university of groningen

University Medical Center Groningen

University of Groningen

Regulation of metabolizing enzymes and transporters for drugs and bile salts in human and rat intestine and liver

Khan, Ansar Ali

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Khan, A. A. (2009). *Regulation of metabolizing enzymes and transporters for drugs and bile salts in human and rat intestine and liver: a study with precision-cut slices*. s.n.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 09-10-2024

Chapter 7

Regulation of Vitamin D Receptor (VDR) Expression in Rat and Human intestine and Liver – Consequences for bile acid detoxification

Ansar A. Khan

Bieuwke S. Dragt

Robert J. Porte

Geny M. M. Groothuis

Submitted

Abstract

The vitamin D receptor (VDR), activated by its ligands, 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) and LCA, regulates the expression of drug metabolizing enzymes and transporters in intestine and liver, but the regulation of VDR expression in intestine and liver is incompletely understood. We studied the regulation of VDR expression at the level of mRNA by ligands for VDR, farnesoid X receptor (FXR), glucocorticoid receptor (GR) and protein kinase C α (PKCα) in rat and human ileum and liver using precision-cut slices. Further, we investigated a possible interaction of chenodeoxycholic acid (CDCA) with LCA and $1,25(OH)_2D_3$ on the regulation of VDR and CYP3A isozymes in rat ileum and liver. The mRNA expression of VDR, CYP3A1 and CYP3A2 was evaluated by qRT-PCR. The expression of VDR mRNA was significantly induced by $1,25(OH)_2D_3$ in rat ileum and liver slices, and human ileum but not in human liver slices. CDCA, but not LCA and the synthetic FXR ligand, GW4064 induced VDR mRNA expression in rat ileum and liver slices. The PKCα activator, phorbol-12-myristate-13-acetate (PMA) induced the expression of VDR in the rat liver, and the induction of VDR by $1,25(OH)_2D_3$ and CDCA was inhibited by the PKC inhibitor, bisindolyl maleimide (Bis I). The GR.ligand, dexamethasone decreased the VDR expression in rat ileum and liver. These results show that the expression of VDR in the rat liver is likely to be regulated by GR and PKCα but not by FXR or VDR activation. The mechanism of induction in the rat and human ileum remains to be elucidated. The VDR mediated induction of CYP3A isozymes in the rat ileum by $1,25(OH)_2D_3$ and LCA was strongly reduced in the presence of CDCA despite the higher VDR expression. Thus, CDCA might potentiate the toxicity of LCA by inhibiting its metabolism and this might be one of the possible mechanisms of increased incidence of colon cancer in populations on high fat diet.

Keywords: Vitamin D receptor**,** cytochrome P450, induction, intestinal slices, liver slices, lithocholic acid

Introduction

The vitamin D receptor (VDR) is a member of the steroid-thyroid hormone nuclear receptor family, NR1I1 and it is expressed predominantly in organs that play an important role in calcium and phosphorous homeostasis such as intestine, kidney, bone and parathyroid glands. VDR is also expressed at a lower level in other tissues, such as liver, muscle and prostate glands (45). VDR regulates the expression of genes involved in calcium and phosphorous metabolism in intestine, kidney and bone (6, 11). Moreover, VDR exhibits a significant homology with PXR and CAR (26) and is reported to modulate xenobiotic metabolism and transport (1, 5, 18, 28, 41, 47). VDR is activated by 1α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) and the toxic bile acid, lithocholic acid (LCA), and its metabolite 3α-keto cholastanic acid (3-KCA), hence VDR is considered as an intestinal bile acid sensor (25). The ligand-activated VDR heterodimerizes with retinoic acid X receptor α (RXRα; NR2B1), and binds to the VDR response elements (VDRE) in the promoters of target genes (35, 39, 41, 48), which are characterized as direct-repeats separated by three nucleotide base pairs (DR3) and everted-repeats with two different direct-repeats separated by six-nucleotide base pairs (ER6) (11, 40).

The regulation of VDR expression is incompletely understood. The VDR ligand, 1,25$(OH)_2D_3$ is reported to up-regulate VDR mRNA expression in mouse fibroblasts, pig kidney cells (LLC-PK1) and human promyelocytic leukemic cells (HL-60) (3, 23, 29). Strom et al. (38) showed that the treatment of vitamin D-deficient Sprague-Dawley rats with 1,25$(OH)_2D_3$ induced VDR mRNA and protein expression in the duodenum within 24 h. Wiese et al (46) found no changes in the mRNA of VDR in mouse fibroblasts *in vitro* and *in vivo* upon treatment with 1,25$(OH)_2D_3$ in intestinal epithelial cells of vitamin D-deficient Sprague-Dawley rats but reported an increase in the half-life of VDR protein. Recently, Healy et al. (12) reported the induction of VDR mRNA in kidneys but not in the duodenum of vitamin-D deficient mice treated with 1,25$(OH)_2D_3$ in the presence of a supplementary calcium diet. Furthermore, glucocorticoids are reported to down regulate the VDR expression in mouse duodenum and up regulated in rat duodenum (13, 14). Protein kinase C α (PKCα) activators such as phorbol-12-myristate-13-acetate (PMA) were reported to up regulate VDR expression in rat osteosarcoma cells (37). However, these results are in contrast to the earlier reports showing a decrease in the expression of VDR in rat osteosarcoma cells by PMA (20, 44). VDR is also up-regulated by parathyroid hormone (PTH) through the activated protein kinase A (PKA) pathway in mouse osteoblast and osteosarcoma cell lines (19). In summary, this data suggests that VDR might be regulated at the level of mRNA and protein in rat, mouse and man by glucocorticoids, PKCα and 1,25$(OH)_2D_3$, but the regulation seems to be species and tissue specific (8).

To obtain more insight in the regulation of VDR mRNA in the human and rat liver and intestine, we performed a systematic study to investigate the direct effects of ligands for VDR (1,25$(OH)_2D_3$ and LCA), FXR (CDCA and GW4064), GR (dexamethasone

(DEX)) as well as those of PKCα activator, PMA on the VDR mRNA expression by using precision-cut slices of rat and human ileum and liver. This *ex-vivo* model was previously shown to be an adequate model to study the regulation of genes of interest by ligands for several NR mediated pathways in the liver (10, 16, 32) and the intestine (18, 27, 42). Moreover, *in vivo*, ligands for several NR's are present simultaneously in the intestine and the portal blood, for instance CDCA, LCA and $1,25(OH)_2D_3$, we investigated a possible interaction of CDCA with LCA and $1,25(OH)_2D_3$ on the regulation of VDR and CYP3A isozymes in rat ileum and liver. Subsequently, we compared the *in vitro* results with those obtained *in vivo* by intraperitoneal treatment (ip) of Wistar rats with $1,25(OH)_2D_3$

