ORIGINAL PAPER

International Journal of Occupational Medicine and Environmental Health 2016;29(2):293–314
http://dx.doi.org/10.13075/ijomeh.1896.00528

EXPRESSION OF CYTOCHROME P450 2C AND 3A IN FEMALE RAT LIVER AFTER LONG-TERM ADMINISTRATION OF GONADOLIBERIN ANALOGS

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Abstract

Objectives: Gonadoliberin (GnRH) analogs may be expected to indirectly modify growth hormone (GH) total concentration and its 24-h secretion profile. As a consequence, changes in the levels of GH may modify the mechanism of sex-dependent cytochromes P450 (CYP450) synthesis, including the expression of transcriptional factors. The aim of the study has been to evaluate the effect of long-term administration of a low dose of GnRH analogs on hepatic expression of CYP2C and CYP3A isoforms, and the transcription factors: pregnane X receptor (PXR), hepatocyte nuclear factor 4α (HNF4α), HNF6 and signal transducers and activators of transcription 5b (STAT5b). **Material and Methods:** The study was carried out on adult female Sprague-Dawley rats during a 3-month treatment with dalarelin (GnRH agonist) and cetrorelix (GnRH antagonist), at a daily intraperitoneal injection (*i.p.*) dose of 6 μg/kg body weight/day, and 1, 2, and 4 weeks after treatment discontinuation. The concentrations of ovarian hormones and GH in the blood serum were determined by radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) method, respectively. Then, the expression of hepatic CYP450s (reverse transcription polymerase chain reaction – RT-PCR, Western blot and immunohistochemistry) and transcription factors (RT-PCR) was evaluated. **Results:** We have found that cetrorelix induces changes in the circadian pattern of GH secretion and enhances GH blood concentrations. These changes may cause increased expression of both, female-specific CYP450s (especially CYP3A9), and HNF4α/HNF6 transcription factors. Decrease in GH blood concentrations, resulting from the effect of dalarelin, may promote inhibition of female-specific CYP2C12 and CYP3A9 isoforms as well as STAT5b transcription factor. Slight changes in sex-independent CYP3A1 protein expression caused by GnRH analogs were also observed. **Conclusions:** In adult female rats, HNF4α/HNF6 and STAT5b seem to be crucial for the regulation of GnRH antagonist/GH- and GnRH agonist/GH-dependent pattern of CYP450 expression, respectively.

Key words:
CYP2C, CYP3A, Liver-enriched transcription factors, Growth hormone, Dalarelin, Cetrorelix

This work was supported by the Institutional Grants of Medical University of Silesia in Katowice No. KNW-2-012/D/4/N, KNW-1-009/08 and KNW-1-004/09, and National Science Center grant No. 2012/07/N/N24/02569 (KNW-6-407/13). Rafał Skowronek is a fellow in the project "DoktoRIS – Scholarship program for innovative Silesia" co-financed by the European Union under the European Social Fund.
Received: December 2, 2014. Accepted: May 9, 2015.
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INTRODUCTION

Enzymes belonging to the superfamily of cytochrome P450 (CYP450) participate in the 1st phase of the metabolism of xenobiotics. Cytochrome P450 2C is the most abundant CYP450 subfamily of rat liver, performing some functions of rat and human CYP3A enzymes [1–3]. Rat CYP2C11 and CYP2C12 are examples of sex-specific liver cytochromes CYP2C [3]. Cytochrome P450 2C11 is a prototypic class I male-specific gene [4] whereas CYP2C12 is a class I female-specific gene [5]. Cytochrome P450 3A subfamily is very important in rodents and humans because it participates in the metabolism of 40–60% of all drugs currently used in medicine [6]. Rat CYP3A1 is an ortholog of human CYP3A4 [1,7]. Cytochrome P450 3A2 is a member of class II male-specific CYP450 that is not recognized in intact females at the protein level [8]. Hypophysectomy conducted in male rats caused 3–4-fold increase in CYP3A2 and CYP3A18 messenger ribonucleic acid (mRNA) expression but it did not affect CYP3A9 mRNA levels or CYP3A-mediated testosterone 2β- or 6β-hydroxylase activity [9]. Cytochrome P450 3A9 (a female-specific member of the CYP3A subfamily) is the "predominant" isoform in the liver of adult female rats because its expression is 3–10 times higher in females as compared to males [3].

