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Decreased Expression of γ-Glutamyltranspeptidase in the Intestinal Cell Line Caco-2 by Inducers of Cytochrome P450 1A1

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ABSTRACT. Our purpose was to investigate whether inducers of cytochrome P450 1A1 (*CYP*1A1), which cause a decreased expression in Caco-2 cells, at both the mRNA and protein levels, of membrane proteins associated with the uptake and transport of hexoses, would also affect the expression of γ-glutamyltranspeptidase (γGT) (EC 2.3.2.2). In Caco-2 clonal TC7 cells grown under standard conditions (25 mM glucose), exposure to β-naphthoflavone (β-NF), 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin, and 3-methylcholanthrene resulted in increased glucose consumption and decreased γGT activity in cells grown to confluence, i.e. when the differentiation is optimum. γGT activity was further analyzed during the time course of differentiation of TC7 cells treated or not with β-naphthoflavone: while γGT activity in untreated cells showed a 10-fold increase from the exponential phase of growth until late postconfluence, γGT activity in β-NF-treated cells, although increasing by 4-fold, remained at a much lower level (<25%). This decreased activity of γGT was associated with a decreased level of γGT mRNA. This inhibiting effect was not dependent on the *CYP*1A1 activity, as it also occurred in the presence of *CYP*1A1 inhibitors such as α-naphthoflavone, 8-methoxypsoralen or ellipticin. It was however dependent on glucose supply as it was not observed when the cells were cultured in low glucose (1 mM). These results raise the question of whether, in Caco-2 cells, *CYP*1A1 inducers or the signal transduction system which controls *CYP*1A1 are involved in the regulation of the expression of γGT through a mechanism involving glucose metabolism. BIOCHEM PHARMACOL **56**;7:817–823, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. Caco-2 cells; γ-glutamyltranspeptidase; inducers of cytochrome P450 1A1; glucose supply; mRNA; cell culture

Since the initial observation of its enterocytic differentiation characteristics [1], the human colon carcinoma cell line Caco-2 has been extensively utilized as an *in vitro* substitute for intestinal epithelial cells. Indeed, Caco-2 cells grown to confluence express most of the structural and functional properties of mature small intestinal enterocytes (for review see [2]). For these reasons, they have been found to be a unique *in vitro* system for investigating the regulation mechanisms involved in and associated with the differentiation and functions of small intestinal enterocytes and epithelium.

It was previously observed that *CYP*1A1‡ was inducible in Caco-2 cells [3,4]. Following these observations, we have recently reported that permanent exposure of Caco-2 cells to several inducers of *CYP*1A1 resulted both in an increased rate of glucose consumption and in marked modifications, at the mRNA and protein levels, of the expression of a number of differentiation-associated proteins involved in the uptake, transport, and metabolism of glucose [5, 6]. These modifications include a decreased expression of the brush border hydrolase sucrase-isomaltase (EC 3.3.1.48), of the hexose transporters SGLT1, GLUT2, and GLUT5 [5], of fructose 1, 6 bisphosphatase and pyruvate kinase [6], and an increased expression of the hexose transporters GLUT1 and GLUT3 [5] and of phosphoenolpyruvate carboxykinase [6], this occurring without modifications of the morphological differentiation of the cells or of the expression of other differentiation-associated proteins such as villin or DPP-IV (EC 3.4.14.5) [5].

The purpose of the present work was to analyze whether inducers of *CYP*1A1 would also modify the expression of a brush border enzyme which is also expressed in Caco-2 cells [7, 8] but not involved in glucose utilization, namely γGT (EC 2.3.2.2). The presence of γGT in the small intestine is well documented [9–11], and its biochemical criteria are well known [12]. In the intestine, γGT is localized in the apical portion of the epithelial cells covering the jejunal

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‡ *Abbreviations:* Ah, aryl hydrocarbon; α-NF, α-naphthoflavone; β-NF, β-naphthoflavone; *CYP*1A1, cytochrome P450 1A1; DMEM, Dulbecco's modified Eagle's medium; DPP-IV, dipeptidylpeptidase-IV; Ell, ellipticin; EROD, 7-ethoxyresorufin-*O*-deethylase; FBS, fetal bovine serum; γ-GT, γ-glutamyltranspeptidase; 3MC, 3-methylcholanthrene; MOP, 8-methoxypsoralen; and TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin

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villi [13] and is thus considered an intestinal brush border enzyme [14]. Functionally, γGT is involved in the degradation of extracellular glutathione, which provides cells with glutathione constituant amino-acids, including cysteine, a limiting factor in intracellular glutathione synthesis [15]. In Caco-2 cells, active transport and translocation of amino-acids have been shown to be dependent on γGT [16].

The experiments reported here were performed, as in previous experiments on the induction of *CYP*1A1 [5, 6], with a clone obtained from a late passage of the Caco-2 cell line, clone TC7. This clone has been shown to be even closer to the small intestinal enterocyte than the parental population as to the level of expression of differentiation-associated proteins [17–19], intestinal uptake of various compounds [20, 21], transepithelial transport properties, and biotransformation of xenobiotics [22].