Materials and methods

Male Wistar (HsdCpb:WU) rats weighing about 230 - 250 g were purchased from Harlan (Horst, The Netherlands) and were allowed to acclimatize in a temperature and humidity controlled room on a 12-h light/dark cycle with food and tap water *ad libitum* for 7 days before experimentation. Pieces of human liver and ileum tissue were obtained as surgical waste from the University Medical Center Groningen with informed consent of the patients/donors. $1,25(OH)_2D_3$ in ethanol was purchased from BIOMOL Research Laboratories, Inc., Plymouth Meeting, PA. Chenodeoxycholic acid (CDCA) and lithocholic acid (LCA) was purchased from Calbiochem, San Diego, California, dexamethasone was from Genfarma bv, Maarssen. Ethanol, methanol, DMSO, polymethyl sulfonyl flouride (PMSF), and dithiothreitol (DTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO); Gentamicin sulfate and Williams medium E with glutamax-I and amphotericin B (Fungizone)-solution were obtained from Gibco (Paisley, UK). GW4064 was purchased from Tocris Bioscience (Bristol, UK). D-Glucose and HEPES were procured from ICN Biomedicals, Inc. (Eschwege, Germany). Low gelling temperature agarose, phorbol-12-myristate-13-acetate (PMA) were purchased from Sigma–Aldrich (St. Louis, MO). RNAeasy mini columns were obtained from Qiagen, Hilden, Germany. Random primers (500 µg/ml), $MgCl_2$ (25 mM), RT buffer (10x), PCR nucleotide mix (10 mM), AMV RT (22 U/µl) and RNasin (40 U/µl) were procured from Promega Corporation, Madison WI, USA. SYBR green and Taq Master Mixes were purchased from Applied Biosystems, Warrington, UK and Eurogentech, respectively. The bisindolyl maleimide I (Bis I) is a generous gift from Professor Dr. H. Meurs, Department of Molecular Pharmacology, University of Groningen. All primers were purchased from Sigma Genosys. All reagents and materials used were of the highest purity that was commercially available.

Experimental protocols. All experimental protocols involving animals were approved by the Animal Ethical Committee of the University of Groningen. Experimental protocols involving human tissue (liver and ileum) were approved by the Medical Ethical Committee of the University Medical Center, Groningen.

Preparation of rat and human intestinal and liver slices. The small intestine, colon and liver were excised from the rat under isoflurane/O_2 anaesthesia. The small intestine and colon were immediately placed into ice-cold Krebs-Henseleit buffer supplemented with 10 mM HEPES, 25 mM sodium bicarbonate and 25 mM D-glucose, pH 7.4 (KHB), saturated with carbogen (95%O_2/5%CO_2) and stored on ice until preparation of the slices. The rat liver was stored in ice-cold UW until slicing. Pieces of human liver tissue were obtained from patients undergoing partial hepactectomy for the removal of carcinoma (PH livers) or from redundant parts of donor livers remaining after split-liver transplantation (Tx livers) as described previously by Olinga et al. (33). Human ileum tissue was obtained as a part of the surgical waste after resection of the ileo-colonic part of the intestine in colon carcinoma patients. After surgical resection, the ileum tissue was immediately placed in ice-cold carbogenated KHB. Human liver and ileum donor characteristics are as reported earlier by Khan et. al. (18). Human liver and ileum slices were prepared within 30 to 60 min after resection. Rat and human intestinal and liver slices were prepared according to the earlier published methods (4, 18, 33, 43).

Induction Studies

Incubation of rat and human intestine slices. Precision-cut slices, prepared from rat intestine (jejunum, ileum and colon) and human ileum were incubated individually in 12-well sterile tissue culture plates (Grenier bio-one GmbH, Frickenhausen, Austria) containing 1.3 ml Williams medium E supplemented with D-Glucose (final concentration of 25 mM), gentamicin sulfate (50 µg/ml) amphotericin / fungizone, (250 µg/ml) and saturated with carbogen. The plates were placed in humidified plastic container kept at 37° C and continuously gassed with carbogen and shaken at 80 rpm. Intestinal slices were incubated with 1,25$(OH)_2D_3$ (5 - 100 nM), CDCA (50 µM), LCA (5 - 10 µM), DEX (1 - 50 µM), GW4064 (1 µM) and PMA (1.6 µM) added as a 100-times concentrated stock solution in ethanol (1,25$(OH)_2D_3$), methanol (CDCA and LCA) or DMSO (DEX, PMA and GW4064). Rat intestinal slices were incubated for 8 h or 12 h, since at 24 h, the expression of villin was found to be decreased. Human ileum slices were incubated for 8 h and 24 h; as villin expression was stable up to 24 h.

Rat ileum slices were also incubated with either 1,25$(OH)_2D_3$ (100 nM) or LCA (10 µM) in the absence or presence of CDCA (1, 30 or 50 µM). Control slices were incubated in Williams medium E (supplemented with D-Glucose and gentamicin sulfate) with 1% ethanol, methanol, DMSO and ethanol or methanol + DMSO without ligands.

From a single rat or human tissue sample, six (rat intestine) or three (human ileum) replicate slices were subjected to each experimental condition. After the incubation, these replicate slices were harvested, pooled and snap-frozen in liquid nitrogen to obtain sufficient total RNA for quantitative real time PCR (qRT-PCR) analysis. The samples were

stored at – 80° C until RNA isolation. These experiments were replicated in 3 to 5 rats and 3 to 5 human ileum donors.

Incubation of rat and human liver slices. Rat and human liver slices (8 mm diameter and 250 µm thick) were incubated individually in sterile six-well tissue culture plates (Grenier bio-one GmbH, Frickenhausen, Austria) containing 3.2 ml Williams medium E supplemented with D-glucose to a final concentration of 25 mM, gentamicin sulfate (50 µg/ml) and saturated with carbogen. The plates were placed in humidified plastic container kept at 37° C and continuously gassed with carbogen and shaken at 80 rpm. Rat liver slices were incubated with 1,25$(OH)_2D_3$ (100 nM), CDCA (50 µM and 100 µM), LCA (50 µM), DEX (50 µM) GW4064 (1 µM) and PMA (1.6 µM) added as a 100-fold concentrated stock solution in ethanol (for 1,25$(OH)_2D_3$), methanol (for CDCA and LCA) or DMSO (for DEX, PMA and GW4064). Human liver slices were incubated with 1,25$(OH)_2D_3$ (100 - 200 nM), CDCA (100 µM), LCA (50 µM) and DEX (50 µM) added as a 100-fold concentrated stock solution in ethanol (for 1,25$(OH)_2D_3$), methanol (for CDCA and LCA) or DMSO (for DEX).

Rat liver slices were incubated in presence of both 1,25$(OH)_2D_3$ (100 nM) and CDCA (50 µM) or PMA (1.6 µM); and with CDCA (50 µM) with PMA (1.6 µM). Furthermore, rat liver slices were incubated in presence of CDCA (50 µM) and 1,25$(OH)_2D_3$ (100 nM) in the presence of the PKC inhibitor, Bis I (3 µM) for 2 h, 4 h and 8 h.

Rat liver slices were incubated for 8 h and 24 h, respectively. Human liver slices were incubated for 24 h. Control rat and human liver slices were incubated in supplemented Williams medium E with 1% ethanol, methanol, DMSO and ethanol or methanol + DMSO without ligands. From a single rat/single human liver donor, three replicate slices that were subjected to identical incubation conditions were harvested, pooled and snap-frozen in liquid nitrogen to obtain sufficient total RNA for qRT-PCR analysis. Samples were stored at – 80° C until RNA isolation. These experiments were replicated in 3 to 5 rats and 4 to 5 human liver donors.

