FEBS Letters 487 (2001) 356–360 FEBS 24444

# Triiodothyronine downregulates the periportal expression of α class glutathione *S*-transferase in rat liver

T. Vanhaecke[a,*], K.O. Lindros[b], T. Oinonen[b], A. Vercruysse[a], V. Rogiers[a]

[a] *Department of Toxicology, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium*
[b] *National Public Health Institute, Alcohol Research Center, P.O. Box 719, 00101 Helsinki, Finland*

Received 25 September 2000; revised 30 November 2000; accepted 30 November 2000

First published online 8 December 2000

Edited by Jacques Hanoune

**Abstract Most drug-metabolizing phase I and phase II enzymes, including the glutathione *S*-transferases (GST), exhibit a zonated expression in the liver, with lower expression in the upstream, periportal region. To elucidate the involvement of pituitary-dependent hormones in this zonation, the effect of hypophysectomy and 3,3′,5-triiodo-L-thyronine ($T_3$) on the distribution of GST was studied in rats. Hypophysectomy increased total GST activity both in the periportal and perivenous liver region. Subsequent $T_3$ treatment counteracted this effect in the perivenous zone. However, analysis for either μ class M1/M2-specific (1,2-dichloro-4-nitrobenzene) or α class A1/A2-specific (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) GST activity revealed that $T_3$ treatment did not significantly affect the perivenous activity of these GST classes. In contrast, $T_3$ was found to significantly counteract the increase of α class GST activity caused by hypophysectomy in the periportal zone. To establish whether this effect was $T_3$-specific, hepatocytes were isolated from either the periportal and perivenous zone by digitonin/collagenase perfusion and cultured either as pyruvate-supplemented monolayer or as co-culture with rat liver epithelial cells. Only in the latter it was found that $T_3$ suppressed the A1/A2-specific GST activity and α class proteins predominantly in periportal cells. The data demonstrate that $T_3$ is an important factor responsible for the low expression of α GST in the periportal region. $T_3$ may be involved in the periportal downregulation of other phase I and II enzymes as well.** 

*Key words:* Glutathione *S*-transferase; Rat liver; Rat hepatocyte co-culture; Triiodothyronine; Periportal downregulation $T_3$

## 1. Introduction

It is well known that many liver genes, including those encoding phase I and phase II biotransformation enzymes, are expressed in a zone-specific manner across the hepatic acinus, the microcirculatory unit of the liver [1,2]. This zonated enzyme expression is thought to be directed by gradients of oxygen, hormones and other mediators, such as substrates and metabolites, that are formed in the blood streaming along the hepatic sinusoids [1,2]. Members of the phase II glutathione *S*-transferase (GST; EC 2.5.1.18) family are predominantly expressed in the perivenous liver region [1,3–5], the area more vulnerable to xenobiotics [1,6,7]. Consequently, understanding the factors that control the heterogeneous expression pattern may reveal mechanisms of zone-specific liver damage [2].

Although endogenous hepatic GST regulation has been studied both in vivo [8–12] and in vitro [13,14], the hormonal regulation of the zonated expression of GST and other biotransformation enzymes has received limited attention. Growth hormone and triiodothyronine ($T_3$) have been found to regulate the zonal expression pattern of the phase I P450 cytochromes (CYPs) 2B and CYP3A genes. The effect of $T_3$ was, however, found to be subgroup- or even isoenzyme-specific. For instance, $T_3$ strongly suppressed CYP3A1/2 exclusively in the periportal region, whereas CYP2B1/2 was equally reduced in both regions [6,15,16]. Since it has been shown both in vivo [10,11] and in vitro [13,14] that $T_3$ is involved in the overall regulation of hepatic GST isoenzymes, we investigated whether $T_3$ also controls their regional distribution. Therefore, in this paper, the effects of hypophysectomy and subsequent $T_3$ treatment on the in vivo zonation of GST activities were studied by analyzing periportal and perivenous cell lysates obtained after zone-restricted digitonin pulse infusion during in situ liver perfusion. The data were compared with in vitro studies based on isolation of periportal and perivenous rat hepatocytes followed by their culture either as pyruvate-supplemented monolayer [17] or as co-culture with bile duct epithelial cells [18].