The expression of hormone-dependent CYP450 has been known to be directly or indirectly regulated by growth hormone (GH) – the main hormonal regulator as well as sex and thyroid hormones, glucocorticoids, insulin, glucagon, cytokines and others [3]. Signal transductions are strictly connected with the activation of numerous DNA-binding proteins involved in the regulation of the expression, primarily at the stage of transcription [10]. Growth hormone activates mainly Janus kinase and signal transducer and activator of transcription (JAK-STAT) signaling pathway, where the key players are STAT proteins, cooperating mostly with hepatocyte nuclear factors (HNF) [11,12]. Some of them, e.g., HNF6, are directly regulated by GH [13].

The molecular mechanism of the expression of some CYP450 also depends on nuclear receptors [14]. Pregnane X receptor (PXR) is involved in the process of transcriptional regulation of the CYP2C as well as the CYP3A subfamily in both, rodents (CYP3A1, CYP3A2, CYP3A9, CYP3A18, CYP3A23) and humans (CYP3A4, CYP3A5, CYP3A7) [15,16]. The mutual dependence among nuclear receptors, other transcription factors and hormones, especially GH and sex hormones, is not well-recognized.

Activation of transcriptional regulators may be directly linked to the 24-h GH secretion profile. Secretion of the GH in male rats is pulsatile in nature [3,17]. Every 3.5–4 h, the blood GH concentration reaches even up to 200 ng/ml but remains almost undetectable (< 1–2 ng/ml) in-between these high values. The average blood plasma GH concentration in female rats is stable at the level of 15–40 ng/ml, with multiple pulses per hour. Growth hormone secretion in humans is also sexually dimorphic [18]. The large nocturnal GH pulses and relatively small pulses during the light phase are observed in men, whereas a more continuous GH secretion may be noted in women [19,20].

The consequence of the above mentioned relations between hormones and CYP450 is that both hormonal pathologies and hormonal pharmacotherapies may influence drug metabolism, significantly modifying treatment effects. The decrease in sex hormone concentrations is an expected treatment result in some gynecological and oncological disorders [21]. It is achieved by a long-term administration of gonadoliberin (GnRH) analogs, namely, GnRH agonists and antagonists. The mechanism of action of these synthetic peptides differs but they both cause pituitary desensitization, resulting in a strong decrease in sex hormone levels in the blood [22].

Owing to recognized relations between estrogens and GH action [23], GnRH analogs may be expected to indirectly

modify the GH total concentration and its 24-h secretion profile [24]. As a consequence, they might influence the mechanism of CYP450 expression, including the expression of transcriptional factors, independently of sex hormones.

In order to investigate the GH-dependent expression of CYP2C and CYP3A subfamilies, we applied a new experimental model, in which changes of the 24-h GH secretion profile in rats occurred under the conditions of a long-term pituitary desensitization caused by application of GnRH agonist and antagonist [25]. We evaluated hepatic expression of CYP2C and CYP3A isoforms in connection with the changes of liver-enriched transcription factors: HNF4α, HNF6, STAT5b (signal transducer and activator of transcription 5b) and PXR receptor, and GH secretory pattern. Twenty-four-hour changes in serum GH concentrations were stimulated by a long-term administration of GnRH agonist – dalarelin, and antagonist – cetrorelix. The changes were observed during a 3-month period of analog administration and 1 month after treatment discontinuation.

MATERIAL AND METHODS

Animals

The study was performed on sexually mature 3-month-old female Sprague-Dawley (SD) rats, weighing 200–220 g, purchased from the Center for Experimental Medicine, Medical University of Silesia in Katowice. During the experiment, the animals were housed in cages, with standard conditions of temperature and humidity. The animals were kept under a 12-h/12-h cycle of either light/dark (7 a.m. – 7 p.m., respectively) with *ad libitum* access to food and water. Each week, the animals were weighed to adjust the proper dose of GnRH analogs.