***In vivo* studies.** Wistar rats were divided into two groups with twelve animals each. In both groups six animals were treated with 1,25 $(OH)_2D_3$ and six served as controls. Treated animals received 1200 pmol/kg/day 1,25 $(OH)_2D_3$ in corn oil by intraperitoneal injection (ip) and corresponding controls received the same volume of corn oil. Group I animals were sacrificed 12 h after the first dose. Group II animals were treated once daily for four days and sacrificed 24 h after the last dose. The small intestine (jejunum and ileum), colon, livers and kidneys were collected in ice-cold phosphate buffered saline (PBS) containing the protease inhibitors PMSF (1 mM) and DTT (0.5 mM). The intestinal segments were flushed with ice-cold PBS with PMS and DTT, and divided into small pieces and snap-

frozen in liquid nitrogen. Pieces of liver and kidney were also snap-frozen in liquid nitrogen. Samples were stored at – 80° C until RNA isolation.

RNA isolation and qRT-PCR. Total RNA was isolated from rat and human intestine and liver samples using RNAeasy mini columns from Qiagen according to the manufacturer's instruction. The RNA concentration and quality were determined by measuring the absorbance at 260 nm, 230 nm and 280 nm using a Nanodrop, ND100 spectrophotometer (Wilmington, DE USA). The ratios of absorbance measured at 260 over 280 and 230 over 260 were found to be above 1.8 in all the samples. About 2 μg of total RNA in 50 μl was reverse-transcribed into template cDNA as reported earlier (18).

qRT-PCR for the rat and human genes of interest was performed either by SYBR Green or Taqman® analysis according to the availability of primer sets; villin and GAPDH were used as house-keeping genes for intestinal and liver samples, respectively. CYP3A1 and CYP3A2 mRNA was analyzed by the SYBR Green detection system and the primer sequences used for villin, GAPDH, CYP3A1 and CYP3A2 analysis were identical to those reported earlier (18, 24). Rat VDR mRNA was analysed by SYBR Green and Taqman® analysis. VDR SYBR green primers are forward (5'-3') TGAAGGCTGCAAAGGTTTCT, and reverse primer (5'-3') TAGCTTGGGCCTCAGACTGT and VDR Taqman primers and probes are forward primer (5'-3') TGACCCCACCTACGCTGACT, reverse primer (5'-3') CCTTGGAGAATAGCTCCCTGTACT and probe (5'-3') FAM-ACTTCCGGCCTCCAGTTCGTATGGAC-TAMRA; β-actin expression was analyzed by Taqman using assay by design primer sets obtained from Applied Biosystems, Warrington, UK. Human villin and GAPDH SYBR Green primer sequences were similar to those reported earlier by us (18). The human VDR primer sequence was: forward primer (5'-3') GGAAGTGCAGAGGAAGCGGAGATG, reverse primer (5'-3') AGTGCTGGGACAGCTCTAGGGTCAC. All primer sets were analyzed using BLASTn to ensure primer specificity for the gene of interest **(http://www.ncbi.nlm.nih.gov/BLAST/)**. For the SYBR Green, ~ 50 ng of cDNA was used in a total reaction mixture of 20 μl. For the Taqman® analysis ~ 250 ng of cDNA was used in a total reaction mixture of 10 μl. The PCR conditions were similar to those described in an earlier report (18). All samples were analyzed in duplicates in 384 well plates using ABI7900HT from Applied Biosystems. Appropriate controls were analyzed to detect potential primer dimer formation and DNA contamination in the samples. Dissociation curves showed a single homogenous product for all the primer sets, except for the rat and human VDR primer set which showed a minor second product. The comparative threshold cycle (C_T) method was used for relative quantification, where C_T is inversely related to the abundance of mRNA transcripts in the initial sample. The mean C_T of the duplicate measurements was used to calculate the difference between the C_T for the gene of interest and that of the reference gene (villin or β-actin for intestine and GAPDH for liver) (ΔC_T), which was compared to the corresponding ΔC_T of the solvent control ($\Delta\Delta C_T$). Data are expressed as fold induction or repression of the gene of interest according

to the formula $2^{-(\Delta\Delta CT)}$.

Statistical analysis. All values were expressed as the mean ± S.E.M. All data were analyzed by paired student's *t*-test using SPSS Version 16 for significant differences between/among the means of treatment. Statistical analysis was performed on fold induction as well as on $\Delta\Delta C_T$ with similar results. The *P* value < 0.05 was considered as significant.

Results

Regulation of VDR expression in rat intestine and liver. The VDR mRNA expression was found to be constant during control incubations of rat jejunum slices and slightly induced in ileum and colon slices (Fig. 1A) but was not influenced by the solvents during 8 h and 12 h of incubation (results not shown). Incubation of rat jejunum, ileum and colon slices with $1,25(OH)_2D_3$ for 12 h did not affect the VDR mRNA expression (Fig. 1B). Incubation of rat ileum slices with $1,25(OH)_2D_3$ for 8 h significantly induced VDR expression (1.7-fold; $P < 0.05$) (Fig. 2). DEX decreased the VDR mRNA expression in the ileum slices (0.6-fold; $P < 0.05$) but the small decrease in jejunum and colon slices was not significant (Fig. 1B). CDCA induced the VDR expression in the ileum slices during 8 h and 12 h incubation (2-2.5-fold; $P < 0.05$), (Figs. 1B and 2), but LCA decreased the VDR expression in the colon slices (0.5-fold; $P < 0.05$) (Fig. 1B). The synthetic FXR ligand, GW4064 and the PKCα agonist, PMA did not affect the VDR expression in the rat ileum slices (Fig. 2). Co-incubation of rat ileum slices with $1,25(OH)_2D_3$ and CDCA induced the VDR expression additively (3-fold; $P < 0.05$) (Fig. 2).

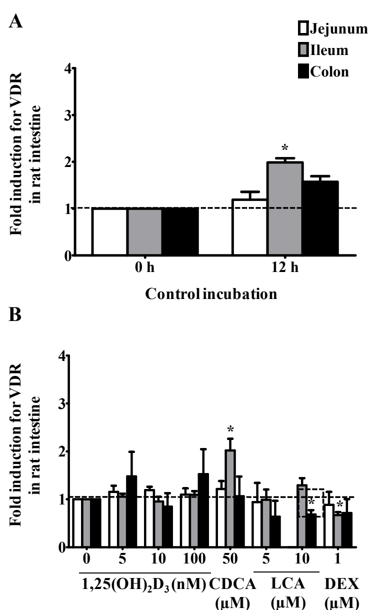

*Figure 1. Slices from rat jejunum, ileum and colon were exposed to control incubation (A), $1,25(OH)_2D_3$ (5-100 nM), LCA (5-10 µM), CDCA (50 µM) and DEX (1 µM) (B), for 12 h, after which total RNA was isolated and mRNA expression of VDR was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with β-actin, and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean ± S.E.M. of 2-3 rats; in each experiment 6 intestinal slices were incubated per condition. Significant differences towards the control incubations are indicated with * P < 0.05.*