MATERIALS AND METHODS

Cell Culture

Caco-2 TC7 clonal cells were isolated as reported [17] and analyzed between passages 20 and 40. Cells were seeded at 12×10^3 cells/cm^2 in 25 or 75 cm^2 T-flasks (Corning Glassworks) and cultured in a 10% CO_2-90% air atmosphere in DMEM (25 mM glucose, 4 mM glutamine, Life Technologies) supplemented with 1% nonessential amino-acids (Life Technologies) and 20% heat-inactivated (30 min, 56°) FBS (Boehringer Mannheim). For growth in low glucose, the same conditions were used, except that DMEM was devoid of glucose, its final concentration in complete medium (1 mM) being supplied by FBS. For treatment with β-NF, TCDD and 3MC, 1000× stock solutions diluted in DMSO were stored at −20°, and freshly added to the culture medium at each medium change, at the same concentrations as previously determined [5]. The final concentration of DMSO (0.1%) was monitored to ensure it would have no effect on any of the parameters in this study. For inhibition of *CYP*1A1 activity, α-NF [23], MOP [24], and Ell [25] were added to the culture medium at the indicated concentrations. From the second day in culture on, the medium was changed daily in all experiments and culture conditions. Cells were regularly verified to be mycoplasmafree.

Preparation of Cell Homogenates and Measurement of Enzymatic Activities

Cells were homogenized (after scraping and centrifugation, 1000 *g*, 10 min) in a Tris-mannitol buffer, pH 7.1, by 30 strokes of glass homogenizer pestle and stored at −20° for a maximum of 3 days with addition of phenylmethylsulfonic acid (PMSF) and benzamidine as protease inhibitors. DPP-IV activity was measured according to Nagatsu et al. [26] in cell homogenates as previously reported [17], using 1.5 mM glycyl-L-proline-4-nitroanilide (Sigma) as substrate. Results are expressed as milliunits per milligram of protein. One unit is defined as the activity that hydrolyzes 1 μmol substrate/min at 37°. Measurement of γGT activities was performed in the same cell homogenates by the method of Szasz [27, 28]. Results are expressed as milliunits per milligram of protein, one unit being defined as the activity which forms 1 μmol amino-5-nitro-2 benzoate per minute, using L-g-glutamylcarboxy-3-nitro-4 anilide (Boehringer) as substrate. Glucose consumption was determined by measuring the concentration of glucose in the medium 16 hr after changes of medium by using the glucose oxidase technique and a Beckman Glucose Analyzer 2.

Measurement of CYP1A1 *Activity*

Cells detached with trypsin were washed three times in PBS$^-$ at 4°, snap-frozen in liquid nitrogen and stored at −80° until analysis. EROD activity was measured by a direct fluorometric assay as described by Burke and Mayer [29]. Microsomal fractions (0.1–0.3 mg/mL) were incubated at 37° in 50 mM Tris/HCl (pH 7.5), 25 mM $MgCl_2$ buffer in the presence of 125 μM NADPH (Boehringer Mannheim), and 2 μM EROD (Sigma). Resorufin (10 pmol) (Aldrich) was added at the end of the assay to calibrate the amount of Resorufin produced. Parameters for fluorescence detection were 522 nm and 586 nm for the excitation and emission wavelengths, using a Jobin Yvon sprectrofluorimeter JYD3. Results are expressed as pmole of Resorufin produced per milligram of microsomal protein per minute.

Protein Measurement

Proteins were measured with the BCA protein assay kit using BSA as standard (Pierce).

Immunofluorescence

Indirect immunofluorescence was performed on frozen cryostat sections of cell layer rolls as previously reported [5, 17], after fixation in 3.5% paraformaldehyde in PBS$^-$ (15 min at room temperature). DPP-IV was detected with mouse mAb 3/775/42 [30] obtained from Dr. H. P. Hauri (Biocenter of the University of Basel, Basel, Switzerland). γGT was detected with a rabbit polyclonal antibody raised against γGT purified from rat kidney [31, 32] obtained from Dr. Y. Laperche (INSERM U99, Créteil, France), and which was observed to cross-react with human small intestinal γGT (data not shown). Double immunofluorescence was performed using anti-mouse and anti-rabbit fluorescein coupled sheep antiglobulins (Institut Pasteur Productions) or rhodamine-coupled sheep antiglobulins (Boehringer Mannheim) as second antibodies.

