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Coordinate regulation of UDP-glucuronosyltransferase UGT1A6 induction by 3-methylcholanthrene and multidrug resistance protein MRP2 expression by dexamethasone in primary rat hepatocytes

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Abstract

Concentration-dependent regulation of 3-methylcholanthrene (MC) inducibility of UDP-glucuronosyltransferase UGT1A6 by the synthetic glucocorticoid, dexamethasone (DEX) was studied. Treatment of cultured rat hepatocytes with MC, 0.1, 1, and 10 μM DEX, and MC combined with DEX, resulted in different induction patterns measured in the intact cells compared to that observed in the microsomes prepared from the same cells. DEX treatment in various concentrations caused a concentration-dependent increase in p-nitrophenol (p-NP) conjugation in intact cells (3-, 4-, and 5-fold over control, respectively), and it positively regulated MC induction (4-, 5-, and 6-fold over control, respectively). In contrast, DEX had smaller effect on microsomal p-NP conjugation (115, 200, 220% of control, respectively) and although MC induction was increased significantly by 0.1 μM DEX (520% of control), but higher concentrations of DEX (10 μM) decreased the degree of induction to 410%. Similar results obtained from *in vivo* experiments showed that at high DEX concentration (100 mg/kg), the rate of MC induction (540%) decreased (420%). Permeabilization of the plasma membrane resulted in a 15-fold increase of p-NP conjugation indicating the importance of transport in the rate of overall p-NP elimination, and the induction pattern was similar to that observed in microsomes isolated from cells. Hyper-osmolarity (405 mOsmol/L) led to a 3-fold decrease of p-NP conjugation, the loss of DEX inducibility and reduction of the MRP2 protein level. Our results suggest coordinated regulation of UGT1A6 inducibility and substrate or product transport by DEX.

Keywords: Rat UGT1A6; MRP2; Dexamethasone; Ah receptor; Hepatocyte

1. Introduction

UDP glucuronosyltransferases (UGTs) catalyse the transfer of glucuronic acid from UDP-glucuronic acid to a wide variety of endogenous and exogenous substrates. As glucuronides are usually less active biologically, more polar and readily excreted from an organism compared to their parent aglycone, the UGTs are considered to be important for the detoxification and elimination of these substances [1,2]. The UGTs have been classified into two families. Members of family 1 conjugate a wide range of substrates, including bilirubin and xenobiotic phenols. UGT1 enzymes are encoded by a unique gene complex, with tandem individual promoters and first exons linked to four common exons by differential splicing. Each UGT exhibits its own unique profile of substrate and tissue specificity and regulation by endogenous and exogenous compounds [3–5].

Recent knowledge concerning regulation of UGTs is still scarce. It is well documented that the expression of many UGTs is influenced by inducers belonging to different classes: polyaromatic hydrocarbons (PAH), phenobarbital, clofibrate, rifampicin, glucocorticoids, antioxidants, etc. [6–10]. In addition, hormonal factors have also been shown to be involved in the regulation of UGT activity [11]. There is evidence to support that UGT1A6 and UGT1A9 are regulated *via* Ah receptor, and recently UGT1A7 was also reported to be under the control of PAH [6,9]. The role of Ah receptor in the regulation of UGT1A1, the only relevant bilirubin conjugating isoform in human, has also been suggested [12]. It was proposed that bilirubin is an endogenous ligand of Ah receptor and

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Abbreviations: UGT, UDP-glucuronosyltransferase; MRP, multidrug resistance protein; DEX, dexamethasone; MC, 3-methylcholanthrene; Ah, aryl hydrocarbon; UDPGA, UDP glucuronic acid; p-NP, *p*-nitrophenol; PAH, polyaromatic hydrocarbons.

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regulates its own metabolism in this way [13]. Prough *et al.* extensively studied the effect of glucocorticoids on expression of genes regulated by the Ah receptors [11]. They proved that at least three of these genes are under the direct control of glucocorticoid receptor.

The coordinated regulation of drug metabolizing enzymes and some of the ATP-dependent transporter proteins including the apical conjugate export pump MRP2 has been described by different groups [15,16]. MRP2 is regarded as a carrier protein of conjugates playing important role in the canalicular secretion of glucuronides [15,17]. The expression of MRP2 has been described to depend on DEX concentration and osmolarity in cultured rat hepatocytes [18–20].

In the present study, UGT1A6 expression on enzyme activity level using p-NP as substrate and on protein level by immunoblot analysis was studied following *in vivo* or *in vitro* treatment with MC and various concentrations of DEX separately and in combination, respectively. MRP2 expression on protein level was also demonstrated by immunoblot analysis. The coordinated regulation of conjugation and transport by DEX was investigated in the intact hepatocytes, and the effect of DEX on the two processes was separated by permeabilization of the plasma membrane and also by the change of osmolarity.