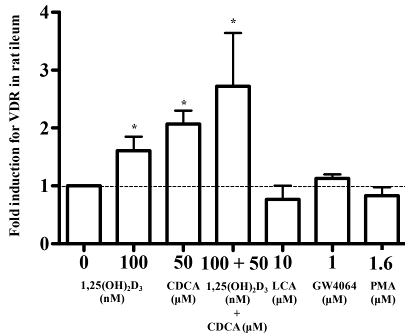

*Figure 2. Rat ileum slices were exposed to 1,25(OH)$_2$D$_3$ (100 nM), CDCA (50 µM), LCA (10 µM), GW4064 (1 µM) and PMA (1.6 µM) and co incubation with 1,25(OH)$_2$D$_3$ (100 nM) and CDCA (1-50 µM) (C) for 8 h, after which total RNA was isolated and mRNA expression of VDR was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with β-actin, and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean ± S.E.M. of 2-5 rats; in each experiment 6 ileum slices were incubated per condition. Significant differences towards the control incubations are indicated with * P < 0.05.*

In the rat liver slices, the VDR mRNA expression was significantly increased after 8 h and 24 h control incubation (Fig. 3A), but was not influenced by the solvents (results not shown). 1,25(OH)$_2$D$_3$, CDCA and PMA significantly induced VDR mRNA expression relative to their respective solvent incubated controls (Figs. 3B and C), whereas LCA and GW4064 had no effect. DEX significantly decreased the VDR mRNA expression in the liver slices (0.8-fold; $P < 0.05$) (Fig. 3B). Co-incubation of rat liver slices with 1,25(OH)$_2$D$_3$ and CDCA showed an additive induction of the VDR expression by 5.4-fold ($P < 0.05$) compared to incubation with the ligands individually (3.5-fold and 2.8-fold, respectively) (Fig. 3B). The PKCα inhibitor, Bis I abolished the induction of VDR mRNA by 1,25(OH)$_2$D$_3$, CDCA and PMA in rat liver slices after 2 h (Fig. 4) but not after 4 h and 8 h incubation (data not shown).

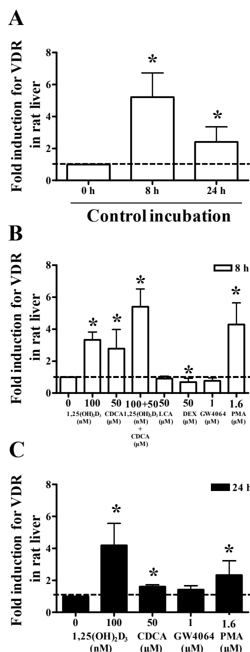

*Figure 3. Rat liver slices were exposed to control incubation; 0 h, 8 h and 24 h (A), 1,25(OH)$_2$D$_3$ (100 nM), CDCA (50 µM), LCA (50 µM), DEX (50 µM), GW4064 (1 µM) and PMA (1.6 µM) and 1,25(OH)$_2$D$_3$ (100 nM) + CDCA (50 µM) for 8 h (B), and 1,25(OH)$_2$D$_3$ (100 nM), CDCA (50 µM), GW4064 (1 µM) and PMA (1.6 µM) for 24 h (C), after which total RNA was isolated and mRNA expression of VDR was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with β-actin, and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean ± S.E.M. of 2-4 rats; in each experiment 3 liver slices were incubated per condition. Significant differences towards the control incubations are indicated with * P < 0.05.*

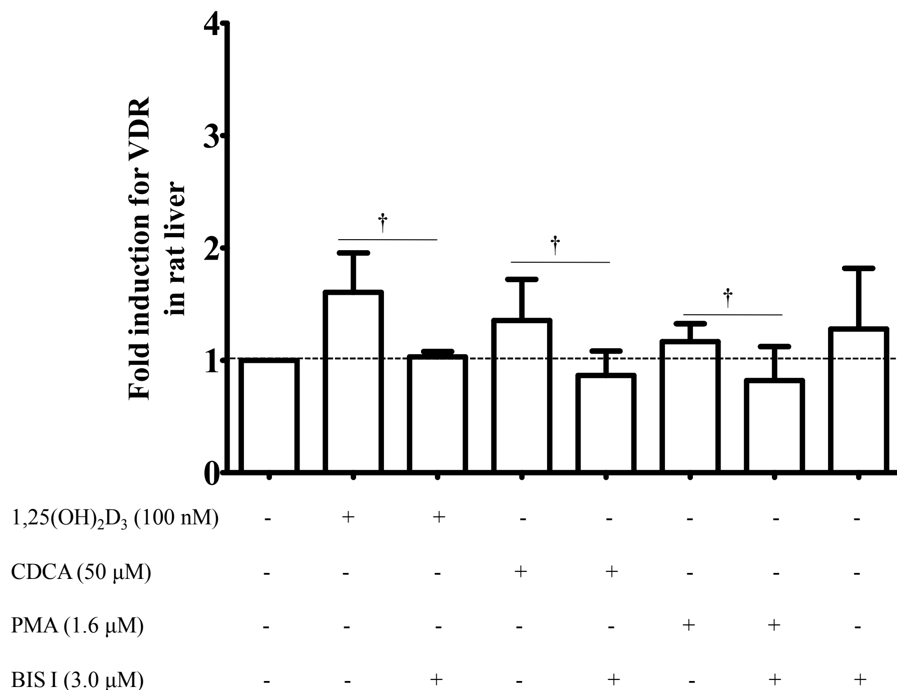

*Figure 4. Rat liver slices were exposed to control incubation, 2 h (A) and incubated with $1,25(OH)_2D_3$ (100 nM), CDCA (50 µM) and PMA (1.6 µM) in the presence or absence of Bis I (3 µM) for 2 h, after which total RNA was isolated and mRNA expression of VDR was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with β-actin, and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean ± S.E.M. of 3 rats; in each experiment 3 liver slices were incubated per condition. Significant differences towards the control incubations are indicated with * $P < 0.05$.*

Regulation of VDR in rat intestine, liver and kidneys – in vivo. The administration of $1,25(OH)_2D_3$ to Wistar rats (1200 pmol/kg/day for 4 days by ip injection) did not affect the VDR mRNA expression in jejunum, ileum, colon and liver. The high variation in the liver data at 12 h was due to the finding that the liver of 1 out of 6 rats showed an induction, whereas no change was observed in the livers of other five rats. In contrast, the VDR mRNA expression was significantly induced in the kidneys after 4 days of treatment (2.5-fold induction; $P < 0.05$).

Regulation of CYP3A isozymes by the VDR ligands $1,25(OH)_2D_3$ and LCA in the presence of CDCA in the rat ileum. The VDR ligands, $1,25(OH)_2D_3$ and LCA, and the FXR ligand, CDCA significantly induced the expression of CYP3A1 (Fig. 6), and $1,25(OH)_2D_3$ and LCA but not CDCA induced CYP3A2 expression in rat ileum after 8 h of incubation (Fig. 7). These results are consistent with our earlier results obtained after 12 h of incubation (18). CDCA strongly decreased the $1,25(OH)_2D_3$ and LCA mediated

induction of CYP3A1 (Fig. 6) and the $1,25(OH)_2D_3$ mediated CYP3A2 induction in a dose-dependent manner (Fig. 7) in rat ileum slices. CDCA did affect the LCA mediated CYP3A2 induction.

