Molecular basis of bilirubin UDP-glucuronosyltransferase induction in spontaneously diabetic rats, acetone-treated rats and starved rats

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INTRODUCTION

Diabetes mellitus leads to a series of metabolic disturbances; they are found not only in the metabolism of carbohydrates, lipids and proteins but also in drug metabolism. Among the phase I drug-metabolizing enzymes both experimentally induced insulin-dependent diabetes and genetically developed insulin-dependent diabetes are associated with increases in the activities of cytochrome P-450 (P450) 1, 2B and 2E [1,2]. The regulation of the steady-state concentration of P450 2E1 in diabetes is an extremely complex process involving different mechanisms [3,4].

Among phase II reactions of biotransformation, glucuronidation is arguably the most important detoxification pathway in all vertebrates, quantitatively [5]. UDP-glucuronosyltransferases (UGTs) are a family of closely related membrane-bound microsomal enzymes with an intravesicular active site [5]. UGTs convert a wide range of xenobiotics and endobiotics to biologically inactive glucuronides that are readily eliminated. Among the endogenous compounds the end product of haem catabolism, bilirubin, is the most extensively studied substrate of UGTs [5]. The requirement for the body to remove bilirubin is paramount, because at high concentrations it causes brain and kidney damage [5]. Several reports have indicated that ethanol enhances the clearance of bilirubin and hepatic haem turnover, resulting in beneficial or preventive effects both in Gilbert’s disease and neonatal hyperbilirubinaemia [6–8]. Moreover it has been shown in rats that bilirubin UDP-glucuronosyltransferase (UGT1A1) activity is induced by ethanol [9]. The UGT multigene family includes 8 different rat UGTs and 14 human UGTs identified by cDNA cloning [10]. A comparison of the deduced amino acid sequences of 26 UGTs cloned from mammalian species has indicated that UGTs can be divided into two families on the basis of amino acid identities [10]. The members of the UGT1 gene family comprises phenol- and bilirubin-metabolizing isofoms, whereas the UGT2 gene family comprises steroid-metabolizing isofoms [5]. The heterogeneity of UGTs has also been well characterized by differential inducibility with various xenobiotics [5].

Because UGT1A1 activity exhibited similar behaviour to that of P450 2E1 by responding to the inductive effect of ethanol, we investigated the molecular changes in UGT1A1 expression and activity in three model systems in vivo in which P450 2E1 was also induced: in the liver of diabetic rats, acetone-treated rats, and rats starved for 96 h. BioBreeding/Worcester (BB/Wor) male rats were chosen as the animal model of human insulin-dependent (Type I) diabetes to avoid possible inductive or repressive effects of highly toxic diabetogenic chemicals such as streptozotocin or alloxan [11,12]. In the present study ketone bodies involving acetone were implicated as being responsible for the modification of UGT1A1 expression and activity.

EXPERIMENTAL

Materials

Alamethicin, bilirubin, Dalton Mark VII-L protein molecular mass markers, direct bilirubin determination kit, glucose UV–visible enzyme (hexokinase) determination kit, Mops, salicylaldehyde and UDP-glucuronic acid (sodium salt) were purchased from Sigma (St. Louis, MO, U.S.A.). Protein assay solution, nitrocellulose membranes and 3MM filter papers were purchased from Bio-Rad Laboratories (Hemel Hempstead, Herts., U.K.). GeneScreen Plus Nylon membrane and [α-32P]dCTP were obtained from Biotechnology Systems, NEN Research Products (Boston, MA, U.S.A.). Horseradish peroxidase (HRP)-conjugated anti-(sheep IgG) from rabbit was purchased from Calbiochem (La Jolla, CA, U.S.A.). HRP-linked anti-(rabbit IgG) from donkey, enhanced chemiluminescence (ECL) Western blotting detection reagents and Hyperfilm were provided by Amer sham International (Little Chalfont, Bucks., U.K.). High Prime DNA labelling kit was purchased from Boehringer Mannheim (Macclesfield, Lancs., U.K.). All other chemicals and reagents were from Sigma and were of analytical-grade purity or better.