2. Materials and methods

2.1. Chemicals

UDP-glucuronic acid, MC, DEX, Williams' Medium E, collagenase, saponin, D-saccharic acid 1,4-lactone were purchased from Sigma, sodium dodecyl sulfate (SDS), methyl propyl ketone were from Merck. Rat polyclonal antibody against UGT1A6 was a generous gift of Dr. B. Burchell (Dundee, Scotland). The anti-MRP2 EAG15 antibody was kindly provided by Dr. D. Keppler (Heidelberg, Germany). All other chemicals were purchased from Reanal.

2.2. Primary hepatocyte cell culture

Hepatocytes were prepared from male Wistar rats (200 g) by *in situ* liver collagenase perfusion [21]. Cell viability (>85%) was determined by trypan blue exclusion. Cells were seeded on collagen-coated dishes (60 mm × 15 mm and 35 mm × 10 mm) at a density of 4.5×10^6 and 1.33×10^6 cells per dish, respectively, in Williams' Medium E containing 10% of foetal calf serum, 100 nM insulin, 2.5 μg/mL amphotericin B, 0.1 mg/mL gentamicin, and 30 nM Na_2SeO_3. Calf serum and amphotericin B were present for the first 24 hr than omitted. Cells were maintained at 37° in a humidified atmosphere of 95% air/5% CO_2. Four hours after plating, and every day thereafter, the medium was changed to Williams' Medium E supplemented with 3.7 μM MC, 0.1, 1, and 10 μM DEX, 3.7 μM MC combined with 0.1, 1, and 10 μM DEX. Control medium contained vehicle, 0.1% DMSO. The induction period lasted for 72 hr. Cells washed twice with PBS were incubated with p-NP for 20 and 40 min, respectively. In some experiments, cells were exposed to hyperosmotic medium (405 mOsmol/L). Osmolarity was changed by alteration of the NaCl concentration of the medium. Hepatocytes were permeabilized by saponin treatment (0.005%) for 5 min. For preparing microsomes, cells were scraped and sonicated on ice followed by centrifugation at 10,000 *g* for 30 min, and the microsomes were sedimented at 100,000 *g* for 60 min. For MRP2 immunoblot analysis, preparation of microsomes was carried out in the presence of 1 mM phenylmethanesulphonyl fluoride. Protein concentration was determined by the method of Lowry, using BSA as protein standard [22]. Microsomes were stored at −70°, and were used for the determination of UGT activity and for Western immunoblot analysis.

2.3. Enzyme assays in cell culture

UGT1A6 activity was determined in intact cell culture using p-NP as substrate [23]. Cells were treated with Williams' Medium E containing p-NP (0.1 mM). p-NP UGT activity was measured by HPLC detection of p-NP-glucuronide peak, using p-NP-glucuronide as a standard [24]. Medium was withdrawn at 10 and 20 min of incubation and was injected directly into the HPLC. The chromatographic equipment consisted of two Isco Model 2350 pumps, an Isco V^4 type UV detector (Isco), a Hypersil ODS 150 mm × 4 mm reversed-phase column with particle size of 3 μm (BST). The mobile phase for determination of p-NP and its glucuronide consisted of 15% acetonitrile, 85% 0.02 M potassium phosphate buffer, pH 1.8, containing 2.5 mM SDS. The flow rate was 1 mL/min, and the eluate was monitored at 305 nm. In medium containing 0.1 μM p-NP, the rate of conjugation was linear up to 60 min. The detection limit for p-NP-glucuronide was 1 μM. In the case of permeabilized cells, the incubation media was supplemented with 4 mM UDP-glucuronic acid and cells were incubated with p-NP for 3 and 6 min [23].

2.4. Enzyme assays in microsomes

Male Wistar rats, five animals per group, were treated with 20 mg/kg MC in corn oil (i.p.), 100 mg/kg DEX in corn oil (p.o.) and MC combined with DEX for 3 days. Control animals were treated with corn oil. Twenty-four hours after the last injection, livers were removed, and microsomes were prepared as described earlier. p-NP conjugation was determined according to the method of Burchell and Weatherhill [25], the incubation mixture contained 0.1 mM p-NP, 2 mM D-saccharic acid 1,4-lactone, 4 mM UDPGA and 0.2 mg of protein in a final

volume of 200 μL. The microsomes were preincubated with 0.05% Triton X-100 for 20 min on ice.

2.5. Western blot analysis

The microsomes were prepared for immunoblot analysis by suspension in sample loading buffer, heated at 100° for 5 min except for MRP2, where the boiling step was omitted. Proteins were separated by SDS–polyacrylamide gel electrophoresis (7.5%). After separation, the proteins were transferred to nitrocellulose membranes, treated with PBS–Tween 20 (0.1%) containing 3% BSA overnight at 4° [26]. The membranes were washed in PBST before addition of primary antibodies, Rat-anti-liver antibody (RAL) (1:3000 dilution of 46.5 mg protein/mL) or rat anti-MRP2 EAG15 (1:10000 dilution), respectively [27]. Following incubation with primary antibodies, the membranes were washed, and incubated with secondary antibody, horseradish peroxidase-conjugated anti-sheep IgG (Calbiochem), or anti-rabbit IgG (Gentest), respectively. Immunoreactive proteins were visualized by chemiluminescence method (Amersham International).