RNA Extraction and Analysis

Total RNA was extracted by the guanidium isothiocyanate method [33]. Samples of total RNA, denatured in 1 M glyoxal, were fractionated by electrophoresis through 1%

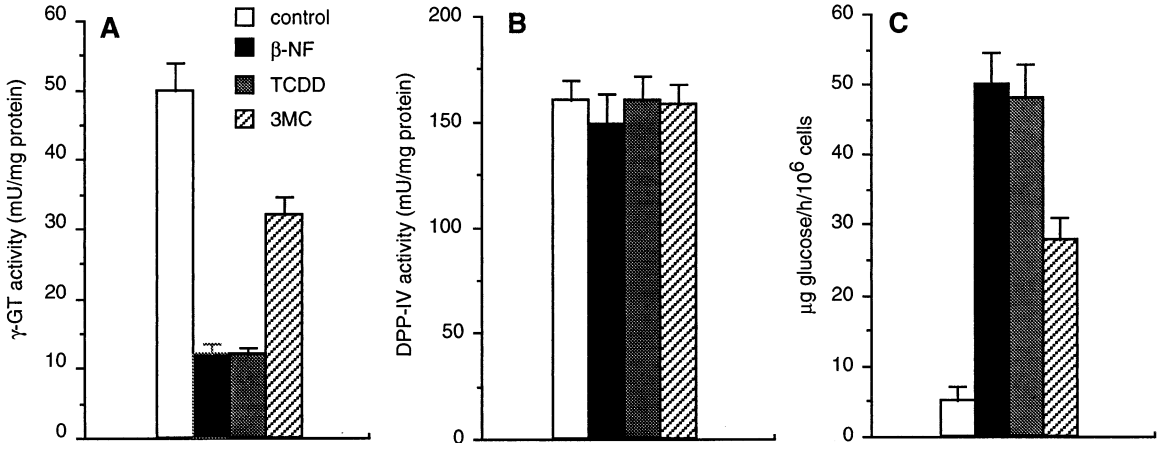

FIG. 1. Activities of (A) γGT, (B) DPP-IV and (C) rate of glucose consumption in postconfluent cultures (Day 20) of TC7 cells cultured in the absence or in the permanent presence of 10 μM β-NF, 1 nM TCDD and 1 μM 3MC. Results are the means and SD of three independent cultures corresponding to three different passages.

agarose gels and transferred onto Hybond N (Amersham) [34]. Prehybridization was performed at 42° in the presence of 50% formamide and hybridization at 42° in the presence of 40% formamide and 10% dextran sulfate. 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°, and once in 0.1× SSC/0.1% SDS for 15 min at 65°. The probes used were DPI-101 [35] for human DPP-IV mRNA and a 963 bp cDNA [36] for human γGT mRNA, obtained from Dr. G. Guellaën (INSERM U99, Créteil, France). To normalize for RNA loading, an antisense oligonucleotide of 22 mers (5′ACGGTATCTGATCGTC TTCGAA3′) specific for 18S sequence was used.

RESULTS

Decreased Activity of γGT in Differentiated Postconfluent Cells Exposed to Inducers of CYP1A1

Because the differentiation of TC7 cells is optimum at late postconfluency [17], γGT activity was measured after 21 days in culture in control cells and in cells treated from the beginning of the culture with β-NF, TCDD, and 3MC, at concentrations which have been shown to induce a high level of *CYP*1A1 mRNA, without modifying the growth and morphological differentiation characteristics of the cells [5]. In cells treated with β-NF, TCDD and 3MC, the γGT activity was decreased to 25%, 25% and 67%, respectively of the values observed in control cells (Fig. 1A). This decrease in γGT activity occurred without modifications of the activity of DPP-IV (Fig. 1B), used here as a *CYP*1A1 independent marker of cell differentiation [5]. As shown in Fig. 1C, treatment with *CYP*1A1 inducers resulted in a marked increase in the rate of glucose consumption.

Time course of γGT Expression in β-NF-treated Cells

In order to further analyze the effect of *CYP*1A1 inducers on the differentiation-related time course of γGT expression, the activities of *CYP*1A1 and γGT were measured at different times in culture in cells treated with β-NF. As shown in Table 1, *CYP*1A1 activity was elevated in β-NF-treated cells, this being concordant with the elevated level of *CYP*1A1 mRNA reported earlier [19]. As shown in Fig. 2A, the activity of γGT in control cells showed a nine-fold increase from the day of confluence on (day 7) up to late postconfluence (day 21). In contrast, γGT activity was always lower in β-NF-treated cells, and the increase at late postconfluence reached only fourfold the values observed at confluence. This effect occurred without modifications of the time course of DPP-IV activity (Fig. 2B). This lower level of γGT activity coincided with a decreased level of expression of the protein, as shown by indirect immunofluorescence of cryostat sections of postconfluent cultures (Fig. 3). The results of γGT mRNA analysis (Fig. 4) show that the inhibiting effect of β-NF on γGT occurred at the mRNA level.

Absence of Relationship between CYP1A1 *Catalytic Activity and the Inhibiting Effect of β-NF of γGT Expression*

In order to analyze whether or not the effect on γGT was dependent on the enzymatic activity of *CYP*1A1, cells were exposed to inhibitors of *CYP*1A1 activity in the presence or

TABLE 1. Expression and inducibility of CYP1A1 activity towards EROD during the time-course in culture of TC7 cells permanently treated with β-NF

Day after seeding	Untreated cells	B-NF-treated cells*
2	ND†	15 ± 2.1
4	ND	18.8 ± 2.1
8	ND	21.1 ± 3
15	ND	29.1 ± 4.3
20	ND	23.05 ± 3.2

*EROD activity (pmole/min/mg of protein) and SD as measured in three different experiments in TC7 cells treated with 10 μM β-NF.