Abbreviations used: BB/Wor, BioBreeding/Worcester rats; ECL, enhanced chemiluminescence; HRP, horseradish peroxidase; RAL, sheep anti-(rat liver UGT) antibody; Rgo, anti-UGT1A1 antibody; P450, cytochrome P-450; UGT, UDP-glucuronosyltransferase; UGT1A1, bilirubin UGT.

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Latency of UGTs is expressed as the difference between the activities in native and permeabilized vesicles and is given as a percentage of the activity observed in fully permeabilized microsomes. Results are means ± S.E.M. for three individual animals. *Statistically significant difference (P < 0.01) from BB/Wor controls.

### Table 1 Effect of diabetes on serum analytes and hepatic microsomal UGT activities

<table>
<thead>
<tr>
<th>Rat type</th>
<th>Serum glucose concentration (mM)</th>
<th>Serum acetone concentration (mM)</th>
<th>Serum direct bilirubin concentration (μM)</th>
<th>p-Nitrophenol UGT activity (nmol/min per mg of protein)</th>
<th>Latency of p-nitrophenol UGT (%)</th>
<th>UGT1A1 activity (nmol/min per mg of protein)</th>
<th>Latency of UGT1A1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BB/Wor control</td>
<td>8.72 ± 0.68</td>
<td>0.37 ± 0.09</td>
<td>2.3 ± 0.2</td>
<td>1.10 ± 0.12</td>
<td>15.95 ± 0.46</td>
<td>93.12 ± 0.65</td>
<td>97.43 ± 0.82</td>
</tr>
<tr>
<td>BB/Wor diabetic</td>
<td>28.24 ± 2.35*</td>
<td>1.34 ± 0.10*</td>
<td>3.6 ± 0.01*</td>
<td>0.89 ± 0.06</td>
<td>16.03 ± 0.31</td>
<td>94.44 ± 0.27</td>
<td>99.02 ± 0.07</td>
</tr>
</tbody>
</table>

### Animal experimentation

Animals received humane care in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (Bethesda, MD, U.S.A.). Male BB/Wor rats were provided by Møllegaard Breeding and Research Centre (Tornbjergvej, Denmark). BB/Wor is an inbred strain of Wistar rat in which approximately 87% of male animals spontaneously develop insulin-dependent diabetes mellitus before the age of 120 days. At the age of approx. 60 days the measurement of urine glucose level was started twice a week with KETO-Diabur Test® 5000 from Boehringer Mannheim. When the urine glucose test became positive, the detection of blood glucose concentration was begun every day by Haemo-Gluko Test® 1.44 from Boehringer Mannheim. When the blood glucose level had reached a level of 14–16 mM a Linplant® insulin implant was inserted under the skin of the animals for the time of delivery from Denmark to Hungary. BB/Wor healthy and insulin-implanted diabetic rats (80 days old; 180–200 g body weight) from the same colony were delivered by Charles River Ltd. (Budapest, Hungary). Animals were fed ad libitum and housed in a rigidly controlled animal room of the Department of Medical Chemistry, Semmelweis University of Medicine (Budapest, Hungary). Blood glucose concentrations were tested each day until the exhaustion of the insulin implant. At that time, blood glucose levels in diabetic rats exceeded 20 mM; they were kept for an additional 3 days in standard circumstances without any insulin supplementation and were then killed.

Male Wistar rats (180–200 g body weight) were provided by Charles River Ltd. and were maintained with free access to standard food. In a series of experiments nourishment was withdrawn from three rats for 96 h, whereas in other experiments three rats were pretreated with acetone added to drinking water (1%, v/v) with free access to standard food for 5 days [3]. Starved and acetone-treated animals were killed after 96 h or 5 days.