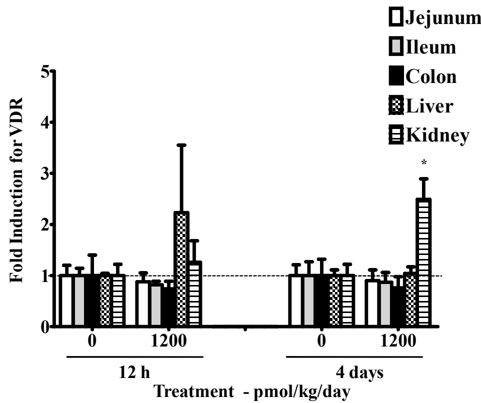

*Figure 5. Wistar rat were treated with with $1,25(OH)_2D_3$ (1200 pmol/kg/day) for 12 h and 4 days by intraperitoneal injection. After 12 h or 24 h of the last dose administered, rats were sacrificed and total RNA was isolated from jejunum, ileum, colon, liver and kidneys and mRNA expression of VDR was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with β-actin, and compared with the control rats treated with vehicle for the same duration of treatment. Results showed mean ± S.E.M. of 6 rats.. Significant differences towards the vehicle treated rats were indicated with * $P < 0.05$.*

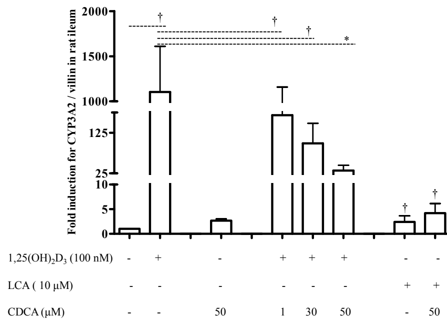

*Figure 6. Rat ileum slices were exposed to $1,25(OH)_2D_3$ (100 nM), CDCA (50 µM) and LCA (10 µM), co incubated with $1,25(OH)_2D_3$ (100 nM) and CDCA (1-50 µM) and co incubated with LCA (10 µM) and CDCA (50 µM) for 8 h, after which total RNA was isolated and mRNA expression of CYP3A1 was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with villin, and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean ± S.E.M. of 3-4 rats; in each experiment 3-6 ileum slices were incubated per condition. Significant differences towards the control incubations are indicated with * $P < 0.05$. "†" denotes in all the experiments but failed to reach statistical significance.*

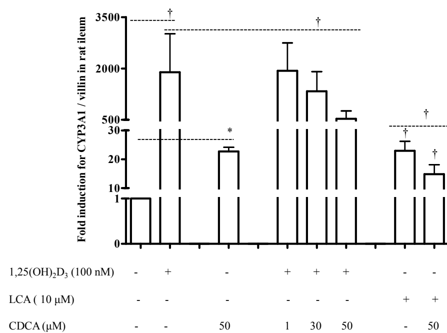

*Figure 7. Rat ileum slices were exposed to $1,25(OH)_2D_3$ (100 nM), CDCA (50 µM) and LCA (10 µM), co incubated with $1,25(OH)_2D_3$ (100 nM) and CDCA (1-50 µM) and co incubated with LCA (10 µM) and CDCA (50 µM) for 8 h, after which total RNA was isolated and mRNA expression of CYP3A2 was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with villin, and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean ± S.E.M. of 3-4 rats; in each experiment 3-6 ileum slices were incubated per condition. Significant differences towards the control incubations are indicated with * $P < 0.05$. "†" denotes decrease in all the experiments but failed to reach statistical significance.*

Regulation of VDR expression in human ileum and liver. The VDR mRNA was clearly detected in the human ileum. In the human ileum slices, the VDR mRNA expression was found to be slightly decreased during 8 h and 24 h control incubation, but was not influenced by the organic solvents (data not shown). Incubation of human ileum slices with 1,25(OH)$_2$D$_3$ induced the VDR expression after 24 h (Fig. 8A). LCA, CDCA and DEX did not affect the VDR expression (Fig. 8A) but PMA induced the VDR expression in the human ileum slices (Fig. 8A). In the human livers, the VDR expression showed high variability and was detectable at a C_T of 33 to 38 whereas in one liver VDR expression could not be detected up to 40 cycles. In the human liver slices, the VDR expression was slightly increased during 24 h incubation but was not influenced by the organic solvents (data not shown). 1,25(OH)$_2$D$_3$ did not affect the VDR expression, whereas CDCA, LCA and DEX decreased the VDR mRNA expression in all the human livers (Fig. 8B).

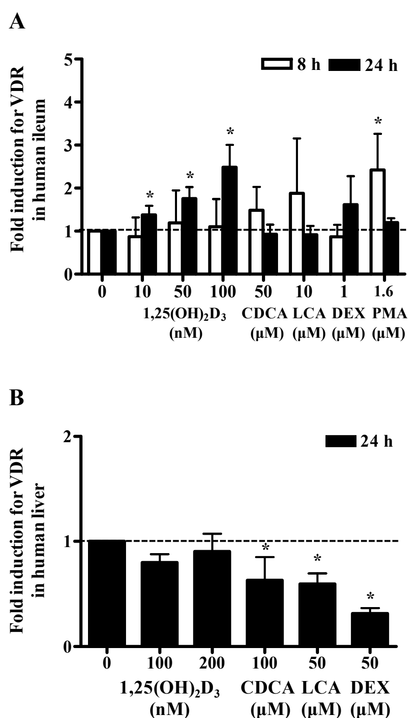

*Figure 8. Human ileum slices were exposed to 1,25(OH)$_2$D$_3$ (10-100 nM), CDCA (50 µM), LCA (10 µM), DEX (1 µM) and PMA (1.6 µM) for 8 h and 24 h (A), and human liver slices were exposed to 1,25(OH)$_2$D$_3$ (100-200 nM), CDCA (100 µM), LCA (50 µM) and DEX (50 µM) for 24 h (B), after which total RNA was isolated and mRNA expression of VDR was evaluated by qRT-PCR. Results were expressed as fold-induction after normalizing with villin (for ileum) or GAPDH (for liver), and compared with the control incubated slices for the same length of time, which was set to unity. Results showed mean ± S.E.M. of 3-4 human ileum and liver donors; in each experiment 3 ileum and liver slices were incubated per condition. Significant differences towards the control incubations are indicated with * $P < 0.05$.*

Discussion

VDR mRNA is expressed along the length of the rat intestine as well as in the liver. In the rat intestine, VDR protein was found to be localized by immunohistochemistry in the epithelial cells, whereas in the rat liver, VDR was localized in the non-parenchymal cells with predominant localization in the cholangiocytes. In the human liver VDR is expressed in both the cholangiocytes and to a lesser extent in the hepatocytes. In the human ileum, the VDR expression is localized in the epithelial cell (Chapter 4).