## 2. Materials and methods

### 2.1. Chemicals

Crude collagenase type I, bovine serum albumin fraction V, insulin, digitonin, L-glutamine, ethacrynic acid (EA), EDTA, guanidinehydrochloride, pyruvate and epoxy-activated Sepharose 6B were purchased from Sigma Chemical Co. (St. Louis, MO, USA). William's medium E (without L-glutamine), fetal bovine serum (FBS) and trypsin-EDTA solution were from Gibco BRL (Brussels, Belgium), hydrocortisone hemisuccinate from Upjohn s.a. (Puurs, Belgium) and amfotericin B from Bristol-Myers-Squibb Belgium n.v. (Brussels, Belgium). 1-Chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) were from Merck Belgolabo (Darmstadt, Germany), 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) and hydroxypropyl-β-cyclodextrin (HPBC, Encapsin™ HPB, 30.221.54) from Janssen Chimica (Beerse, Belgium), glutathione from Boehringer Mannheim

*Corresponding author. Fax: (32)-2-4774582.
E-mail: tamaravh@vub.vub.ac.be

*Abbreviations:* CDNB, 1-chloro-2,4-dinitrobenzene; CYP, cytochrome P450; DCNB, 1,2-dichloro-4-nitrobenzene; EA, ethacrynic acid; GST, glutathione *S*-transferase; HPBC, hydroxypropyl-β-cyclodextrin; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; $T_3$, 3,3′,5-triiodo-L-thyronine

0014-5793/01/$20.00 
PII: S0014-5793(00)02353-X

Table 1
Effect of $T_3$ on GST activities measured by CDNB, DCNB, NBD-Cl and EA in periportal and perivenous rat hepatocytes either cultured as pyruvate-supplemented monolayer or co-cultured with rat liver epithelial cells for 4 days

| Substrate | GST isoenzyme activities (% of control value) | | | |
|---|---|---|---|---|
| | Co-culture | | Pyruvate-supplemented monoculture | |
| | Periportal | Perivenous | Periportal | Perivenous |
| CDNB | 74.9 ± 5.3* | 106.0 ± 4.4 | 89.2 ± 16.4 | 89.8 ± 25.1 |
| DCNB | 99.0 ± 12.4 | 75.2 ± 17.2 | 82.4 ± 4.0 | 109.7 ± 15.1 |
| NBD-Cl | 53.9 ± 5.7** | 103.7 ± 14.1 | 86.2 ± 16.2 | 91.5 ± 7.1 |
| EA | 94.0 ± 27.4 | 74.3 ± 4.0 | 83.2 ± 7.9 | 99.9 ± 3.7 |

Mean values ± S.D. of three independent periportal and perivenous culture experiments are presented. Statistical difference between corresponding periportal or perivenous control values (0.02% w/v HPBC) is given by * or ** for $P < 0.05$ or $P < 0.01$, respectively (unpaired Student's *t*-test).

(Germany) and 3,3′,5-triiodo-L-thyronine ($T_3$) from Fluka Chemie (Bornem, Belgium). All other chemicals were readily available commercial products.

### 2.2. Animals

Hypophysectomized and sham-operated outbred male Sprague Dawley rats were obtained from Møllegaard, Ejby, Denmark. The rats were hypophysectomized or sham-operated at the age of 5 or 7 weeks and allowed to stabilize for 1 week. The rats received buprenorphin (Temgesic 0.3 mg/ml) at 0.15 mg/kg immediately after surgery. The animals were fed a commercial R3 laboratory diet (Ewos, Södertälje, Sweden) and water ad libitum. The animals were: (a) controls (sham-hypophysectomized rats, (b) hypophysectomized rats and (c) hypophysectomized rats injected daily intraperitoneal with $T_3$ (50 μg/kg) for the last 7 days. The effect of hypophysectomy and $T_3$ treatment was monitored by weighing the rats. All animal experiments were approved by the Institutional Animal Care and Use Committee at Alko-Group Ltd., Finland.

### 2.3. Collection of periportal and perivenous cell lysates

Periportal and perivenous cell lysates were obtained during in situ digitonin perfusion as described previously [15]. Briefly, periportal cells were lysed by infusion of 3.5 mM digitonin via the vena porta and the lysate was collected by immediate retrograde flushing. Subsequently, perivenous cell lysates were obtained by infusing digitonin via the upper vena cava followed by antegrade flushing. The length of the digitonin pulse, which determines the penetration depth, was empirically determined to allow the digitonin to lyse approximately one-fourth to one-third of the cells along the plate in either the proximal or distal part of the sinusoid. The activity of the periportal marker enzyme alanine aminotransferase (EC 2.6.1.2), which was assayed to verify the zone-specificity of the cell lysates [16], was found to be 7.6-fold higher in periportal samples than in perivenous samples, indicating clear acinar separation [6]. For GST activity measurements, aliquots (200 μl) of cell lysates were immediately frozen in liquid nitrogen and stored at −80°C until assayed.