### Preparation and treatment of rat liver microsomes

Liver microsomal vesicles were prepared from male BB/Wor and male Wistar rats as previously described [13]. Microsomes were resuspended at a concentration of 10–20 mg/ml protein in 20 mM Mops, pH 7.2, containing 100 mM KCl, 20 mM NaCl and 3 mM MgCl₂. The suspensions were frozen and maintained under liquid nitrogen until used. In some series of experiments vesicles were permeabilized by alamethicin at its optimally activating concentration (0.05 mg/mg of protein).

### Production of antibodies

Anti-(p450 2E1) was kindly provided by Professor Arthur Cederbaum (The Mount Sinai Medical Center, New York, NY, U.S.A.). The polyclonal antibody was raised in rabbits against P450 2E1 purified from hamster liver. This antibody recognizes rat, mouse and human P450 2E1 proteins. Anti-(rat liver UGT) antibody (RAL) was raised in sheep against glucuronosyltransferase protein purified from rat, as described previously [13]. Anti-UGT1A1 antibody (R₉₀) was raised in White Lop-eared rabbits by the method described by Burchell [14] by using highly purified rat liver UGT1A1 [15].

### Western blotting

Denatured microsomal protein samples (10–20 μg) and low-molecular-mass protein markers were loaded on 0.1% SDS/7.5% (w/v) polyacrylamide gels and electrophoresis was performed as detailed previously [16]. The protein samples were then electrophoretically blotted to nitrocellulose filters by the method of Towbin et al. [17]. After completion of the blot, non-specific sites on the membranes were blocked with PBS-T [PBS (pH 7.4)/0.1% (v/v) Tween 20] containing 7.5% (w/v) lactalbumin (in the form of low-fat dried milk powder) for a minimum of 1 h at room temperature. The membranes were then incubated, with gentle agitation, at room temperature with the following antibodies (and their optimal dilutions in PBS-T containing lactalbumin): anti-(p450 2E1) (1:200), RAL (1: 30000) or R₉₀ (1:500). The incubation time was between 1 and 2 h depending on the antibody used. Detection of primary antibody binding was performed with a suitably diluted specific HRP-linked secondary antibody followed by ECL-directed exposure to Hyperfilm in accordance with the manufacturer’s instructions (Amersham).

### RNA extraction and Northern blotting with a UGT1A1 probe

Total RNA was extracted from the liver of each rat by the acid guanidinium thiocyanate method [18]. Human genomic DNA was subjected to PCR with primers corresponding to bases 388–408 (forward) and 675–696 (reverse) in the UGT1A1 exon. The 188 bp fragment (AF16) obtained was purified on a Chroma-Spin 100 column (Clontech, CA, U.S.A.) and diluted to a concentration of approx. 25 ng/μl before being denatured and labelled with [α-32P]dCTP by using the High Prime DNA labelling kit. This was then used to probe the Northern blots. Northern
Measurement of metabolites

For the determination of p-nitrophenol UDP-glucuronosyltransferase activities, native and permeabilized rat liver microsomes were incubated in the presence of 5 mM UDP-glucuronic acid and 100 μM p-nitrophenol for 30 min at 37 °C. Incubations were terminated by the addition of 0.05 vol. of 100% (w/v) trichloroacetic acid. p-Nitrophenol-UDP-glucuronosyltransferase activities were measured spectrophotometrically on the basis of aglycone disappearance from trichloroacetic acid-soluble supernatants, as described previously [20]. As bilirubin is a light-sensitive compound, all the experiments involving bilirubin were performed in the dark. Intact and alamethicin-permeabilized microsomes were incubated in the presence of 10 mM UDP-glucuronic acid and 122 μM bilirubin for 30 min at 37 °C; the reactions were terminated by the addition of ice-cold 2 M glycine/HCl, pH 2.7. The direct bilirubin contents of microsomal suspensions and rat sera were determined with a direct bilirubin detection kit by using bilirubin calibrator solutions on the basis of the reaction between direct bilirubin and diazotized sulphanilic acid [21]. Serum glucose concentrations were measured by the UV–visible hexokinase method [22]. Serum acetone concentrations were determined on the basis of the Urbach reaction [23].