†ND, not detectable.

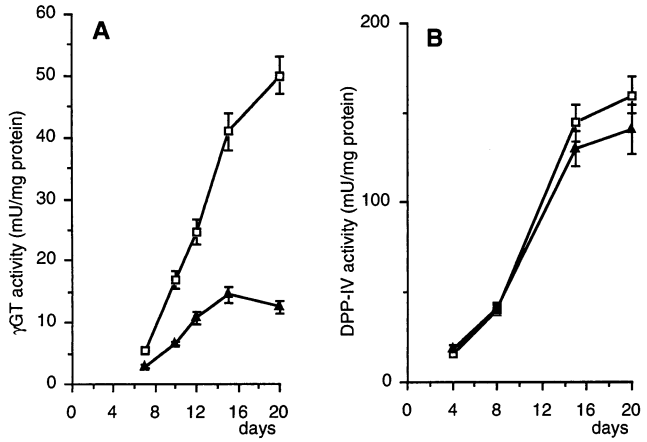

FIG. 2. Activities of (A) γGT, and (B) DPP-IV during the time course in culture of TC7 cells grown in the absence (□) or permanent presence (▲) of 10 μM β-NF. Results are the means and SD of three independent cultures corresponding to three different passages.

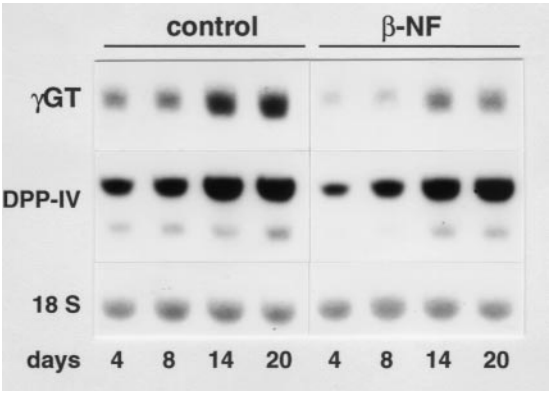

FIG. 4. Northern blot analysis of γGT and DPP-IV during the time course in culture of TC7 cells grown in 25 mM glucose in the absence or presence of 10 μM β-NF. The same quantified amount of total RNA (20 μg) was loaded in each lane. RNAs from control and treated cells were electrophoresed and transferred onto a single membrane, which was successively hybridized with each probe (see Materials and Methods).

not of β-NF. As shown in Fig. 5, treatment with the inhibitors resulted in appearance of *CYP*1A1 mRNA and enhancement of the effect of β-NF on the *CYP*1A1 mRNA (Fig. 5A), this being concomitant with a decreased level of γGT activity (Fig. 5B) and the absence of detectable *CYP*1A1 activity (Fig. 5C).

Glucose Supply-dependent Inhibition of γGT βGT β-NF

It was previously shown that in TC7 cells grown under standard culture conditions, i.e. in the presence of 25 mM of glucose, treatment with β-NF resulted in a tenfold increase in the rate of glucose consumption associated with