### 2.4. Isolation and culture of periportal and perivenous rat hepatocytes

Intact hepatocytes from outbred adult male Sprague Dawley rats weighing 200–250 g (Iffa Credo Brussels, Belgium) were isolated from either the periportal or the perivenous region of the liver acinus by using zone-specific destruction with digitonin infusion as described above, followed by collagenase perfusion and isolation of cells from the intact acinar regions [19,20]. The activity of the perivenous marker enzyme glutamine synthetase (GS; EC 6.3.1.2) was assayed [21] to verify the zone-specificity of the freshly isolated hepatocytes [1,21]. GS activity in freshly isolated perivenous hepatocytes was 33-fold higher than in periportal cells, which is in agreement with previous studies [22,23] and thus demonstrates the different acinar origin of the cell populations to be cultured. After testing for cell integrity by trypan blue exclusion, hepatocytes were seeded in medium containing 10% FBS (v/v). After 4 h incubation at 37°C in an atmosphere of 95% air and 5% $CO_2$ at a relative humidity of 100%, monolayer cultures [17] and co-cultures with rat liver epithelial cells of primitive biliary origin [24] were set up. 24 h after cell plating and every day thereafter, all cultures were incubated with serum-free medium supplemented with 0.1 μM $T_3$ incorporated in 0.02% w/v HPBC (final concentration) in phosphate-buffered saline [14]. Control cultures contained 0.02% (w/v) of the delivery system alone. The vehicle HPBC has no effect on GST expression [14]. Samples were taken at days 4 and 7 of culture.

### 2.5. GST isoenzyme activities

The GST activity was measured spectrophotometrically using CDNB and DCNB as broad spectrum and μ class M1/M2-specific substrates, respectively [25]. The α A1/A2-specific and π class GST activities were determined using NBD-Cl [26] and EA [27] as substrates.

Table 2
Effect of $T_3$ on the GST subunit profile of periportal and perivenous rat hepatocytes either cultured as pyruvate-supplemented monolayer or co-cultured with rat liver epithelial cells for 4 days

| Subunits | Subunit concentration (μg/mg cytosolic protein) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Periportal | | Perivenous | | Periportal | | Perivenous | |
| | Control | $T_3$-treated | Control | $T_3$-treated | Control | $T_3$-treated | Control | $T_3$-treated |
| α Class | | | | | | | | |
| A1 | 4.9 ± 2.1 | 1.7 ± 0.1* | 3.4 ± 1.1 | 2.7 ± 0.6 | 6.3 ± 1.3 | 5.8 ± 0.3 | 6.7 ± 0.7 | 7.1 ± 0.6 |
| A2 | 2.2 ± 0.5 | 2.0 ± 0.4 | 1.8 ± 1.0 | 2.9 ± 0.8 | 5.2 ± 1.3 | 5.1 ± 0.3 | 5.7 ± 1.1 | 5.0 ± 2.0 |
| A3 | 2.7 ± 0.6 | 1.4 ± 0.1* | 2.3 ± 1.8 | 2.5 ± 1.0 | 4.7 ± 2.4 | 5.6 ± 0.1 | 5.5 ± 0.5 | 4.8 ± 1.2 |
| Subtotal | 10.7 ± 3.7 | 5.5 ± 0.7* | 7.8 ± 3.2 | 8.5 ± 1.2 | 16.9 ± 2.4 | 17.0 ± 0.6 | 18.6 ± 1.7 | 17.4 ± 6.3 |
| μ Class | | | | | | | | |
| M1 | 4.2 ± 2.4 | 2.6 ± 1.4 | 4.5 ± 2.9 | 5.0 ± 1.7 | 6.0 ± 1.1 | 5.3 ± 0.5 | 7.3 ± 0.5 | 7.0 ± 2.6 |
| M2 | 6.0 ± 3.3 | 3.2 ± 1.6 | 6.6 ± 3.1 | 7.2 ± 1.3 | 4.6 ± 1.7 | 3.3 ± 0.6 | 7.2 ± 0.8 | 4.6 ± 4.3 |
| M3 | 0.4 ± 0.2 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.2 | 0.3 ± 0.2 | 0.3 ± 0.1 | 0.2 ± 0.2 | 0.3 ± 0.3 |
| Subtotal | 10.5 ± 5.6 | 6.0 ± 3.1 | 11.4 ± 6.0 | 12.5 ± 2.8 | 11.0 ± 2.6 | 8.8 ± 1.2 | 14.7 ± 1.5 | 11.9 ± 6.9 |
| π Class | | | | | | | | |
| P1 | 5.1 ± 1.0 | 3.3 ± 0.2* | 5.0 ± 1.3 | 6.3 ± 0.2 | 4.6 ± 3.2 | 4.7 ± 0.8 | 8.0 ± 2.7 | 6.5 ± 1.0 |
| Total | 26.3 ± 10.6 | 14.8 ± 3.9 | 24.3 ± 10.1 | 27.2 ± 3.8 | 32.5 ± 4.1 | 30.5 ± 1.0 | 41.3 ± 4.6 | 35.9 ± 12.9 |