Miscellaneous

The protein concentration of microsomes was determined by using the Bio-Rad protein assay solution with BSA as standard in accordance with the manufacturer’s instructions. All results are expressed as means ± S.E.M. Statistical analysis was performed with Student’s t-test.

RESULTS

Induction of UGT1A1 in spontaneously diabetic BB/Wor rats

Liver microsomes prepared from healthy and spontaneously diabetic BB/Wor rats were assayed after a period of 3 days without insulin treatment. At that time diabetic animals demonstrated many of the features of the disease: polydipsia, polyuria and excessive weight loss. Serum glucose and acetone concentrations were also indicative of diabetes mellitus (Table 1). The serum glucose concentration became extremely high in diabetic animals. Acetone levels in diabetic sera were elevated significantly compared with those of controls, indicating severe ketonaemia. At the same time, higher conjugated bilirubin concentrations in serum were observed in diabetic rats (Table 1), whereas total bilirubin concentrations remained unaltered in both groups (results not shown). When hepatic microsomes prepared from BB/Wor animals were analysed with a P450 2E1-specific polyclonal antibody, the concentration of this cytochrome was increased in diabetes relative to controls (Figure 1A), in agreement with previous observations [3]. Western blot analysis with sheep RAL, which recognizes two isoforms of the UGT1 family (54 kDa UGT1A1 and 53 kDa phenol UGT) and two isoforms of UGT2 family (52 kDa androsterone UGT and 50 kDa testosterone UGT) [13], exhibited the increased expression of UGT1 isoforms in liver microsomes prepared from diabetic rats, whereas UGT2 isoforms were not induced (Figure 1B). Immunoblots of rat liver microsomes by using a specific rabbit antibody against UGT1A1 (R19) confirmed that UGT1A1 was induced in diabetes (Figure 1C). UGT activities were determined towards p-nitrophenol or bilirubin aglycone substrates in intact and alamethicin-permeabilized rat liver microsomes prepared from control and diabetic BB/Wor animals. UGT activities were increased up to 7-fold towards bilirubin, whereas p-nitrophenol glucuronidation was not increased significantly in either impermeabilized or alamethicin-activated diabetic microsomes (Table 1). Total RNA, isolated from livers of healthy and diabetic BB/Wor rats to determine whether UGT1A1 induction occurs at the mRNA level in diabetes, was subjected to Northern hybridization with a UGT1A1 cDNA probe, as described. The autoradiograph showed that UGT1A1 mRNA concentration was increased in samples prepared from diabetic rats compared with control rats (Figure 2). Therefore the diabetic condition might cause increased...
Table 2  Effect of acetone treatment and starvation on serum analytes and hepatic microsomal UGT activities

Three untreated rats, three rats treated with acetone for 5 days and three rats starved for 96 h were killed. Concentrations of glucose, acetone and conjugated bilirubin were measured in the serum of control, acetone-treated and fasted rats as described in the text. Microsomes were also prepared from all of these animals. p-Nitrophenol UGT and UGT1A1 activities were determined in the presence or absence of alamethicin (0.05 mg/mg of protein) in each microsomal preparation as detailed in the Experimental section. For the definition of latency see the legend to Table 1. Values are means ± S.E.M. for three individual animals. Statistically significant differences from untreated controls: *P < 0.05, **P < 0.02, ***P < 0.01.

<table>
<thead>
<tr>
<th>Rat type</th>
<th>Serum glucose concentration (mM)</th>
<th>Serum acetone concentration (mM)</th>
<th>Serum direct bilirubin concentration (µM)</th>
<th>p-Nitrophenol UGT activity (nmol/min per mg of protein)</th>
<th>Latency of p-nitrophenol UGT (µM)</th>
<th>UGT1A1 activity (nmol/min per mg of protein)</th>
<th>Latency of UGT1A1 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.56 ± 0.43</td>
<td>0.33 ± 0.06</td>
<td>2.1 ± 0.2</td>
<td>0.41 ± 0.16</td>
<td>16.08 ± 0.28</td>
<td>97.45 ± 1.00</td>
<td>0.006 ± 0.001</td>
</tr>
<tr>
<td>Acetone treated</td>
<td>9.38 ± 0.70</td>
<td>1.69 ± 0.07***</td>
<td>3.9 ± 0.3***</td>
<td>0.39 ± 0.07</td>
<td>16.43 ± 0.23</td>
<td>97.60 ± 0.45</td>
<td>0.020 ± 0.002***</td>
</tr>
<tr>
<td>Starved for 96 h</td>
<td>6.49 ± 0.22*</td>
<td>0.95 ± 0.19***</td>
<td>1.2 ± 0.05**</td>
<td>0.40 ± 0.08</td>
<td>16.20 ± 0.02</td>
<td>97.53 ± 0.52</td>
<td>0.028 ± 0.005***</td>
</tr>
</tbody>
</table>