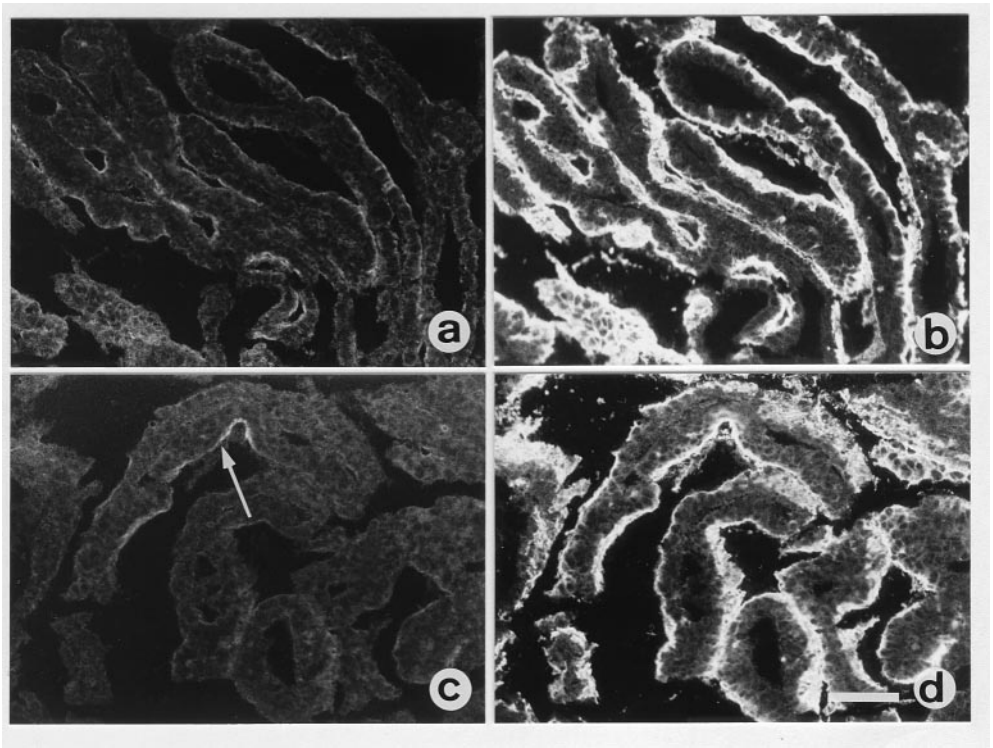

FIG. 3. Double labeling immunofluorescence of (a, c) rhodamine-labeled γGT, and (b, d) fluorescein-labeled DPP-IV in cryostat sections of the cell layer of postconfluent TC7 cells (Day 20) cultured in the absence (a, b) or presence (c, d) of 10 μM β-NF. Note that, in untreated control cells, γGT is present on the totality of the apical brush border, as substantiated by its colocalization with DPP-IV. In contrast, in β-NF-treated cells, only a limited number of cells (arrow) express γGT. The lower intensity of staining of γGT, as compared to DPP-IV, is most likely due to the fact that the antibody has been raised against rat γGT. Bar = 100 μm.

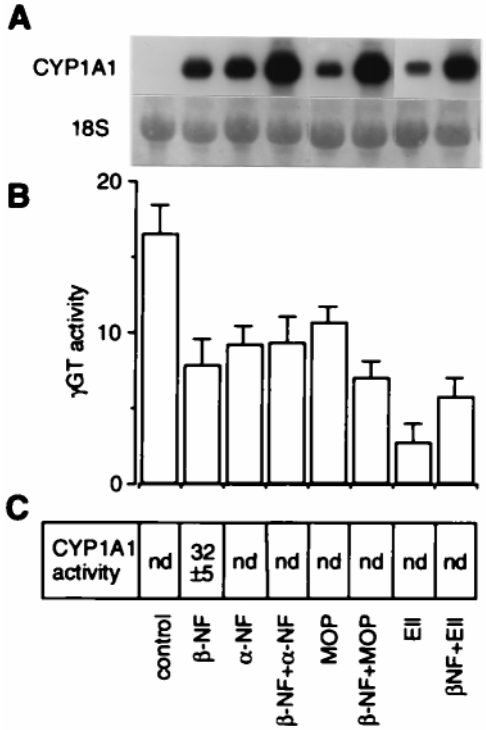

FIG. 5. Effect of inhibitors of CYP1A1 activity on the expression of γGT and CYP1A1. The cells were analyzed on Day 10 of the culture (i.e. during the period when the expression of γGT increased, see Fig. 2) after a 48-hr treatment with α-NF (20 μM), MOP (200 μM) or Ell (20 μM) in the presence or not of β-NF (10 μM). (A) Northern blot analysis of CYP1A1 mRNA; (B) γGT activity in the cell homogenates; (C) CYP1A1 activity toward EROD: nd, not detectable. Results are from a single experiment. Enzyme activities are the means and SD of triplicate measurements.

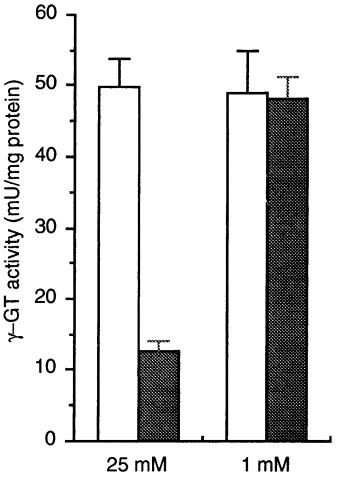

FIG. 6. Glucose-dependent effect of β-NF on the activity of γGT. TC7 cells were grown, from the day of seeding on, in high (25 mM) or low (1 mM) glucose medium in the absence (open bars) or presence (solid bars) of 10 μM β-NF and analyzed after confluence (Day 20). Results are the means and SD of 3 independent cultures corresponding to three different passages.

a marked decrease in the level of expression of several membrane proteins associated with uptake and transport of sugars such as sucrase-isomaltase and hexose transporters GLUT2, GLUT5 and SGLT1 [5]. It was also observed that artificially decreasing glucose consumption by growing the cells in low glucose medium (1 mM) had no effect on the inducibility of CYP1A1, but that under such glucose supply conditions, the β-NF-dependent increase in CYP1A1 expression was not associated with changes in the level of expression of sucrase-isomaltase and hexose transporters [5]. As shown in Fig. 6, this was also true for γGT: indeed, unlike cells cultured in 25 mM glucose, the level of γGT activity was not modified when β-NF-treated TC7 cells were cultured in low glucose.