Mean values ± S.D. of three independent periportal and perivenous culture experiments are presented. Statistical difference between corresponding periportal or perivenous control values (0.02% w/v HPBC) is given by * for $P < 0.05$ (unpaired Student's *t*-test).

The protein contents in the cell lysates and the cytosolic fractions of freshly isolated and co-cultured periportal and perivenous hepatocytes were measured using a 'Bio-Rad protein assay kit' (Bio-Rad, Brussels, Belgium) with bovine serum albumin as a standard. To be able to express the results versus hepatocyte proteins, correction for the helper epithelial cell proteins was carried out as described previously [28].

### 2.6. High performance liquid chromatography (HPLC) analysis of GST subunits

GST proteins were determined by reversed phase HPLC [29], after purification of the samples with affinity chromatography on a glutathione-Sepharose 6B column as described earlier [30,31].

## 3. Results and discussion

### 3.1. Effect of hypophysectomy and $T_3$ treatment on zonation of GST isoenzyme activities

In agreement with previous results obtained by our groups [3,31], we found a general perivenous dominance ($P < 0.01$) of GST activities measured by CDNB, DCNB and NBD-Cl in control cell lysates. After hypophysectomy, CDNB and DCNB activities were increased equally in both liver regions (increases of 35% and 25%, respectively) (Fig. 1). Consequently, the perivenous/periportal ratio for both GST activities remained unaltered. Treatment of hypophysectomized rats with $T_3$ restored the CDNB activity partially and the DCNB activity totally, to control levels. However, no zone-specific effect of $T_3$ on CDNB or DCNB activity was observed. In contrast, hypophysectomy increased the NBD-Cl activity, which reflects the α class A1/A2 activity, 2-fold more in the periportal than the perivenous zone. This resulted in a significantly ($P < 0.05$) decreased perivenous/periportal ratio as compared to controls. $T_3$ treatment completely restored the NBD-Cl activity both in periportal and perivenous regions to those observed in controls (Fig. 1). Consequently, the perivenous/periportal ratio was also normalized ($P < 0.05$). Thus, although hypophysectomy and subsequent $T_3$ treatment affect the overall expression of GST similarly in both zones, the effect on GST α A1/A2 activity appears to be zone-specific.

### 3.2. Effect of $T_3$ treatment on zonation of GST isoenzyme expression in cultures of periportal and perivenous rat hepatocytes

To establish the specificity of the $T_3$ effect observed in vivo, periportal and perivenous rat hepatocytes were isolated and investigated during culture. Hepatocytes were cultured either in the presence of pyruvate [17] or together with bile duct epithelial cells [18].

In freshly isolated perivenous hepatocyte populations, higher levels of CDNB ($P < 0.05$), DCNB ($P < 0.05$) and NBD-Cl (not significant) enzyme activities were measured as compared to freshly isolated periportal cells (Figs. 2 and 3). In contrast to monocultures (Fig. 3), hepatocytes in co-culture maintained their in vivo periportal < perivenous CDNB, DCNB and NBD-Cl activity differences for up to 4 days (Fig. 2). These differences disappeared, however, after 7 days. Results obtained by using EA as substrates suggested that the π class GST is equally distributed in the acinus.