Figure 3  Expression of microsomal P450 2E1 and UGT proteins in the liver of normal rats and of rats fasted for 96 h

Microsomes were prepared from control and starved animals; samples (10 or 20 µg) of microsomal protein were denatured and subjected to SDS/PAGE. Proteins were then transferred to nitrocellulose filters as described in the Experimental section. Filters were subjected to immunoreaction with anti-(p450 2E1) antibody (A), RAL (B) or R10 (C). The detection of primary antibody binding was performed as described in the legend to Figure 1. Lanes 1–3 contained microsomes from three normal rats; lanes 4–6 contained microsomes from three fasted animals.

Figure 4  Immunoblot analysis of microsomal P450 2E1 and UGT proteins prepared from the liver of control and acetone-treated rats

Western blot analysis of denatured hepatic microsomal protein samples (10 or 20 µg per vial) prepared from control and acetone-treated rats was performed as described in the legends to Figures 1 and 2 by using anti-(p450 2E1) antibody (A), RAL (B) or R10 (C). The detection of primary antibody binding was executed as described in the legend to Figure 1. Lanes 1–3 contained microsomes from three individual control livers; lanes 4–6 contained microsomes from various acetoic livers.

transcription from the UGT1A1 gene leading to the enhanced production of UGT1A1 protein and a parallel increase in enzyme activity, although the results do not rule out additional mRNA stabilization or protein stabilization.

Alterations of UGT1A1 expression and activity in starvation and acetone treatment

Liver microsomes were prepared from acetone-pretreated Wistar rats or from Wistar rats fasted for 96 h. Acetone pretreatment or starvation can imitate some clinical and/or metabolic changes that occur in diabetes. Serum glucose levels significantly decreased after 96 h of starvation (Table 2) simultaneously with severe weight loss (results not shown). Acetone concentrations were increased in both fasted and acetone-pretreated animals, resulting from the accumulation of ketones (Table 2). A moderate elevation of direct bilirubin (bilirubin glucuronide) concentration in the serum was observed in acetone-pretreated rats but decreased in starved rats (Table 2). Total bilirubin concentrations were not altered in either group of animals (results not shown). In agreement with earlier findings [3], the results obtained with anti-(p450 2E1) antibody clearly indicated an increase in the expression of P450 2E1 both in starvation and after acetone administration (Figures 3A and 4A). Bilirubin UGT and phenol UGT activities were assayed in the absence or presence of an optimally activating concentration of pore-forming alamethicin (0.05 mg/mg of microsomal protein) in each preparation of microsomes, to detect any possible difference between the expressions of the two UGT1 isoforms recognized by RAL. Only bilirubin glucuronidation was found to be enhanced in microsomes prepared from acetonemic (3.5-fold) and fasted (6.6-fold) rats, whereas p-nitrophenol glucuronidation was not altered in either experimental group (Table 2). Probing the liver microsomal proteins with RAL clearly indicated an increase in the amount of the UGT1 isoforms both in acetone-treated or starved rats over the controls (Figures 3B and 4B). The increase in UGT1A1 protein level was also observed by Western blotting.
from three normal livers; lanes 4–6 contained RNA from three acetonic livers. were stained with ethidium bromide to show equal loadings of RNA. Lanes 1–3 contained RNA autoradiography of total RNA (20 μg/sample) isolated from control and starved rats was separated by formamide/agarose gel electrophoresis and blotted to GeneScreen Plus Nylon membrane as detailed in the text. (A) The membrane was hybridized with [α-32P]dCTP-labelled UGT1A1 cDNA probe and autoradiography was used to detected UGT1A1 mRNA. (B) Staining with ethidium bromide indicated equal loadings of RNA. Lanes 1–3 contained RNA from three individual controls; lanes 4–6 contained RNA from various tasted animals.