3. Results

Primary rat hepatocytes were treated with various concentrations of DEX ranging from 0.1 to 10 μM and 3.7 μM MC separately and in combination for 72 hr. The UGT1A6 activity was determined using p-NP as substrate. The rate of conjugation was measured both in intact cells by HPLC analysis of the medium (Fig. 1A) and in the microsomes prepared from the induced cells (Fig. 1B). Enzyme activity data obtained from experiments with intact cells indicate that DEX enhanced p-NP glucuronidation in a concentration-dependent manner (275, 350, and 504% of the control, respectively). Basal rate of conjugation was not influenced markedly by MC treatment alone (150%), however, it was positively modulated by the presence of DEX, and the potentiation by DEX showed concentration dependence (443, 502, and 628% of the control, respectively). In contrast, the effect of DEX on p-NP conjugation measured in the microsomes prepared from the same induced hepatocytes showed a distinctly different pattern. The level of induction by DEX was much less pronounced than that observed in intact cells. Only a 2-fold induction was detected even at the highest DEX concentration (10 μM). It was surprising that the concentration dependence for DEX potentiation of MC induction showed a biphasic response in contrast to the data obtained in intact cells. At 0.1 μM concentration, DEX increased MC induction to 3-fold of basal induction, and the rate of induction was not increased by higher DEX concentrations, moreover, in 10 μM concentration the potentiation effect of DEX decreased.

In addition to experiments carried out *in vitro*, the effect of DEX, MC, and their combination was observed *in vivo*

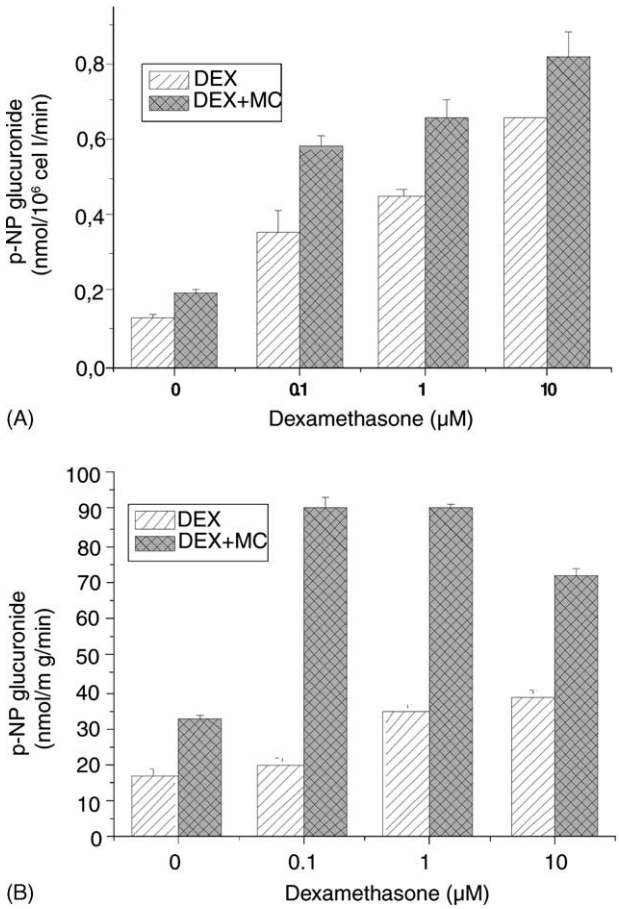

Fig. 1. Alteration of MC inducibility of p-NP conjugation by DEX in primary culture of rat hepatocytes (A) and in the microsomes prepared from the same cells (B). Hepatocytes were treated with 0.1, 1, and 10 μM DEX, 3.7 μM MC and DEX combined with MC, respectively, for 72 hr. Glucuronidation was measured in the presence of 0.1 mM p-NP at 10 and 20 min of incubation by HPLC (A). Microsomes were prepared from the cells and UGT1A6 activity was determined in 200 μL of 0.1 mM Tris–HCl buffer containing 1 mM p-NP, 2 mM D-saccharic acid 1,4-lactone, 4 mM UDP-glucuronic acid and 0.2 mg of microsomal protein. Substrate disappearance was measured spectrophotometrically (B). Data are given as means ± SD of three separate experiments that were performed in triplicate.

as well. Male Wistar rats were treated with 20 mg/kg of MC (in corn oil, i.p.), 100 mg/kg of DEX (in corn oil, p.o.) and MC in combination with DEX, respectively, for 3 days. p-NP conjugation was measured in the microsomes prepared from the livers 24 hr after the last injection. *In vivo* DEX treatment had no effect on p-NP conjugation, MC treatment resulted in a 5-fold increase, while the combination of the two inducers reduced the induction to 4-fold (Fig. 2). Exactly the same results were obtained *in vitro* (Fig. 1B) by changing the DEX concentration from 0.1 μM that is commensurate with physiologic glucocorticoid serum level to 10 μM in the presence of MC.