Exposure of periportal and perivenous hepatocyte co-cultures to $T_3$ for 4 days (Table 1) clearly decreased the CDNB activity in periportal cell cultures, as a consequence of a 50% reduction ($P < 0.01$) of the A1/A2 activity. This finding corresponds well with the in vivo effect of $T_3$ on periportal NBD-

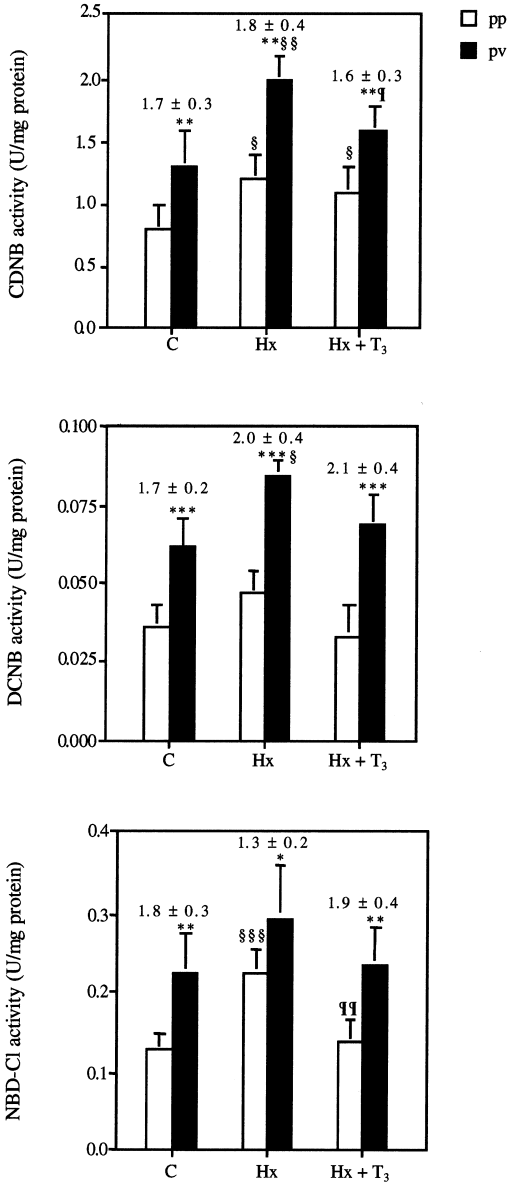


Fig. 1. GST activities measured by CDNB (broad spectrum), DCNB (μ class M1/M2-specific) and NBD-Cl (α class A1/A2-specific) in periportal (pp) and perivenous (pv) cell lysates of male adult rat liver under control conditions (C) and after hypophysectomy (Hx) and subsequent $T_3$ treatment (Hx+$T_3$). Results are expressed as means ± S.D. ($n = 5$ for each treatment group). Values above the bars indicate pv/pp ratios. Statistical difference between pp and pv values within the treatment group, as calculated by the paired Student's *t*-test, is given by *, ** or *** for $P < 0.05$, $P < 0.01$ or $P < 0.001$, respectively. Statistical difference between Hx or Hx+$T_3$ values and control values from a similar acinar region is given by § or §§ for $P < 0.05$ or $P < 0.01$, respectively (unpaired Student's *t*-test). Statistical difference between Hx and Hx+$T_3$ values from the same acinar region is given by ¶ or ¶¶ for $P < 0.05$ or $P < 0.01$, respectively (unpaired Student's *t*-test).

Cl activity, described earlier. As a result of the decrease in NBD-Cl activity in the periportal region, the NBD-Cl activity was twice as high ($P < 0.05$) in $T_3$-treated perivenous hepatocytes. These data were confirmed by HPLC analysis of GST subunits (Table 2). In periportal co-cultures treated with $T_3$, the amount of α class proteins was reduced to about 50% as compared to corresponding controls, mainly due to significant

effects on subunits A1 and A3 ($P < 0.05$). Importantly, $T_3$ treatment had no effect on co-cultured perivenous hepatocytes.

The absence of effects of $T_3$ treatment on GST isoenzyme activities (Table 1) or subunits (Table 2) in hepatocytes grown in pyruvate-supplemented monocultures confirms previous observations that responsiveness to $T_3$ is maintained in co-cultures [13,32] but not in monocultures [33,34]. This demonstrates the importance of using the correct culture conditions for studies of in vivo phenomena during in vitro conditions.