The animals were divided into 6 control groups (K groups) and 12 experimental groups (6 rats in each). Control rats, at the moment of decapitation, were in the proestrus phase of the reproductive cycle (confirmed by the cytological analysis of vaginal smears during 2 consecutive 4-day cycles). Once a day, the animals from the experimental groups were administered GnRH analogs intraperitoneally at a dose of 6 μg/kg body weight (b.w.), dissolved in 0.9% sodium chloride. Drug administration started when the females were in the proestrus phase. Animals from groups D (dalarelin) received subcutaneous injection of dalarelin acetate (BAPEX, Ryga, Latvia; a gift from prof. F. Ryszka (Biochefa, Sosnowiec, Poland)). Animals from groups C (cetrorelix) were administered cetrorelix acetate (ChemPep, Inc., Wellington, USA).

Both analogs were administered for: 1 month (group ID and IC), 2 months (group IID and IIC) and 3 months (group IIID and IIIC). The rats from the control groups were concurrently administered placebo (0.9% sodium chloride) for 1 month (IK), 2 months (IIK) and 3 months (IIIK). After the specified period of time (1, 2 or 3 months) of analog or placebo administration, and 1 week (groups IIIK+1, IIID+1, IIIC+1), 2 weeks (groups IIIK+2, IIID+2, IIIC+2) and 4 weeks (groups IIIK+4, IIID+4, IIIC+4) after the 3-month treatment with each analog, the animals were sectioned to excise the liver and to draw a probe of blood.

The study was accepted by the Local Ethics Committee.

Hormonal assays

During autopsy, blood from the left ventricle of the heart was drawn and allowed to clot. Then, it was centrifuged in MPW 360 centrifuge for 15 min, 3000 revolutions per minute (rpm), and the obtained serum was kept at –20°C. The concentrations of steroid hormones: 17β-estradiol, progesterone and testosterone were determined by radioimmunoassay (RIA), according to the producer's protocol (Immunotech, Marseille, France).

The daily profile of the total GH concentration was evaluated after 2 and 3 months of GnRH analogs administration and 1 and 2 weeks after its discontinuation. In order to set the 24-h profile of the GH concentration,

200 µl of the blood from the rat tail vessels was drawn at closely fixed hours (9 a.m., 10:30 a.m., noon, 1:30 p.m., 3 p.m., 4:30 p.m., 6 p.m., 9 p.m., midnight, 3 a.m., 6 a.m., 7:30 a.m.) to the test-tube containing 20 µl of heparin. Next, test-tubes were centrifuged in MPW 360 centrifuge for 15 min, 2000 rpm. The achieved plasma was kept at –20°C until biochemical analyses. Growth hormone concentration in the blood serum was assessed by means of the enzyme-linked immunosorbent assay (ELISA) method (Rat GH Enzyme Immunoassay Kit, category No. A05104, SPI-bio), according to the protocol provided by the manufacturer.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated from the liver by acid guanidinium thiocyanate/phenol/chloroform extraction using the commercially available kit (Trizol TM) [26]. Ribonucleic acid was reversely transcribed into complementary deoxyribonucleic acid (cDNA). Reverse transcription (RT) reaction was carried out at 42°C for 1 h. The polymerase chain reaction (PCR) mixture contained primers specific for the investigated rat genes and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) reference gene (Table 1).

Amplification was carried out by 26 repeated cycles at 94°C for 1 min, 54–66°C (gradient temperature) for 1.5 min, and 72°C for 1 min on the Programmable Thermal Controller PTC-200 (MJ Research, Inc., Watertown, MA, USA). An aliquot of each reaction mixture was subjected to electrophoresis in a 2% agarose gels. Gels were stained with ethidium bromide and analyzed qualitatively (base pairs) and semi-quantitatively by densitometry (One D-scan software, Scanalytics).