Figure 5 Northern blot analysis of UGT1A1 mRNA in the liver of control rats and of rats starved for 96 h

Total RNA (20 μg per sample) isolated from control and starved rats was separated by formamide/agarose gel electrophoresis and blotted to GeneScreen Plus Nylon membrane as indicated in the text. (A) The membrane was hybridized with [α-32P]dCTP-labelled UGT1A1 cDNA probe and autoradiography of total RNA (20 μg/sample) isolated from the livers of control and acetone-treated rats was performed as described in the legend to Figures 2 and 5. (B) RNA samples were stained with ethidium bromide to show equal loadings of RNA. Lanes 1–3 contained RNA from three normal livers; lanes 4–6 contained RNA from three acetone-treated rats.

Figure 6 Effect of acetone treatment on the UGT1A1 mRNA content of the liver

(A) Northern blot analysis, hybridization with [α-32P]dCTP-labelled UGT1A1 cDNA probe and autoradiography of total RNA (20 μg/sample) isolated from the livers of control and acetone-treated rats were performed as described in the legends to Figures 2 and 5. (B) RNA samples were stained with ethidium bromide to show equal loadings of RNA. Lanes 1–3 contained RNA from three normal controls; lanes 4–6 contained RNA from three acetone-treated rats.

with R₉₀ in microsomes prepared from acetone-treated and starved animals, suggesting the specific induction of UGT1A1 in starvation and acetonemia (Figures 3C and 4C). Northern blotting of RNA samples with the UGT1A1 cDNA probe indicated an increase in UGT1A1 mRNA levels in liver from starved rats, but UGT1A1 mRNA concentrations were apparently unchanged in livers from acetone-treated rats (Figures 5 and 6). In starvation the increased UGT1A1 activity was due at least in part to the stimulation of UGT1A1 gene transcription and the consequent increase in the translation of the enzyme protein, whereas in acetone treatment the UGT1A1 protein only was affected, leading to a higher activity of UGT1A1 enzyme.

DISCUSSION

In the present study an enhanced activity of UGT1A1 was observed after diabetes, starvation and acetone treatment, whereas the other isofrom of the UGT1 gene family (phenol UGT) remained unaltered (Tables 1 and 2). This increase in UGT1A1 caused a slight increase in serum bilirubin glucuronide concentration in diabetes and acetone treatment, but not in starvation (Tables 1 and 2). The UDP-glucose supply for the synthesis of UDP-glucuronic acid and consequently glucuronidation is dependent on hepatic glycogen stores [24]. In severe deprivation of food intake, the UDP-glucose supply might be insufficient for bilirubin conjugation despite the induction of microsomal UGT1A1.

Glucuronidation catalysed by UGTs is dependent on the transport of UDP-glucuronic acid into the hepatic endoplasmic reticulum from the cytosol [5]. Therefore the stimulation of bilirubin glucuronidation in all cases could be due to altered membrane permeability/transport activity and/or to the direct induction of the enzyme. In a previous observation the activity of phenol UGT has been found to be deficient in freshly prepared intact microsomes from streptozotocin-induced diabetic male rats [25]. In other studies differences have not been observed for the serum disappearance, glucuronidation or biliary excretion of bilirubin in streptozotocin-induced diabetic compared with normal rats [26]. Increased membrane constraint has been suggested as the mechanism responsible for the lower activity of certain UGT1 isoforms [25]. In contrast with previous findings, the latency of both phenol UGT and UGT1A1 was not influenced by all those pathological stages produced in the present study (Tables 1 and 2). Consequently, the increased membrane constraint could be due to the effect of streptozotocin.