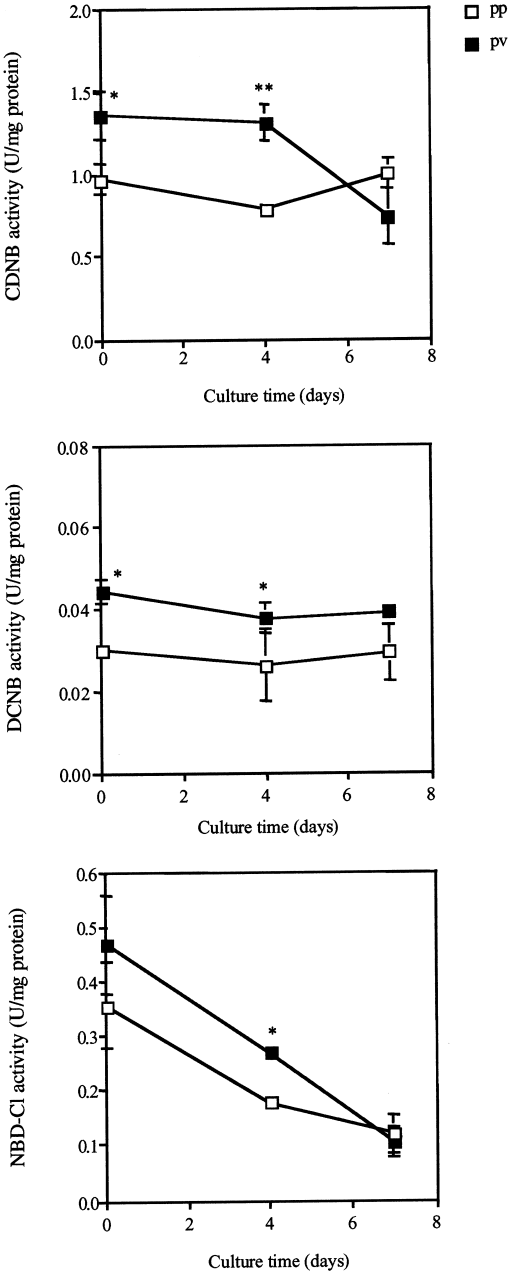


Fig. 2. GST activities measured by CDNB (broad spectrum), DCNB (μ class M1/M2-specific) and NBD-Cl (α class A1/A2-specific) in periportal (pp) and perivenous (pv) rat hepatocytes co-cultured with rat liver epithelial cells for 7 days. Mean values ± S.D. of three independent pp and pv culture experiments are presented. Statistical difference between corresponding pp and pv values is given by * or ** for $P < 0.05$ or $P < 0.01$, respectively (unpaired Student's *t*-test).