We report here for the first time the induction of VDR mRNA in the rat and human ileum by 1,25$(OH)_2D_3$. Incubation of rat ileum slices with 1,25$(OH)_2D_3$ for 8 h significantly induced the VDR expression by 1.7-fold; $P < 0.05$ (Fig. 2), but this effect appeared to be transient, since at 12 h, induction of VDR was not found (Fig. 1B). However, *in vivo* VDR induction was not found after 12 h and 4 days of treatment in any of the regions of the intestine (Fig. 5). Recently, Chow et al. found an induction of VDR in the jejunum but not in the ileum of Sprague-Dawley rats treated with a high dose of 1,25$(OH)_2D_3$ (2560 pmol/kg/day) for four days (2). In the human ileum slices, similar to the rat ileum, 1,25$(OH)_2D_3$ induced the VDR expression (Fig. 8A). To the best of our knowledge this is the first data on VDR induction by 1,25$(OH)_2D_3$ in man. Furthermore, 1,25$(OH)_2D_3$ induced the VDR expression in rat liver slices (Fig. 3B). However, treatment of Wistar rats with 1,25$(OH)_2D_3$ (1200 pmol/kg/day) either for 12 h or 4 days did not induce the VDR expression in the rat liver (Fig. 5) but Chow et al. found induction of VDR in the livers of Sprague-Dawley rats at high dose of 1,25$(OH)_2D_3$ (2560 pmol/kg/day) (un published observation). The induction of VDR expression observed in the kidneys (Fig 5) was also found by Wiese et al. (46), Healy (2005) and Chow et al (2) and showed that the dose was high enough to give a response in the kidney.

To investigate whether the concomitant increase in bile salt concentration in the portal blood due the increased absorption of bile salts in the rat ileum by the 1,25$(OH)_2D_3$ mediated induction of ASBT (2) could explain the absence of an effect *in vivo* in the liver, we co-incubated liver slices with 1,25$(OH)_2D_3$ and CDCA. We found an additive effect on the VDR induction *in vitro* (Fig. 3B). Surprisingly, LCA, a high affinity ligand for VDR, and a low affinity ligand for PXR and FXR did not induce VDR expression in rat ileum and liver slices (Figs. 1B, 2 and 3B). In contrast, CDCA, which is a high affinity ligand for FXR and a low affinity ligand for VDR, induced the VDR expression in the rat ileum and liver slices (Figs. 1B, 2 and 3B). To the best of our knowledge, this effect of CDCA was not reported before. The lack of induction of VDR by LCA, and the finding that the CDCA, which exhibits moderate affinity to VDR compared to LCA induced VDR in ileum and liver slices (Figs. 2 and 3B). These results suggest that CDCA and 1,25$(OH)_2D_3$ mediate the VDR expression by a VDR-independent pathway. This is further supported by the finding that both 1,25$(OH)_2D_3$ and LCA showed an induction of the expression of the well known VDR target genes, CYP3A1 and CYP3A2 (Figs. 6 and 7), which is consistent with our earlier findings (18).

Species differences were observed for the regulation of VDR expression by LCA and CDCA. In contrast to rat ileum and liver, where CDCA increased the VDR expression, CDCA did not affect the VDR expression in human ileum slices (Fig. 8A) and decreased VDR expression in human liver slices (Fig. 8B). LCA did not affect the VDR expression in the human ileum, similar to the rat ileum, but decreased the VDR expression in the human liver, in contrast to the lack of effect in the rat liver. These observations emphasize the species and organ specific regulation of VDR. This cannot be explained by a lack of uptake

of the ligands into the cells of the ileum or liver as we have previously found up regulation of SHP expression by CDCA, and up regulation of OSTα and OSTβ by LCA and CDCA in human ileum and human liver slices (17).

To further gain insight in the nuclear receptor pathways involved in the $1,25(OH)_2D_3$ and CDCA mediated VDR induction in the rat ileum and liver slices, we incubated the rat ileum and liver slices with the synthetic FXR ligand, GW4064 and the PKCα ligand, PMA, as CDCA is known to interact with FXR, but also activates PKCα. Furthermore, PKCα is reported to up regulate VDR expression (20, 22, 34, 37). The FXR ligand, GW4064 did not affect the VDR expression in rat ileum and liver slices (Figs. 2, 3B and 3C), but induced the short heterodimer protein (SHP) (A.A. Khan et al., un published observation), as expected for FXR ligands (9), suggesting that the FXR pathway is intact in the slices and that the FXR is not involved in the regulation of VDR. In contrast, PMA induced the VDR expression in the rat liver slices, confirming the role of PKCα in the regulation of VDR expression in rat liver slices. Furthermore, the induction of VDR by CDCA, PMA and $1,25(OH)_2D_3$ was inhibited by the PKCα inhibitor, Bis I during 2 h of incubation (Fig. 4), which indicates that the effect of CDCA and $1,25(OH)_2D_3$ in the liver might be mediated via the PKCα pathway. Bis I mediated inhibition of VDR induction by CDCA and $1,25(OH)_2D_3$ in rat liver slices was not observed for 4 h and 8 h incubation. This might be probably related to the rapid metabolism of Bis I. Also in the human ileum, PKCα seems to be involved in the regulation of VDR expression (Fig. 8A). The lack of effect of PMA on the expression of VDR in the rat ileum is difficult to explain with the current data, but the PMA effect was tested at one incubation time point only. Further studies are needed to confirm the role of PKCα in the human liver and rat ileum. The GR ligand DEX decreased the VDR expression in rat ileum, rat liver and human liver slices (Figs. 1B, 3B and 8B), but not in human ileum slices (Fig. 8A). Our results show for the first time that the expression of VDR in rat ileum and liver and human liver is decreased in the presence of glucocorticoids. A repression of VDR by glucocorticoids was reported before for the mouse intestine (13), but not for the liver, and is in contrast to the earlier reports in rat intestine in vivo (14)

Further, we investigated the effect of the CDCA mediated induction of VDR in rat ileum on the regulation of CYP3A isozymes: CYP3A1 and CYP3A2, since these enzymes are reported to be induced by VDR ligands, $1,25(OH)_2D_3$ (18) and LCA (Chapter 3) in rat ileum slices. Hence, a synergistic increase in the induction of CYP3A1 and CYP3A2 was expected for $1,25(OH)_2D_3$ and LCA in rat ileum slices in the presence of CDCA due to the induction of VDR. Such an additive effect was reported earlier for $1,25(OH)_2D_3$ mediated induction of CYP24A1 in rat osteosarcoma cells after pretreatment with PTH for 4 h, (19). In contrast to the expected increase in CYP3A1 and CYP3A2 induction, CDCA strongly decreased the $1,25(OH)_2D_3$ mediated induction of CYP3A1 and CYP3A2 in a dose dependent manner (Figs. 6 and 7) and also decreased the LCA mediated induction of CYP3A1 (Figs. 6 and 7) but not that of CYP3A2 (Fig. 7). The CDCA mediated repression

of CYP3A induction by VDR ligands in rat ileum suggest that the toxicity of LCA might be potentiated in the presence of CDCA by inhibiting its metabolism, which is predominantly mediated by feed forward induction of CYP3A1 and CYP3A2 in rat intestine and CYP3A4 in human ileum by VDR (18) and (Chapter 3). Whether this interaction also occurs in human ileum is currently under investigation. This might be of importance in people consuming high fat diet, which is associated with an increase in the faecal excretion of bile acids (36), including LCA and CDCA. LCA is implicated as a carcinogenic agent in the intestine and as a cholestatic agent in the liver of rats and man (7, 15, 21, 30), because it forms DNA adducts and DNA strand breaks and inhibits the DNA repair enzyme, DNA polymerase β (31). Thus, CDCA might potentiate the LCA mediated colon carcinogenesis by repressing the VDR-liganded induction of CYP3A isozymes by LCA. This might be one of the possible mechanisms of increased incidence of colon cancer in populations consuming high fat diet.