The elevation in the activity of UGT1A1 can be explained by induction of the enzyme. This suggestion was confirmed by Western blot analysis that demonstrated the increases in UGT1A1 protein levels (Figures 1, 3 and 4). It has been shown previously that alkyl ketones can activate UGT towards 2-aminoanisole in Gunn rat liver and Wistar rat liver [27]. The accumulation of ketones, indicated by the increase in serum acetone concentrations in each situation, suggests a role for ketone bodies in the induction of UGT1A1 (Tables 1 and 2). The larger amount of UGT1A1 mRNA was detected only in diabetes and starvation (Figures 2 and 5) by Northern hybridization. Therefore ketones (acetone) alone might directly activate the enzyme or stabilize the UGT1A1 protein (Figure 6), in agreement with earlier studies [28]. Although glucose deprivation and/or the consequent hormonal alterations in diabetes and starvation seem to be sufficient to induce UGT1A1 at the level of mRNA (Figures 2 and 5), ketones might have an additional role in increasing the activity of UGT1A1 in these two pathological conditions.

One of the major factors that affects UGT expression and hence glucuronidation reactions is ontogeny [5]. It has been shown that phenol UGT protein is the only detectable UGT in fetal rat liver, whereas bilirubin and other UGT isoforms appear postnatally [29]. Extensive studies on rats have demonstrated that glucuronidation of endogenous compounds such as bilirubin develops immediately after birth and does not exhibit any postnatal decline [5]. The increase in UGT1A1 activity is continuous after birth and reaches a maximum at approx. 1 month of age [5]. Among human UGT enzyme activities and proteins there is only one isoform, which catalyses the glucuronidation of 5-hydroxytryptamine present in the fetus at levels comparable with those in the adult [30,31]. The remaining UGTs, which conjugate bilirubin and other aglycones, are present at less than 20% of adult levels in fetal liver [31]. UGT1A1 activity gradually develops postnatally [30,31]. Assays in vitro of UGT activity in human biopsy samples, and studies in vivo, have revealed that newborns have an impaired ability to glucuronidate bilirubin compared with adult subjects. Unconjugated hyperbilirubinaemia in newborn infants is due to an inadequacy to glucurononate bilirubin [32]. The factors that regulate the ontogenetic expression of UGT1A1 are as yet poorly understood,
although corticosteroids [33] and thyroid hormones [34] have been implicated in the development surge of UGT1A1 after birth.

The hormonal/metabolic alterations observed in diabetes and starvation are comparable to the postnatal metabolic state. The acute interruption of maternal glucose transfer to the fetus at delivery imposes an immediate need to mobilize endogenous glucose. In all mammalian species there is a 3–5-fold abrupt increase in glucagon concentration within minutes to hours of birth. Insulin, however, usually falls initially and remains in the basal range for several days [35,36]. Acting in unison, these hormonal changes at birth mobilize glucose via glycogenolysis and gluconeogenesis, activate lipolysis and promote ketogenesis [35,36]. In conclusion, it is tempting to suggest that the sudden shortage of neonatal blood glucose after birth leads to a decrease in insulin/glucagon ratio and a consequent ontogenic induction of UGT1A1. Moreover, the metabolic changes (mainly the enhanced ketogenesis) might contribute to the direct activation and/or stabilization of UGT1A1 protein. Because haem oxygenase can be induced by hypoglycaemia [37], creating a need for greater bilirubin glucuronidation to handle the increased bilirubin load, the up-regulation of UGT1A1 activity might be beneficial in glucose-deprived situations.

We thank Mrs. Valéria Szénási for her skilful technical assistance during the experiments, and Professor Arthur Cederbaum (The Mount Sinai Medical Center, New York, NY, U.S.A.) for the anti-(p450 2E1) polyclonal antibody. This work was supported by OTKA (Országos Tudományos Kutatási Alap), Hungary, and by the Ministries of Welfare, and Education, Hungary.

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