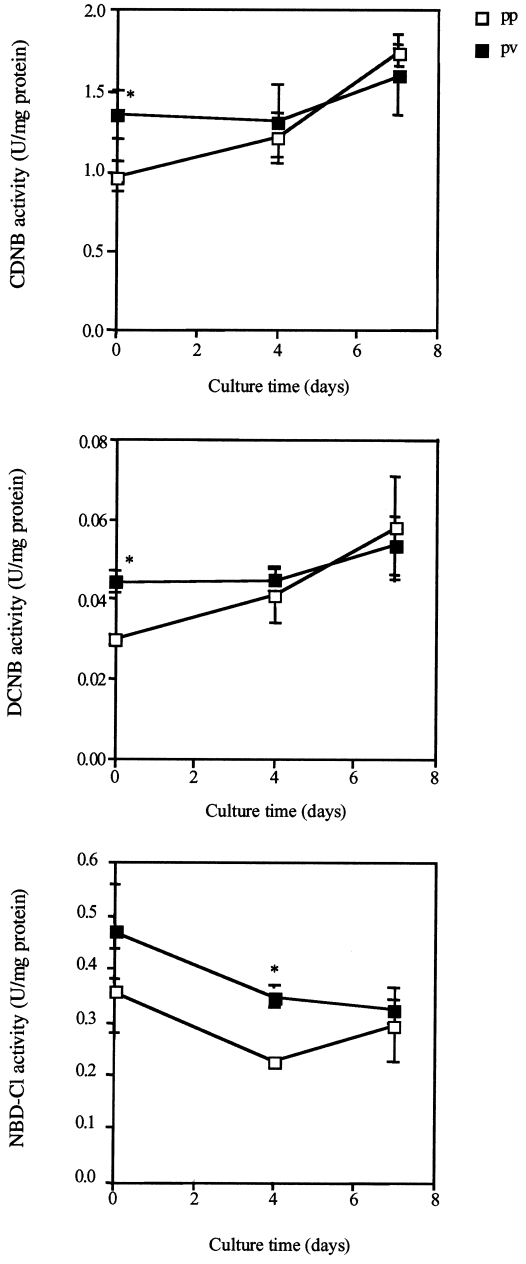


Fig. 3. GST activities measured by CDNB (broad spectrum), DCNB (μ class M1/M2-specific) and NBD-Cl (α class A1/A2-specific) in 7-day-old pyruvate-supplemented monolayer cultures of periportal (pp) and perivenous (pv) rat hepatocytes. Mean values ± S.D. of three independent pp and pv culture experiments are presented. Statistical difference between corresponding pp and pv values is given by * for $P < 0.05$ (unpaired Student's *t*-test).

Previous in vivo studies on hormonal gradients are scarce. There are reports on gradients for the growth factor EGF and several hormones, including insulin, glucagon and thyroxine [1,2]. However, the interpretation of zonal effects of hormones is complicated by their binding to specific serum proteins and by the complex relation between their uptake and degradation in the sinusoid [1,2].

To our knowledge, periportal and perivenous rat hepatocytes co-cultured with epithelial cells have not been investigated previously. This model should provide a useful in vitro model for studying hormonal control of zonated expression of

liver genes. Our data strongly suggest that $T_3$ downregulates the expression of GST α proteins, in particular GSTA1 and GSTA3, and that this explains the low GST activity in the periportal liver region. The zone-specificity of this effect suggests the existence of a sinusoidal gradient, either of $T_3$ uptake or of the $T_3$ receptor. The fact that the periportal responsiveness to $T_3$ is maintained in isolated cells suggests that the $T_3$ receptor is expressed predominantly in the periportal region. Alternatively, $T_3$ might specifically affect some GST α regulating transcription factor(s) that are unevenly distributed within the hepatic acinus.

In conclusion, the present results show that $T_3$ downregulates the periportal expression of GST α, while other GST subunits are unaffected. We hypothesize that $T_3$ also is responsible for other liver genes that exhibit lower expression in the periportal region.

*Acknowledgements:* The authors gratefully thank Mrs. G. De Pauw, Mrs. E. Desmedt, Ms. Gunilla Rönnholm, Ir. André Callaerts and Ir. Walter Sonck for their skilful technical assistance. This work was supported by grants from the National Fund for Scientific Research (FWO), Belgium, and the Research Council (OZR) of the Vrije Universiteit Brussel, Belgium.