Glucuronidation of p-NP was also measured in saponin treated hepatocytes in the presence of 4 mM UDPGA (Fig. 3). Permeabilization of plasma membrane enhanced the rate of p-NP glucuronidation 15-fold over the intact

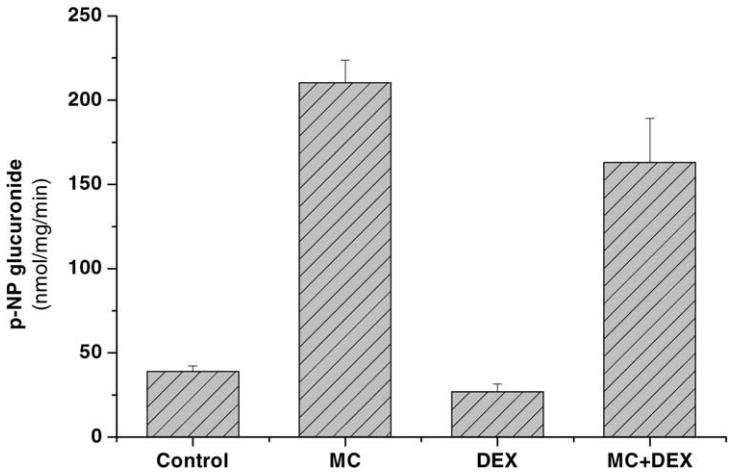

Fig. 2. *In vivo* induction of p-NP conjugation by DEX and MC separately and in combination. Male Wistar rats, five animals per group were treated with 20 mg/kg MC in corn oil (i.p.), 100 mg/kg DEX in corn oil (p.o.) and MC combined with DEX, respectively, for 3 days. Microsomes were prepared 24 hr after the last treatment, and UGT1A6 activity was measured. Refer to Fig. 1B for experimental details.

cells. The administration of UDPGA to the non-permeabilized cells did not increase the rate of conjugation (data not shown). The rate of induction by DEX treatment in the permeabilized cells showed very similar pattern to that obtained in the microsomes of *in vitro* treated cells. DEX treatment had little effect, treating the cells with the highest DEX concentration (10 μM) resulted in only a 2-fold increase in the rate of conjugation.

The great increase in the rate of p-NP glucuronidation following permeabilization of plasma membrane indicates that the transport might be of determining importance in the overall p-NP elimination from the cell. Primary rat hepatocytes were treated with DEX, MC, and MC + DEX in norm-osmotic medium for 48 hr. Thereafter, they were exposed to hyper-osmotic (405 mOsmol/L) medium containing the inducers at the same concentration. The metabolism of p-NP to its glucuronide conjugate was measured following a 24-hr hyper-osmotic incubation. In the medium of cells kept under hyper-osmotic conditions, the amount of p-NP-glucuronide decreased to 30% of control cells (Fig. 4). The rate of metabolism was not affected by DEX treatment at either concentration. MC treatment resulted in a 175% increase in the amount of p-NP-glucuronide, and the coadministration of MC with DEX (0.1, 1, and 10 μM) slightly enhanced p-NP elimination (167, 202, and 256% of untreated, respectively).

The UGT1A6 protein expression after 72 hr of induction by MC and DEX is shown in Fig. 5. The induction pattern of the amount of UGT1A6 protein and the enzyme activity data of the microsomes (Fig. 1B) was similar. MC treat-

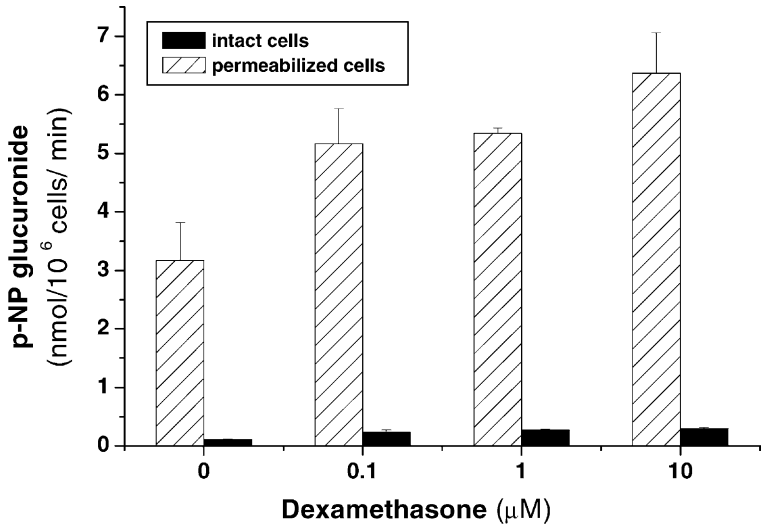

Fig. 3. Alteration of MC inducibility of p-NP conjugation by DEX in intact and saponin-treated primary rat hepatocytes. Treatment of hepatocytes and determination of UGT1A6 activity in intact cells were carried out as described in Fig. 1A. Hepatocytes were permeabilized after 72 hr of induction with 0.005% saponin for 5 min. In the case of permeabilized cells, the incubation medium contained 4 mM UDP-glucuronic acid, and the incubation time was 3 and 6 min, respectively. Data are given as means ± SD of three separate experiments that were performed in triplicate.