DISCUSSION

The results reported here show that, as previously observed for membrane proteins such as sucrase-isomaltase and hexose transporters GLUT2, GLUT5 and SGLT1 [5], CYP1A1 inducers cause a decreased expression of γGT in TC7 at both the mRNA and protein level. This effect is concomitant with the induction of CYP1A1 mRNA, but independent of CYP1A1 activity as substantiated by the results obtained with inhibitors of the catalytic activity of CYP1A1. As with the former membrane proteins, the effect on γGT is closely dependent on glucose supply and utilization. The effect is observed at 25 mM glucose, a concentration which allows a reproducible 5- to 10-fold increase in glucose consumption under exposure to inducers of CYP1A1 [5]. It is not observed in low glucose (1 mM), a condition in which the increase in glucose consumption is rapidly limited by its unavailability [5].

In our present state of knowledge, there is no indication of which mechanisms are responsible for the decreased expression of γGT in Caco-2 cells treated with CYP1A1 inducers when grown in high glucose. To our knowledge, little is known of the effects of CYP1A1 inducers on glucose utilization, except for modifications of glucose transport in adipose tissue and brain in the mouse [37]. Similarly, very little is known as to the role of inducers of CYP1A1 and glucose in the regulation of γGT. In primary cultures of rat hepatocytes, γGT activity is not modified by CYP1A1 inducers such as 3MC and β-NF [38], but interestingly enough it is repressed by high glucose [39].

It is clear that the results obtained in TC7 cells with γGT are very similar to those observed with sucrase-isomaltase and the hexose transporters. This strongly suggests that the mechanisms involved in the glucose-dependent repression of these proteins by CYP1A1 inducers should be the same. That the effects observed on γGT do not depend on the CYP1A1 activity *per se*, but are concomitant with the induction of CYP1A1 mRNA suggests that either the effect is an unknown consequence of the inducers themselves or that it depends on the signal transduction system of CYP1A1.

The signal transduction mechanism involved in the xenobiotic-dependent activation of the *CYP1A1* gene has been extensively analyzed, primarily in the liver (for review

see [40]). It is accepted that, to induce *CYP1A1* transcription, ligands first bind to the Ah cytosolic receptor. A 90-kDa heat-shock protein (hsp90) associated with the Ah receptor is then released, which results in dimerization of the receptor with the basic helix-loop-helix factor Arnt, nuclear translocation of the Ah receptor-Arnt complex, and binding of both proteins to xenobiotic-responsive elements in the 5′ region of the *CYP*1A1 gene. Further experiments are in progress to investigate whether the Ah receptor or Arnt is involved in the effects observed in Caco-2 cells. In this respect, mutant variants of hepatoma cell lines which are defective in *CYP*1A1 expression [41, 42], Ah receptor or Arnt [43] should help to answer the question, depending on whether or not they express γGT.

It is most likely that the repressive effect of *CYP*1A1 inducers on γGT is glucose-dependent, whatever the mechanism responsible for the increased glucose consumption associated with the treatment with inducers of *CYP*1A1 [5]. In the case of sucrase-isomaltase, it has been shown that the repressive role of glucose on the transcription of the gene involves a glucose-dependent region of the promoter of the gene [44]. Whether a similar regulation is also present in γGT remains to be elucidated.

Taken together, the present results, along with those previously reported on the effects of *CYP*1A1 inducers on membrane proteins associated with the uptake and transport of glucose [5] and glucose metabolism enzymes [6], raise the question of whether, in addition to their role in the metabolism of xenobiotics, inducers of *CYP*1A1 or the *CYP*1A1 transduction system also play a major role in cell physiology. Whether this is restricted to Caco-2 cells, is specific to intestinal cells, or is a more general physiological function remains to be investigated.