In conclusion, we have studied the regulation of VDR mRNA expression in rat and human ileum and liver and found that $1,25(OH)_2D_3$, DEX, CDCA and LCA are involved in its regulation. Moreover, we found prominent species and organ differences in the regulation of VDR by bile acids and $1,25(OH)_2D_3$. The regulation of VDR by $1,25(OH)_2D_3$ and CDCA is not likely to be mediated by VDR or FXR activation but we found indications that in rat liver and human ileum, PKCα seems to be involved, which could not be confirmed for the rat ileum and human liver. In contrast to what was observed in the rat liver, CDCA and LCA decreased the VDR expression in human livers. Further, glucocorticoids decreased the VDR expression in rat and human liver and also in rat ileum but not in human ileum, which indicates a cross talk between VDR and GR. In addition, we identified a possible novel mechanism of enhancement of LCA mediated toxicity in ileum by potential inhibition of VDR mediated induction of CYP3A isozymes by the simultaneous presence of CDCA and LCA in the rat ileum. Further studies are in progress to investigate whether a similar mechanism plays a role in the regulation of CYP3A4 in human ileum.

Acknowledgments

The authors thank Professor Dr. H. Meurs (Dept. of Molecular pharmacology, University of Groningen .) for the generous gift of the PKCα inhibitor, bisindolyl maleimide I (BIS I), Dr. Vincent B. Nieuwenhuijs (University Medical Center, Groningen) for providing the human ileum tissue.

References

1.**Chen X, Chen F, Liu S, Glaeser H, Dawson PA, Hofmann AF, Kim RB, Shneider BL, and Pang KS.** Transactivation of rat apical sodium-dependent bile acid transporter and increased bile acid transport by 1alpha,25-dihydroxyvitamin D3 via the vitamin D receptor. *Mol Pharmacol* 69: 1913-1923, 2006.

2.**Chow ECY, Maeng HJ, Liu S, Khan AA, Groothuis GMM, and Pang KS.** 1a,25-Dihydroxyvitam D_3 Triggered Vitamin D Receptor and Farnesoid X Receptor-like Effects in Rat Intestine and Liver *In Vivo*. *Biopharmaceutics and Drug Disposition*, 2009.
3.**Costa EM, Hirst MA, and Feldman D.** Regulation of 1,25-dihydroxyvitamin D3 receptors by vitamin D analogs in cultured mammalian cells. *Endocrinology* 117: 2203-2210, 1985.
4.**de Kanter R, Monshouwer M, Meijer DK, and Groothuis GM.** Precision-cut organ slices as a tool to study toxicity and metabolism of xenobiotics with special reference to non-hepatic tissues. *Curr Drug Metab* 3: 39-59, 2002.
5.**Echchgadda I, Song CS, Roy AK, and Chatterjee B.** Dehydroepiandrosterone sulfotransferase is a target for transcriptional induction by the vitamin D receptor. *Mol Pharmacol* 65: 720-729, 2004.
6.**Evans RM.** The steroid and thyroid hormone receptor superfamily. *Science* 240: 889-895, 1988.
7.**Fisher MM, Magnusson R, and Miyai K.** Bile acid metabolism in mammals. I. Bile acid-induced intrahepatic cholestasis. *Lab Invest* 25: 88-91, 1971.
8.**Goff JP, Reinhardt TA, Beckman MJ, and Horst RL.** Contrasting effects of exogenous 1,25-dihydroxyvitamin D [1,25-(OH)2D] versus endogenous 1,25-(OH)2D, induced by dietary calcium restriction, on vitamin D receptors. *Endocrinology* 126: 1031-1035, 1990.
9.**Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, Maloney PR, Willson TM, and Kliewer SA.** A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 6: 517-526, 2000.
10.**Graaf IA, Groothuis GM, and Olinga P.** Precision-cut tissue slices as a tool to predict metabolism of novel drugs. *Expert Opin Drug Metab Toxicol* 3: 879-898, 2007.
11.**Haussler MR, Mangelsdorf DJ, Komm BS, Terpening CM, Yamaoka K, Allegretto EA, Baker AR, Shine J, McDonnell DP, Hughes M, and et al.** Molecular biology of the vitamin D hormone. *Recent Prog Horm Res* 44: 263-305, 1988.
12.**Healy KD, Frahm MA, and DeLuca HF.** 1,25-Dihydroxyvitamin D3 up-regulates the renal vitamin D receptor through indirect gene activation and receptor stabilization. *Arch Biochem Biophys* 433: 466-473, 2005.
13.**Hirst M and Feldman D.** Glucocorticoid regulation of 1,25(OH)2vitamin D3 receptors: divergent effects on mouse and rat intestine. *Endocrinology* 111: 1400-1402, 1982.
14.**Hirst M and Feldman D.** Glucocorticoids down-regulate the number of 1, 25-dihydroxyvitamin D3 receptors in mouse intestine. *Biochem Biophys Res Commun* 105: 1590-1596, 1982.
15.**Javitt NB.** Cholestasis in rats induced by taurolithocholate. *Nature* 210: 1262-1263, 1966.
16.**Jung D, Elferink MG, Stellaard F, and Groothuis GM.** Analysis of bile acid-induced regulation of FXR target genes in human liver slices. *Liver Int* 27: 137-144, 2007.
17.**Khan AA, Chow ECY, Porte RJ, Pang KS, and Groothuis GMM.** Expression and regulation of the bile acid transporter, OSTα-OSTβ in rat and human intestine and liver *Biopharmaceutics and drug disposition* 30: 241-258, 2009.
18.**Khan AA, Chow ECY, Van Loenen-Weemaes A, Porte RJ, Pang KS, and Groothuis GMM.** Comparison of effects of VDR versus PXR, FXR and GR ligands on the regulation of CYP3A isozymes in rat and human intestine and liver. *European Journal of Pharmaceutical Sciences* 37: 115-125, 2009.
19.**Krishnan AV, Cramer SD, Bringhurst FR, and Feldman D.** Regulation of 1,25-dihydroxyvitamin D3 receptors by parathyroid hormone in osteoblastic cells: role of second messenger pathways. *Endocrinology* 136: 705-712, 1995.
20.**Krishnan AV and Feldman D.** Activation of protein kinase-C inhibits vitamin D receptor gene expression. *Mol Endocrinol* 5: 605-612, 1991.
21.**Kulkarni MS, Cox BA, and Yielding KL.** Requirements for induction of DNA strand breaks by lithocholic acid. *Cancer Res* 42: 2792-2795, 1982.
22.**Le M, Krilov L, Meng J, Chapin-Kennedy K, Ceryak S, and Bouscarel B.** Bile acids stimulate PKCalpha autophosphorylation and activation: role in the attenuation of prostaglandin E1-induced cAMP production in human dermal fibroblasts. *Am J Physiol Gastrointest Liver Physiol* 291: G275-287, 2006.
23.**Lee Y, Inaba M, DeLuca HF, and Mellon WS.** Immunological identification of 1,25-dihydroxyvitamin D3 receptors in human promyelocytic leukemic cells (HL-60) during homologous regulation. *J Biol Chem* 264: 13701-13705, 1989.
24.**Mahnke A, Strotkamp D, Roos PH, Hanstein WG, Chabot GG, and Nef P.** Expression and inducibility of cytochrome P450 3A9 (CYP3A9) and other members of the CYP3A subfamily in rat liver. *Arch Biochem Biophys* 337: 62-68, 1997.
25.**Makishima M, Lu TT, Xie W, Whitfield GK, Domoto H, Evans RM, Haussler MR, and Mangelsdorf DJ.** Vitamin D receptor as an intestinal bile acid sensor. *Science* 296: 1313-1316, 2002.
26.**Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, and Evans RM.** The nuclear receptor superfamily: the second decade. *Cell* 83: 835-839, 1995.