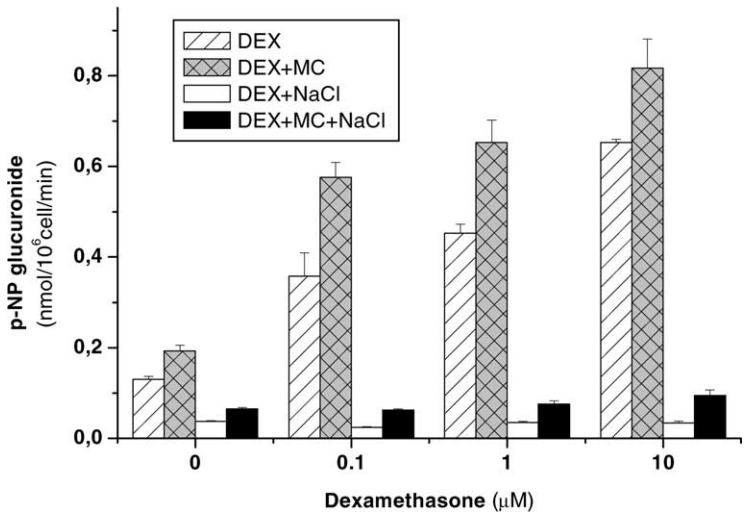

Fig. 4. Alteration of MC inducibility of p-NP conjugation by DEX in primary rat hepatocytes maintained under norm-osmotic and hyper-osmotic conditions. Cells were incubated in norm-osmotic medium (305 mOsmol/L) containing 0.1, 1, and 10 μM DEX, 3.7 μM MC, and DEX combined with MC for 36 hr and subsequently were exposed to hyper-osmotic medium (405 mOsmol/L) containing the inducers at the same concentration for 24 hr. Glucuronidation was measured as described in Fig. 1A. Data are given as means ± SD of three separate experiments that were performed in triplicate.

ment enhanced both the UGT1A6 protein level and the enzyme activity to 175% of the control. DEX at 1 μM had only a slight effect (139%). DEX increased MC induction on protein level, the potentiation was not dependent on DEX concentration (204, 225, and 220% of the control). DEX enhanced MRP2 protein level to 140 and 240% at 0.1 and 10 μM concentration, respectively. Under hyper-osmotic conditions MRP2 protein level decreased (Fig. 6).

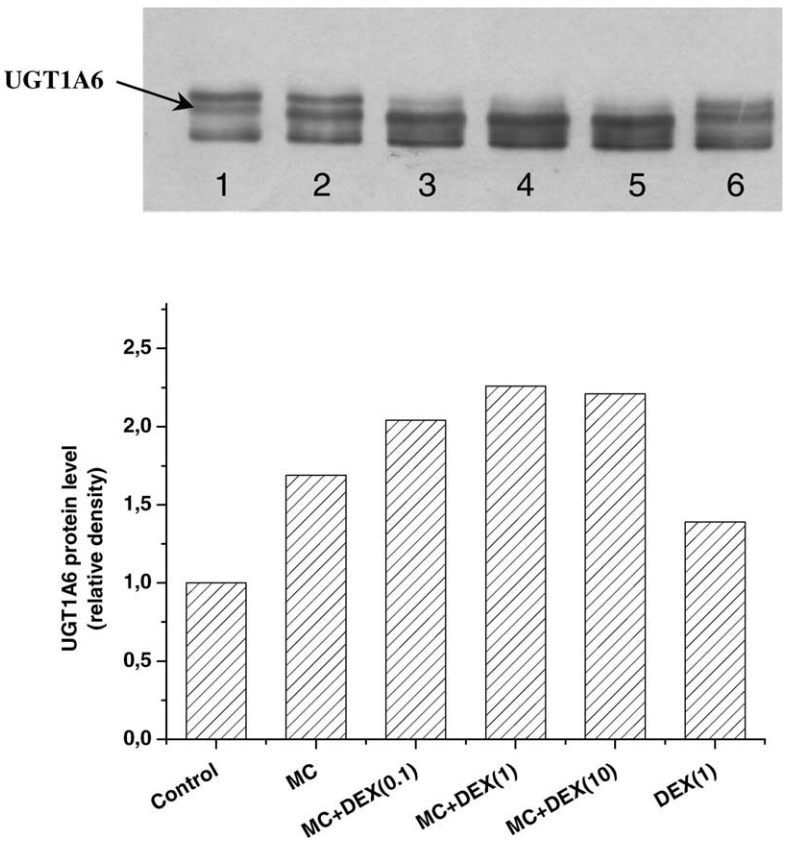

Fig. 5. Immunoblot analysis of UGT1A6 induction by DEX and MC separately and in combination. Hepatocytes were treated with 0.1, 1, and 10 μM DEX, 3.7 μM MC, and DEX combined with MC, respectively, for 72 hr. Microsomes were prepared, fractionated, blotted, and reacted with specific primary antibodies (RAL). Each line contained 15 μg of protein. Relative levels of UGT1A6 were determined by UN-SCAN-IT digitizing Software (Silk Scientific). Data shown are representative of three independent experiments.