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References

1. Pinto M, Robine–Léon S, Appay MD, Kedinger M, Triadou N, Dussaulx E, Lacroix B, Simon–Assmann P, Haffen K, Fogh J, and Zweibaum A, Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol Cell* **47**: 323–330, 1983.
2. Zweibaum A, Laburthe M, Grasset E, and Louvard D, Use of cultured cell lines in studies of intestinal cell differentiation and function. In: *Intestinal Absorption and Secretion. Handbook of Physiology, section 6. The Gastrointestinal System*. (Eds. Field M and Frizzell RA), pp. 223–255. Am Physiol Soc, Bethesda, 1991.
3. Boulenc X, Bourrié M, Fabre I, Roque C, Joyeux H, Berger Y, and Fabre G, Regulation of cytochrome P450IA1 gene expression in a human intestinal cell line, Caco-2. *J Pharmacol Exp Ther* **263**: 1471–1478, 1992.
4. Rosenberg DW, and Leff T, Regulation of cytochrome P450 in cultured human colonic cells. *Arch Biochem Biophys* **300**: 186–192, 1993.
5. Carrière V, Barbat A, Rousset M, Brot–Laroche E, Dussaulx E, Cambier D, de Waziers I, Beaune P, and Zweibaum A, Regulation of sucrase-isomaltase and hexose transporters in Caco-2 cells: A role for cytochrome P-4501A1? *Am J Physiol (Gastrointest Liver Physiol 33)* **270**: G976–G986, 1996.
6. Rodolosse A, Carrière V, Chantret I, Lacasa M, Zweibaum A, and Rousset M, Glucose-dependent transcriptional regulation of the human sucrase-isomaltase (SI) gene. *Biochimie* **79**: 119–123, 1997.
7. Jumarie C, and Malo C, Caco-2 cells cultured in serum-free medium as a model for the study of enterocytic differentiation *in vitro*. *J Cell Physiol* **149**: 24–33, 1991.
8. Howell S, Kenny AJ, and Turner AJ, A survey of membrane peptidases in two human colonic cell lines, Caco-2 and HT-29. *Biochem J* **284**: 595–601, 1992.
9. Kottgen E, Reutter N, and Gerok W, Two different γ-glutamyl transferases during development of liver and small intestine: A fetal (sialo) and adult (asialo) glycoprotein. *Biochem Biophys Res Comm* **72**: 61–66, 1976.
10. Marathe GV, Nash B, Hashemeyer RH, and Tate SS, Ultrastructural localization of gammaglutamyl transpeptidase in rat kidney and jejunum. *FEBS Lett* **107**: 436–440, 1979.
11. Menard D, Malo C, and Calvert R, Development of γ-glutamyl transpeptidase activity in mouse small intestine: Influence of cortisone and thyroxine. *Bio Neonate* **40**: 70–77, 1981.
12. Matsuda Y, Tsuji A, and Katunuma N, Membrane bound γ-glutamyl transpeptidase, its structure, biosynthesis and degradation. In: *Advances in Enzyme Regulation* (Ed. Weber G), pp. 103–109. Pergamon Press, New York, 1983.
13. Curthoys NP, and Shapiro R, γ-glutamyl transpeptidase in intestinal brush border membranes. *FEBS Lett* **58**: 230–237, 1975.
14. Kuno T, Matsuda Y, and Katunuma N, The conversion of the precursor form of γ glutamyl transpeptidase to its subunit form B takes place in brush border membranes. *Biochem Biophys Res Comm* **28**: 3573–3579, 1983.
15. Hanigan MH, and Ricketts WA, Extracellular glutathione is a source of cysteine for cells that express γ-glutamyl transpeptidase. *Biochemistry* **32**: 6302–6306, 1993.
16. Smith TK, Gibson CL, Howlin BJ, and Pratt JM, Active transport of aminoacids by γ-glutamyl transpeptidase through Caco-2 cell monolayers. *Biochem Biophys Res Comm* **178**: 1028–1035, 1991.
17. Chantret I, Rodolosse A, Barbat A, Dussaulx E, Brot–Laroche E, Zweibaum A, and Rousset M, Differential expression of sucrase-isomaltase in clones isolated from early and late passages of the cell line Caco-2: Evidence for a glucose-dependent negative regulation. *J Cell Sci* **107**: 213–225, 1994.
18. Mahraoui L, Rodolosse A, Barbat A, Dussaulx E, Zweibaum A, Rousset M, and Brot–Laroche E, Presence and differential expression of SGLT1, GLUT1, GLUT2, GLUT3 and GLUT5 hexose-transporter mRNAs in Caco-2 cell clones in relation to cell growth and glucose consumption. *Biochem J* **298**: 629–633, 1994.
19. Carrière V, Lesuffleur T, Barbat A, Rousset M, Dussaulx E, Costet P, de Waziers I, Beaune P, and Zweibaum A, Expression of cytochrome P-450 3A in HT29-MTX cells and Caco-2 clone TC7. *FEBS Lett* **355**: 247–250, 1994.
20. Jumarie C, Campbell PG, Berteloot A, Houde M, and Denizeau F, Caco-2 cell line used as an *in vitro* model to study cadmium accumulation in intestinal epithelial cells. *J Membr Biol* **158**: 31–48, 1997.
21. Tranchant T, Besson P, Hoinard C, Delarue J, Antoine JM, Couet C, and Gore J, Mechanisms and kinetics of α-linolenic acid uptake in Caco-2 clone TC7. *Biochem Biophys Acta* **1345**: 151–161, 1997.
22. Caro I, Boulenc X, Rousset M, Meunier V, Bourrié M, Julian B, Joyeux H, Roques C, Berger Y, Zweibaum A, and Fabre G,