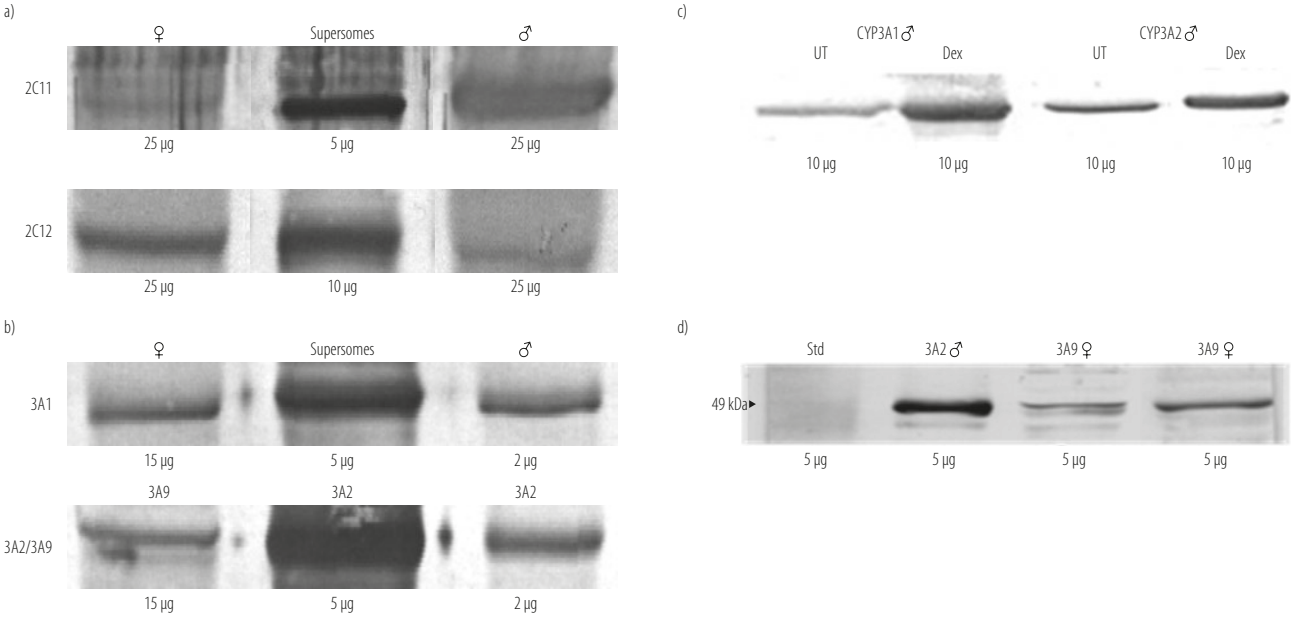
Western blotting

In order to assess CYP450 and PXR protein expressions, microsomal and nuclear fractions were isolated from liver homogenates, respectively [27]. The protein content was determined according to Bradford [28]. The samples of 5–10 µg of protein were subjected to polyacrylamide gel (8%) electrophoresis in the presence of sodium dodecyl sulphate [29]. Then, the proteins were blotted electrophoretically onto polyvinylidene difluoride (PVDF)

Table 1. Primers used for polymerase chain reaction (PCR)

Gene	Primer sequence		Hybridization temperature [°C]	Product size [bp]
	forward	reverse		
CYP2C11	5'-TGC CCC CTT TTT ACG AGG CT	5'-GGA ACA GAT GAC TCT GAA TTC T	60.0	249
CYP2C12	5'-TAT AAA CTC AAT ACG TTC TGA G	5'-TTT TAC ATT AAC TTC AGA AAC TG	60.0	262
CYP3A1	5'-CCG CCT GGA TTC TGT GCA GA	5'-TGG GAG GTG CCT TAT TGG GC	62.9	203
CYP3A2	5'-TTG ATC CGT TGC TCT TGT CA	5'-GGC CAG GAA ATA CAA GAC AA	54.0	323
CYP3A9	5'-GGA CGA TTC TTG CTT ACA GG	5'-ATG CTG GTG GGC TTG CCT TC	61.0	373
PXR	5'-GAC GGC AGC ATC TGG AAC TAC	5'-TGA TGA CGC CCT TGA ACA TG	56.0	112
HNF4α	5'-CGG GCT GUC ATG AAG AAA G	5'-AGC GCA TTA ATG GAG GGT AGG	60.0	101
HNF6	5'-AAG CCC TGG AGC AAA CTC AA	5'-CCA CAT CCT CCG GAA AGT CTC	60.0	51
STAT5b	5'-GCA ATG ATT ACA GCG GCG AGA	5'-CAA AGG CGT TGT CCC AGA GG	60.0	299
GAPDH	5'-GTG AAC GGA TTT GGC CGT ATC G	5'-ATC ACG CCA CAG CTT TCC AGA GG	66.0	543

CYP – cytochrome; PXR – pregnane X receptor; HNF – hepatocyte nuclear factor; STAT – signal transducers and activators of transcription; GAPDH – glyceraldehyde-3-phosphate dehydrogenase; bp – base pair.