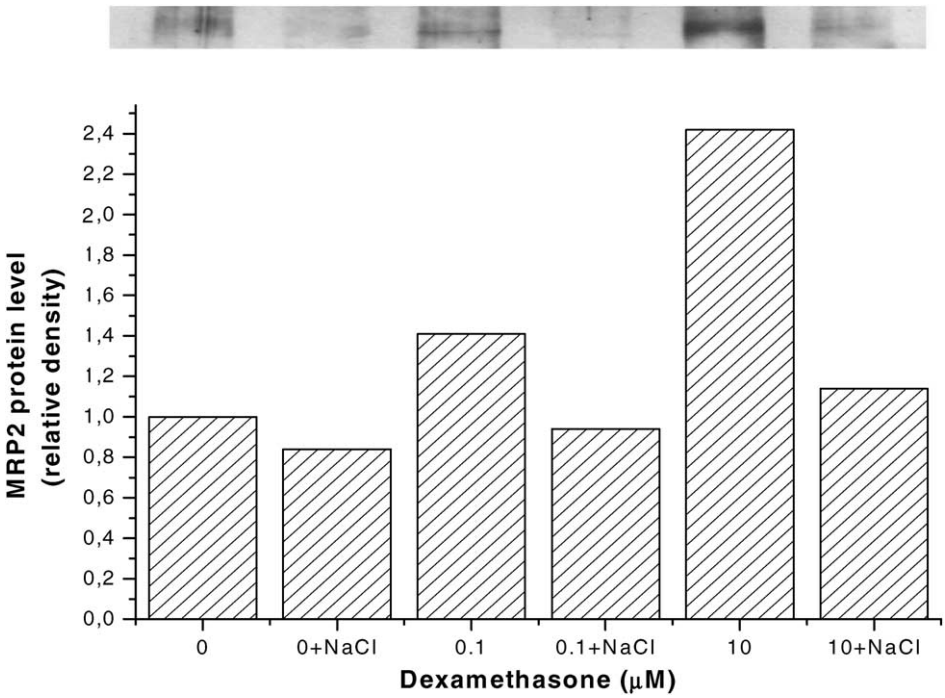

Fig. 6. Effect of DEX and hyper-osmolarity on MRP2 protein level. Hepatocytes were treated with 0.1 and 10 μM DEX for 72 hr. After 48-hr treatment, some dishes were exposed to hyper-osmotic media for 24 hr. Microsomes were prepared, fractionated, blotted, and reacted with specific primary antibodies (EAG15). Each line contained 17 μg of protein. Relative levels of MRP2 were determined by UN-SCAN-IT digitizing Software (Silk Scientific). Data shown are representative of two independent experiments.

4. Discussion

The results reported in the present study suggest the coordinate regulation of UGT1A6 and the transport of its glucuronide from the rat hepatocyte by DEX. The simultaneous induction of metabolizing enzymes and transport proteins by different types of inducers has been published, and it may be important in chemoprotection against xenobiotics [15,16]. In previous reports it was clearly demonstrated that some of the genes of the Ah battery, including UGT1A6 is under the direct control of glucocorticoid receptor [11,14]. It has been shown that although DEX itself has little effect on the expression of these genes, it up-regulates PAH induction; however, in some cases (GST Ya_1, ALDH3) negative regulation by DEX ($\leq$1 μM) was also demonstrated. The concentration dependence of DEX on UGT1A6 induction by PAH has not been investigated. The effect of DEX on p-NP conjugation measured in intact cells compared to that in the microsomes from DEX treated cells seems to be different. In the microsomes, DEX considerably up-regulated UGT1A6 induction at 0.1 μM concentration, but higher DEX concentrations did not result in further increase in the rate of conjugation. On the contrary, in the intact cells DEX up-regulated MC induction in a concentration-dependent manner, and DEX treatment itself caused pronounced concentration-dependent increase of p-NP conjugation. An explanation for this discrepancy might be that in the case of intact cells the amount of p-NP-glucuronide is influenced by both UGT1A6 expression and transport activity. However, the rate of microsomal conjugation of p-NP depends only on UGT1A6 expression in the presence of excess UDP-glucuronic acid following Triton X-100 treatment. To prove the importance of the transport in p-NP elimination and to clarify the role of DEX in this process, the following experiments were performed: (1) permeabilization of plasma membrane of the hepatocytes by saponin treatment, (2) down-regulation of MRP2 by hyper-osmotic incubation, (3) *in vivo* treatments of rats by MC and DEX separately and in combination.