27.**Martignoni M, Groothuis G, and de Kanter R.** Comparison of mouse and rat cytochrome P450-mediated metabolism in liver and intestine. *Drug Metab Dispos* 34: 1047-1054, 2006.
28.**McCarthy TC, Li X, and Sinal CJ.** Vitamin D receptor-dependent regulation of colon multidrug resistance-associated protein 3 gene expression by bile acids. *J Biol Chem* 280: 23232-23242, 2005.
29.**McDonnell DP, Mangelsdorf DJ, Pike JW, Haussler MR, and O'Malley BW.** Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. *Science* 235: 1214-1217, 1987.
30.**Narisawa T, Magadia NE, Weisburger JH, and Wynder EL.** Promoting effect of bile acids on colon carcinogenesis after intrarectal instillation of N-methyl-N'-nitro-N-nitrosoguanidine in rats. *J Natl Cancer Inst* 53: 1093-1097, 1974.
31.**Ogawa A, Murate T, Suzuki M, Nimura Y, and Yoshida S.** Lithocholic acid, a putative tumor promoter, inhibits mammalian DNA polymerase beta. *Jpn J Cancer Res* 89: 1154-1159, 1998.
32.**Olinga P, Elferink MG, Draaisma AL, Merema MT, Castell JV, Perez G, and Groothuis GM.** Coordinated induction of drug transporters and phase I and II metabolism in human liver slices. *Eur J Pharm Sci* 33: 380-389, 2008.
33.**Olinga P, Merema MT, Hof IH, De Jager MH, De Jong KP, Slooff MJ, Meijer DK, and Groothuis GM.** Effect of cold and warm ischaemia on drug metabolism in isolated hepatocytes and slices from human and monkey liver. *Xenobiotica* 28: 349-360, 1998.
34.**Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, and Lehmann JM.** Bile acids: natural ligands for an orphan nuclear receptor. *Science* 284: 1365-1368, 1999.
35.**Prufer K, Racz A, Lin GC, and Barsony J.** Dimerization with retinoid X receptors promotes nuclear localization and subnuclear targeting of vitamin D receptors. *J Biol Chem* 275: 41114-41123, 2000.
36.**Reddy BS.** Diet and excretion of bile acids. *Cancer Res* 41: 3766-3768, 1981.
37.**Reinhardt TA and Horst RL.** Phorbol 12-myristate 13-acetate and 1,25-dihydroxyvitamin D3 regulate 1,25-dihydroxyvitamin D3 receptors synergistically in rat osteosarcoma cells. *Mol Cell Endocrinol* 101: 159-165, 1994.
38.**Strom M, Sandgren ME, Brown TA, and DeLuca HF.** 1,25-Dihydroxyvitamin D3 up-regulates the 1,25-dihydroxyvitamin D3 receptor in vivo. *Proc Natl Acad Sci U S A* 86: 9770-9773, 1989.
39.**Thompson PD, Hsieh JC, Whitfield GK, Haussler CA, Jurutka PW, Galligan MA, Tillman JB, Spindler SR, and Haussler MR.** Vitamin D receptor displays DNA binding and transactivation as a heterodimer with the retinoid X receptor, but not with the thyroid hormone receptor. *J Cell Biochem* 75: 462-480, 1999.
40.**Thompson PD, Jurutka PW, Whitfield GK, Myskowski SM, Eichhorst KR, Dominguez CE, Haussler CA, and Haussler MR.** Liganded VDR induces CYP3A4 in small intestinal and colon cancer cells via DR3 and ER6 vitamin D responsive elements. *Biochem Biophys Res Commun* 299: 730-738, 2002.
41.**Thummel KE, Brimer C, Yasuda K, Thottassery J, Senn T, Lin Y, Ishizuka H, Kharasch E, Schuetz J, and Schuetz E.** Transcriptional control of intestinal cytochrome P-4503A by 1alpha,25-dihydroxy vitamin D3. *Mol Pharmacol* 60: 1399-1406, 2001.
42.**van de Kerkhof EG, de Graaf IA, de Jager MH, Meijer DK, and Groothuis GM.** Characterization of rat small intestinal and colon precision-cut slices as an in vitro system for drug metabolism and induction studies. *Drug Metab Dispos* 33: 1613-1620, 2005.
43.**van de Kerkhof EG, Ungell AL, Sjoberg AK, de Jager MH, Hilgendorf C, de Graaf IA, and Groothuis GM.** Innovative methods to study human intestinal drug metabolism in vitro: precision-cut slices compared with ussing chamber preparations. *Drug Metab Dispos* 34: 1893-1902, 2006.
44.**van Leeuwen JP, Birkenhager JC, Buurman CJ, van den Bemd GJ, Bos MP, and Pols HA.** Bidirectional regulation of the 1,25-dihydroxyvitamin D3 receptor by phorbol ester-activated protein kinase-C in osteoblast-like cells: interaction with adenosine 3',5'-monophosphate-induced up-regulation of the 1,25-dihydroxyvitamin D3 receptor. *Endocrinology* 130: 2259-2266, 1992.
45.**Walters MR.** Newly identified actions of the vitamin D endocrine system. *Endocr Rev* 13: 719-764, 1992.
46.**Wiese RJ, Uhland-Smith A, Ross TK, Prahl JM, and DeLuca HF.** Up-regulation of the vitamin D receptor in response to 1,25-dihydroxyvitamin D3 results from ligand-induced stabilization. *J Biol Chem* 267: 20082-20086, 1992.
47.**Xu Y, Iwanaga K, Zhou C, Cheesman MJ, Farin F, and Thummel KE.** Selective induction of intestinal CYP3A23 by 1alpha,25-dihydroxyvitamin D3 in rats. *Biochem Pharmacol* 72: 385-392, 2006.
48.**Yasmin R, Williams RM, Xu M, and Noy N.** Nuclear import of the retinoid X receptor, the vitamin D receptor, and their mutual heterodimer. *J Biol Chem* 280: 40152-40160, 2005.