A1 gene (19, 35).

Among the modifications associated with the increased glucose utilization observed in TC7 cells exposed to β-NF and CoCl₂, the increase in PFK, LDH, and PDH and the decrease in Fru(1,6)P₂ase mRNAs are consistent with the increase of glycolysis observed in these cells. However, it must also be noted that the glucose-dependent regulation of PEPCK and PK is the inverse of that observed in the liver (10, 16, 20) or in skeletal muscle cells (28) and does not correlate with an increase of glycolysis. Further studies are needed to determine whether this different behavior is a characteristic of intestinal cells or depends on the neoplastic status of TC7 cells.

That the observed effects are associated with the induction of CYP1A1 mRNA but do not depend on the catalytic activity of the enzyme can be concluded from the results obtained with the inhibitors of CYP1A1 activity, CoCl₂ or hypoxia. This raises the question of whether the signal-transduction pathway responsible for CYP1A1 induction is directly or indirectly involved in the modifications of the parameters of glucose utilization observed in the cells.

The molecular mechanisms that control CYP1A1 induction and, more particularly, the involvement of the AHR and ARNT protein are now well documented (32). It has also been recently reported that ARNT is involved in the cellular response to hypoxia (39, 42). However, nothing is known of an involvement of its transduction system in any of the metabolic effects and changes observed in TC7 cells.

One hypothesis is that these metabolic effects could be the consequence of a cascade of events triggered by a signal that remains to be determined, which results in an increase of glycolysis, which in turn modifies the expression of genes shown to be glucose dependent (7, 29). The increase of glycolysis may result from a direct role of ARNT on the regulation of genes of glycolytic and gluconeogenic pathways via its dimerization with HIF-1α and binding to HRE. Such HRE have been described in GLUT1, LDH, and PFK (33). ARNT can also dimerize with AHR and interact with XRE sites after treatment with xenobiotics (see Ref. 32 for review). Such XRE sites have been reported to be implicated in the response to hypoxia as well (14). When both hypoxia and treatment with xenobiotics are combined, AHR and HIF-1α may compete for the recruitment of ARNT. Such a competition has been recently described in Hep G2 cells. Pretreatment of the cells with CoCl₂ for 16 h before administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for an additional 8 h reduced the TCDD-dependent induction of CYP1A1 (15). There was no such competition in our experiments; we observed an amplification of the effects not only on CYP1A1 but also on SI and Fru(1,6)P₂ase expression when cells permanently treated with β-NF were exposed to CoCl₂. Interestingly, we did not find any XRE or HRE sites in the region of the promoter of the SI gene responsible for its glucose-dependent expression or in the promoter region of the Fru(1,6)P₂ase gene. Modification of expression of these genes by β-NF treatment or hypoxic conditions could therefore be a consequence of a preliminary increase of glycolysis.

Some of the modifications observed could also be a consequence of the action of the drugs on mitochondria. Indeed, the increase of glycolysis in CoCl₂-treated cells may be explained by the fact that CoCl₂ inhibits the functions of the mitochondrial respiratory chain by its action on heme synthesis and degradation (18, 20), and therefore the hypoxic cells increase glycolysis to compensate for the loss of ATP production that occurs when the mitochondria are not functional. The effects of CYP1A1 inducers on mitochondria are not documented. However, if such an effect exists, the mitochondrial target must differ between β-NF and CoCl₂, since cell viability differs between both conditions according to the duration of the treatment.

At present, in TC7 cells treated with β-NF or hypoxia, the mechanism responsible for the increase in glucose utilization and for the coordinate variations of expression of CYP1A1 mRNA and genes associated with glucose utilization remains to be elucidated. A possible implication of AHR or ARNT cannot be excluded.

This work was supported in part by Université Paris XI and contract 1393 from the Association pour la Recherche sur le Cancer. V. Carrière is supported by a fellowship from the Association de Secours des Amis des Sciences, and A. Rodolosse is supported by a fellowship from the Ministère de l’Éducation Nationale de la Recherche et de la Technologie.

Address for reprint requests: V. Carrière, INSERM U178, 16 Ave. Paul-Vaillant-Couturier, 94807 Villejuif Cedex, France.

Received 15 October 1997; accepted in final form 2 March 1998.

REFERENCES
Hypoxia and CYP1A1 induction in Caco-2 cells

Firth, J. D., B. L. Ebert, C. W. Pugh, and P. J. Ratcliffe.


Jaiswal, A. K.

Halpert, J. R., F. P. Guengerich, and M. A. Correla.