1. The up-regulation of MRP2 expression by DEX has been shown in rat hepatocytes [19,20]. *In vitro* and *in vivo* evidences demonstrated that DEX treatment led to a marked increase of hepatic amounts of MRP2 mRNA [18]. In accord with these findings, our present results also support that DEX positively regulates MRP2 expression. MRP2 is considered to play essential role in the transport of conjugates in drug metabolism process [17]. The concentration dependence of p-NP conjugation on DEX observed in intact cells may be due to alteration of transport processes, e.g. enhanced MRP2 expression. Permeabilization of the plasma membrane allows separation of the effect of DEX on UGT1A6 and transport activity. Providing excess of UDP-glucuronic acid after permeabilization of the membrane enables one to investigate the effect of various DEX concentrations on UGT1A6 expression in the hepatocytes. The

induction profile obtained in saponin treated cells was the same as that obtained in the microsomes. All these findings are in good agreement with previous studies [11,14] describing that DEX itself only slightly affects UGT1A6 expression, but in small concentration (0.1 μM) up-regulates the induction of UGT1A6 by PAH. In higher concentration (10 μM) negative response was observed. The concentration-dependent up-regulation of p-NP conjugation observed in intact cells can be, at least, partly due to the enhanced transport processes, e.g. the enhanced MRP2 protein level.

Immunoblot experiments support the finding that DEX at 0.1 μM increases the inductor effect of MC, but at higher concentrations no further increase in UGT1A6 protein level can be detected.

2. Another line of evidence concerning the role of MRP2 in p-NP elimination is the negative effect of the enhanced osmolarity on p-NP conjugation. As shown by Kubitz *et al*. [20] MRP2 expression is regulated by cell osmolarity. In their experiments, hyper-osmolarity down-regulated MRP2 at the mRNA and protein level by 46%. In our experiments hyper-osmolarity decreased p-NP conjugation by 70%, and simultaneously significantly reduced MRP2 protein level. Although MC inducibility of conjugation was still observed, the effect of DEX diminished.
3. Prough *et al*. [11] demonstrated that glucocorticoid regulation of PAH induction occurs *in vivo* as well. The developmental changes in the *in vivo* response to glucocorticoid treatment are due to the difference in the basal circulating levels of these steroids in different ages. In adult rats, the physiological glucocorticoid concentration is sufficient to potentiate MC induction, that is the reason for the finding that UGT1A6 enzyme activity in the liver of MC-treated animals showed the same rate of induction as the microsomes of MC + 0.1 μM DEX-treated hepatocytes. The combination of DEX and MC treatment *in vivo* corresponds to the combined MC + 10 μM DEX treatment *in vitro*, resulting in the same reduced scale of induction.

In conclusion, the difference between *in vitro* and *in vivo* results suggest that DEX treatment itself has little effect on UGT1A6 expression, however, UGT1A6 gene is under the control of the glucocorticoid receptor. At small concentration, DEX (≤0.1 μM) positively regulates PAH induction of UGT1A6 similarly that shown for CYP1A1 [14]. At high concentration, DEX (10 μM) negatively regulates MC induction both *in vitro* and *in vivo*. In addition, DEX is a potent inducer of MRP2, and the induction pattern shows concentration dependence. Therefore, at least, two steps in the elimination of p-NP and probably of many other xenobiotics are under the control of glucocorticoids. The effect of DEX on the rate of the overall elimination process measured in the intact hepatocytes is the result of coordinated induction of the two proteins.

References

[1] Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Bélanger A, Fournel-Gigleux S, Green M, Hum DW, Iyanagi T, Lancet D, Louisot P, Magdalou J, Chowdhury JR, Ritter JK, Schachter H, Tephly TR, Tipton KF, Nebert DW. The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. Pharmacogenetics 1997;7:255–69.

[2] Meech R, Mackenzie PI. Structure and function of uridine diphosphate glucurinosyltransferases. Clin Exp Pharmacol Physiol 1997;24:907–15.

[3] Guengerich FP, Parikh A, Johnson EF, Richardson TH, von Wachenfeldt C, Cosme J, Jung F, Strassburg CP, Manns MP, Tukey RH, Prichard M, Fournell-Gigleux S, Burchell B. Heterologous expression of human drug-metabolizing enzymes. Drug Metab Dispos 1997;2:1234–41.

[4] Owens IS, Ritter JK, Yeatman MT, Chen F. The novel UGT1 gene complex links bilirubin, xenobiotics, and therapeutic drug metabolism by encoding UDP-glucuronosyltransferase isoenzymes with a common carboxyl terminus. Pharmacokinet Biopharm 1996;24:491–508.

[5] Burchell B, McGurk K, Brierley CH, Clarke DJ. UDP-glucuronosyltransferases. In: Guengerich FP, editor. Comprehensive toxicology, vol. 3. Biotransformation, 1997. p. 401–35.

[6] Bock KW, Gschaidmeier H, Heel H, Lehmköster T, Münzel PA, Bock-Hennig BS. Functions and transcriptional regulation of PAH-inducible human UDP-glucuronosyltransferases. Drug Metab Rev 1999;31:411–22.