Characterization of a newly isolated Caco-2 clone (TC-7), as a model of transport processes and biotransformation of drugs. *Int J Pharmac* **116**: 147–158, 1995.
23. Wilhelmsson A, Whitelax ML, Gustafsson J-A, and Poellinger L, Agonistic and antagonistic effects of α-naphthoflavone on dioxin receptor function. *J Biol Chem* **269**: 19028–19033, 1994.
24. Tassaneeyakul W, Birkett DJ, Veronese ME, McManus ME, Tukey RH, Quattrochi LC, Gelboin HV, and Miners JO, Specificity of substrate and inhibitor probes for human cytochromes P450 1A1 and 1A2. *J Pharmacol Exp Ther* **265**: 401–407, 1993.
25. Kurl RN, DePetrillo PB, and Olnes MJ, Inhibition of Ah (dioxin) receptor transformation by 9-hydroxy ellipticine. *Biochem Pharmacol* **46**: 1425–1433, 1993.
26. Nagatsu T, Hino M, Fuyamada H, Hayakawa T, Sakakibara S, Nakagawa Y, and Takemoto T, New chromogenic substrates for X-prolyl dipeptidyl aminopeptidase. *Anal Biochem* **74**: 466–476, 1976.
27. Szasz G, γ-glutamyltranspeptidase. In: *Methods of Enzymatic Analysis*. (Ed. Bergmeyer HU), pp. 715–720. Verlag Chemie GmbH, Weinheim, 1974.
28. Persijn JP, and van der Slik W, A new method for the determination of γ-glutamyltransferase in serum. *J Clin Chem Clin Biochem* **14**: 421–427, 1976.
29. Burke MD, and Mayer RT, Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal metabolism and cytochrome P450 binding of phenoxazone and a homologous series of its N-alkyl ether alkoxy resorufin. *Chem Biol Interact* **45**: 243–427, 1983.
30. Hauri HP, Sterchi EE, Bienz D, Fransen MA, and Marxer A, Expression of intracellular transport of microvillus membrane hydrolases in human intestinal epithelial cells. *J Cell Biol* **101**: 838–851, 1985.
31. Finidori J, Laperche Y, Hauguenauer–Tsapis R, Barouki R, Guellaen G, and Hanoune J, *In vitro* biosynthesis and membrane insertion of γ-glutamyltranspeptidase. *J Biol Chem* **259**: 4687-, 1984.
32. Beaudoin AR, Grondin G, and Laperche Y, Immunocytochemical localization of γ-glutamyltranspeptidase, GP-2 and amylase in the rat exocrine pancreas: The concept of zymogen granule membrane recycling after exocytosis. *J Histochem Cytochem* **41**: 225–233, 1993.
33. Chirgwin JM, Przybylan AE, McDonald RJ, and Rutter WJ, Isolation of biologically active RNA from sources enriches in ribonuclease. *Biochemistry* **18**: 5294–5299, 1979.
34. Thomas PS, Hybridization of denatured mRNA and small DNA fragments transferred to nitrocellulose. *Proc Natl Acad Sci USA* **77**: 5201–5205, 1980.
35. Darmoul D, Lacasa M, Chantret I, Swallow D, and Trugnan G, Isolation of cDNA probe for the human intestinal diptidylpeptidase IV and assignment of the gene locus DPPIV to chromosome 2. *Ann Hum Genet* **54**: 191–197, 1990.
36. Pawlak A, Cohen EH, Octave JN, Schweickhardt R, Wu SJ, Bulle F, Chikhi N, Baik JH, Siegrist S, and Guella'91n G, An alternatively processed mRNA specific for γ-glutamyl transpeptidase in human tissues. *J Biol Chem* **265**: 3256–3262, 1990.
37. Liu PC and Matsumura F, Differential effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the "adipose-type" and "brain type" glucose transporters in mice. *Mol Pharmacol* **47**: 65–73, 1995.
38. Edwards AM, Glistak ML, Lucas CM, and Wilson PA, 7-ethoxycoumarin deethylase activity as a convenient measure of liver drug metabolizing enzymes: Regulation in cultured rat hepatocytes. *Biochem Pharmacol* **33**: 1537–1546, 1984.
39. Edwards AM, Regulation of γ-glutamyltranspeptidase in rat hepatocyte monolayer cultures. *Cancer Res* **42**: 1107–1115, 1982.
40. Gonzalez FJ, Liu SY, and Yano M, Regulation of cytochrome P450 genes: Molecular mechanisms. *Pharmacogenetics* **3**: 51–57, 1993.
41. Hankinson O, Andersen RD, Birren BW, Sander F, Negishi M, and Nebert DW, Mutations affecting the regulation of transcription of the cytochrome P1-450 gene in the mouse Hepa-1 cell line. *J Biol Chem* **260**: 1790–1795, 1985.
42. Kimura S, Smith HH, Hankinson O, and Nebert DW, Analysis of two benzo [a]pyrene-resistant mutants of the mouse hepatoma Hepa-1 P(1)450 gene via cDNA expression in yeast. *EMBO J* **6**: 1929–1933, 1987.
43. Hankinson O, The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol* **35**: 307–340, 1995.
44. Rodolosse A, Chantret I, Lacasa M, Chevalier G, Zweibaum A, Swallow D, and Rousset E, A limited upstream region of the human sucrase-isomaltase gene confers glucose-regulated expression on a heterologous gene. *Biochem J* **315**: 301–306, 1996.