## References

[1] Gebhardt, R. (1992) Pharmacol. Ther. 53, 275–354.
[2] Oinonen, T. and Lindros, K.O. (1998) Biochem. J. 329, 17–35.
[3] Kera, Y., Sippel, H., Penttilä, K.E. and Lindros, K.O. (1987) Biochem. Pharmacol. 36, 2003–2006.
[4] Redick, J.A., Jakoby, W.B. and Baron, J. (1982) J. Biol. Chem. 257, 15200–15203.
[5] Sippel, H., Lindros, K.O. and Oinonen, T. (1996) Pharmacol. Toxicol. 79, 80–86.
[6] Oinonen, T., Mode, A., Lobie, P.E. and Lindros, K.O. (1996) Biochem. Pharmacol. 51, 1379–1387.
[7] Lindros, K.O. (1997) Gen. Pharmacol. 28, 191–196.
[8] Mankowitz, L., Castro, V.M., Mannervik, B., Rydström, J. and DePierre, J.W. (1990) Biochem. J. 265, 147–154.
[9] Staffas, L., Mankowitz, L., Söderström, M., Blanck, A., Porsch-Hällström, I., Sundberg, C., Mannervik, B., Olin, B., Rydström, J. and Depierre, J.W. (1992) Biochem. J. 286, 65–72.
[10] Kelley, M.K. and Bjeldanes, L.F. (1995) Food Chem. Toxicol. 33, 129–137.
[11] Beckett, G.J., Boyd, R., Beddows, S.E. and Hayes, J.D. (1988) Biochem. Pharmacol. 37, 3201–3204.
[12] Meyer, D.J., Harris, J.M., Gilmore, K.S., Coles, B., Kensler, T.W. and Ketterer, B. (1993) Carcinogenesis 14, 567–572.
[13] Coecke, S., Vanhaecke, T., Foriers, A., Phillips, I.R., Vercruysse, A., Shephard, E.A. and Rogiers, V. (2000) J. Endocrinol. 166, 363–371.
[14] Vanhaecke, T., Derde, M.-P., Vercurysse, A. and Rogiers, V. (2000) Biochem. Pharmacol. (in press).
[15] Oinonen, T., Nikkola, E. and Lindros, K.O. (1993) FEBS Lett. 327, 237–240.
[16] Oinonen, T. and Lindros, K.O. (1995) Biochem. J. 309, 55–61.
[17] Vanhaecke, T., Vercruysse, A. and Rogiers, V. (1997) Toxicol. In Vitro 11, 435–441.
[18] Guguen-Guillouzo, C., Clément, B., Baffet, G., Beaumont, C., Morel-Chany, E., Glaise, D. and Guillouzo, A. (1983) Exp. Cell Res. 43, 47–54.
[19] Lindros, K.O. and Penttilä, K.E. (1985) Biochem. J. 228, 757–760.
[20] Quistroff, B. (1985) Biochem. J. 229, 221–226.
[21] Gebhardt, R. (1998) Isolation of periportal and pericentral hepatocytes, in: Cytochrome P450 Protocols. Series in: Methods in Molecular Biology (Phillips, I.R. and Shephard, E.A., Eds.), Vol. 107, pp. 319–339, Humana Press Inc., Totowa, NJ.
[22] Gebhardt, R., Gaunitz, F. and Mecke, D. (1994) Adv. Enz. Reg. 34, 27–56.
[23] Quistorff, B. and Grunnet, N. (1987) Biochem. J. 243, 87–95.
[24] Vanhaecke, T., De Smet, K., Beken, S., Pauwels, M., Vercruysse, A. and Rogiers, V. (1998) Rat hepatocyte cultures. Conventional monolayer cultures and cocultures with rat liver epithelial cells, in: Cytochrome P450 Protocols. Series in: Methods in Molecular Biology (Phillips, I.R. and Shephard, E.A., Eds.), Vol. 107, pp. 311–317, Humana Press Inc., Totowa, NJ.
[25] Habig, W.H., Pabst, M.J. and Jakoby, W.B. (1974) J. Biol. Chem. 249, 7130–7139.
[26] Ricci, G., Caccuri, A.M., Lo Bello, M., Pastore, A., Piemonte, F. and Federici, G. (1994) Anal. Biochem. 218, 463–465.
[27] Habig, W.H. and Jakoby, W.B. (1981) Methods Enzymol. 77, 398–405.
[28] Rogiers, V., Vandenberghe, Y., Callaerts, A., Verleye, G., Cornet, M., Mertens, K., Sonck, W. and Vercruysse, A. (1990) Biochem. Pharmacol. 40, 1701–1706.
[29] Vandenberghe, Y., Foriers, A., Rogiers, V. and Vercruysse, A. (1990) Biochem. Pharmacol. 39, 685–690.
[30] Vandenberghe, Y., Glaise, D., Meyer, D.J., Guillouzo, A. and Ketterer, B. (1988) Biochem. Pharmacol. 37, 2482–2485.
[31] Vanhaecke, T., Lindros, K.O., Oinonen, T., Vercruysse, A. and Rogiers, V. (2000) Drug Metab. Dispos. (in press).
[32] Coecke, S., Debast, G., Phillips, I.R., Shephard, E.A. and Rogiers, V. (1998) Biochem. Pharmacol. 56, 1047–1051.
[33] Gebhardt, R., Fitzke, H., Fausel, M., Eisenmann-Tappe, I. and Mecke, D. (1990) Cell Biol. Toxicol. 6, 365–378.
[34] Nishii, Y., Hashizume, K., Ichikawa, K., Takeda, T., Kobayashi, M., Nagasawa, T., Katai, M., Kobayashi, H. and Sakurai, A. (1993) Endocr. J. 40, 399–404.