[7] Pritchard M, Fournel-Gigleux S, Siest G, Mackenzie P, Magdalou J. A recombinant phenobarbital-inducible rat liver UDP-glucuronosyltransferase (UDP-glucuronosyltransferase 2B1) stably expressed in V79 cells catalyzes the glucuronidation of morphine, phenols and carboxylic acids. Mol Pharmacol 1994;45:42–50.

[8] Metz RP, Ritter JK. Transcriptional activation of the UDP-glucuronosyltransferase 1A7 gene in rat liver by aryl hydrocarbon receptor ligands and oltipraz. J Biol Chem 1998;273:5607–14.

[9] Ritter JK, Kessler FK, Thomson MT, Grove AD, Auyeung DJ, Fisher RA. Expression and inducibility of the human bilirubin UDP-glucuronosyltransferase UGT1A1 in liver and cultured primary hepatocytes: evidence for both genetic and environmental influences. Hepatology 1999;30:476–84.

[10] Li YQ, Prentice DA, Howard ML, Mashford ML, Wilson JS, Desmond PV. Alcohol up-regulates UDP-glucuronosyltransferase mRNA expression in rat liver and in primary rat hepatocyte culture. Life Sci 2000;66:575–84.

[11] Prough RA, Linder MW, Pinaire JA, Xiao G, Falkner KC. Hormonal regulation of hepatic enzymes involved in foreign compound metabolism. FASEB J 1996;10:1369–77.

[12] Li YQ, Prentice DA, Howard ML, Mashford ML, Desmond PV. Bilirubin and bile acids may modulate their own metabolism via regulating uridine diphosphate-glucuronosyltransferase expression in the rat. J Gastroenterol Hepatol 2001;15:865–70.

[13] Phelan D, Winter GM, Rogers WJ, Lam JC, Denison MS. Activation of the Ah receptor signal transduction pathway by bilirubin and biliverdin. Arch Biochem Biophys 1998;357:155–63.

[14] Xiao GH, Pinaire JA, Rodrigues AD, Prough RA. Regulation of the Ah gene battery via Ah receptor-dependent and independent processes in cultured adult rat hepatocytes. Drug Metab Dispos 1995;23:642–50.

[15] Bock KW, Eckle T, Ouzzine M, Fournel-Gigleux S. Coordinate induction by antioxidants of UDP-glucuronosyltransferase UGT1A6 and the apical conjugate export pump MRP2 (multidrug resistance protein 2) in Caco-2 cells. Biochem Pharmacol 2000;59:467–70.

[16] Lin JUN, Chiba M, Chen IVV, Nishime JA, deLuna FA, Yamazaki M, Lin YJ. Effect of dexamethasone on the intestinal first pass metabolism of indinavir in rats: evidence of cytochrome P-450 A and P-glycoprotein induction. Drug Metab Dispos 1999;27:1187–93.

[17] Keppler D, Arias IM. Introduction: transport across the hepatocyte canalicular membrane. FASEB J 1997;11:15–8.

[18] Courtois A, Payen L, Guillouzo A, Fardel O. Up-regulation of multidrug resistance-associated protein 2 (MRP2) expression in rat hepatocytes by dexamethasone. FEBS Lett 1999;459:381–5.
[19] Kubitz R, Wettstein M, Warskulat U, Haussinger D. Regulation of the multidrug resistance protein 2 in the rat liver by lipopolysaccharide and dexamethasone. Gastroenterology 1999;116:401–10.
[20] Kubitz R, Warskulat U, Schmitt M, Haussinger D. Dexamethasone- and osmolarity-dependent expression of the multidrug resistance protein 2 in cultured rat hepatocytes. Biochem J 1999;340:585–91.
[21] Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol 1976;13:2983.
[22] Lowry H, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.
[23] Bánhegyi G, Garzó T, Fulceri R, Benedetti A, Mandl J. Latency is the major determinant of UDP-glucuronosyltransferase activity in isolated hepatocytes. FEBS Lett 1993;328:149–52.
[24] Jemnitz K, Veres ZS, Monostory K, Vereczkey L. Glucuronidation of thyroxin in primary monolayer cultures of rat hepatocytes: in vitro induction of UDP-glucuronosyltranferases by methylcholanthrene, clofibrate and dexamethasone alone and in combination. Drug Metab Dispos 2000;28:34–7.
[25] Burchell B, Weatherhill P. 4-Nitrophenol UDPglucuronyltransferase. In: Jakoby WB, editor. Methods in enzymology, vol. 77. New York: Academic Press, 1981. p. 169–76.
[26] Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrilamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350–4.
[27] Büchler M, König J, Brom M, Kartenbeck J, Spring H, Horie T, Keppler D. cDNA cloning of the hepatocyte canalicular isoform of the multidrug resistance protein, cMrp, reveals a novel conjugate export pump deficient in hyperbilirubinemic mutant rats. J Biol Chem 1996;271:15091–8.