Supersomes, cDNA-expressed rat isoforms: CYP2C11, CYP2C12, CYP3A1 and CYP3A2 (a and b) as well as microsomes from dexamethasone-induced (Dex) and non-induced (UT) males (c) were used as positive controls. Dex rats were administered dexamethasone twice at a daily intraperitoneal injection (i.p.) of the dose at 30 mg/kg body weight (b.w.). In females CYP3A9 protein was assessed using anti-CYP3A2 antibody. Anti-CYP3A2 antibody binds CYP3A9 but its band migrates slower than CYP3A2 during gel electrophoresis. Cytochrome 3A2 was not identified in female intact rats (d). The amount of microsomal protein per lane (2–25 µg) is also shown. Standard (Std) molecular weight marker (Bio Rad). The position of molecular weight 49 kDa band is marked.

Fig. 1. Immunodetection of: a) CYP2C, b) CYP3A isoforms in the liver microsomes of adult male and female rats with the use of supersomes as positive controls, c) CYP3A1 and CYP3A2 isoforms in the liver microsomes of adult dexamethasone-induced (Dex) and non-induced (UT) male rats, d) CYP3A2 and CYP3A9 isoforms in the liver microsomes of intact adult male and female rats

membrane (Millipore) and afterwards microsomal isoforms of CYP450 and nuclear PXR were identified with Western blot immunoassay.

Polyclonal antibodies to: CYP2C11, CYP2C12 (Abcam), CYP3A1, CYP3A2 (Chemicon) and PXR (Santa Cruz) were developed in the rabbit and goat. Bound primary antibodies were detected with goat anti-rabbit or mouse anti-goat secondary antibody conjugated with alkaline phosphatase and the reaction was developed with BCIP/NBT (5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium) liquid substrate system (Sigma), according to the manufacturer's instruction.

Cytochrome P450 3A9 protein was assessed using anti-CYP3A2 antibody because anti-CYP3A9 antibody was not available commercially. Cytochrome P450 3A9 immunodetection is possible because of high homology with male isoform CYP3A2. However, its band migrates slower than CYP3A2 during gel electrophoresis [30]. Supersomes, cDNA-expressed rat isoforms: CYP2C11, CYP2C12, CYP3A1 and CYP3A2 (Corning), as well as dexamethasone-induced microsomes were used as a standard (Figure 1). Molecular weight markers were purchased from Bio Rad. Intensities of stained bands in gels were analyzed for densitometric differences with One D-scan software (Scanalytics).

Immunohistochemistry

Expression and localization of CYP450 and PXR proteins in zones of liver acini were visualized with avidin-biotin

complex (ABC) [31]. Tissue samples taken from the left liver lobe were fixed in 10% neutral-buffered formalin, subsequently passed through graded alcohol solutions and xylenes, and finally embedded in paraffin blocks. Slices of 5 μm in thickness were placed on silane-coated slides, deparaffinized and rehydrated. For quenching the endogenous peroxidase activity, tissue sections were blocked with 1.5% (v/v) H_2O_2 for 10 min. In the case of PXR, to make antigens more accessible, demascation in the oven (850 W, pH = 6, 3 min) was performed. Before incubation with the primary antibody, the sections were washed 2 times (5 min each) in TBS (Tris-buffered saline) and pretreated with 1% BSA (bovine serum albumin) for 30 min to block places of nonspecific binding of antibodies.

Appropriate primary antibodies (anti-CYP2C11 and anti-CYP2C (Abcam), anti-CYP3A1 and anti-CYP3A2 (Chemicon) and anti-PXR (Santa Cruz)) were added, and the sections were incubated for 22 h at 4°C. This step was followed by incubation with proper biotinylated secondary antibody. Final visualization was achieved by using the avidin-biotin-peroxidase complex kit (Vectastain ABC Kit, Vector Laboratories), freshly prepared DAB (3,3'-diaminobenzidine) and hydrogen peroxide according to the protocol provided by the manufacturer. Appropriate negative controls were done. Levels of expression of the studied proteins were determined by densitometry (Image ProPlus software). The documentation of immunohistochemical reactions was performed with the SSC-DC58AP camera (Sony) coupled with the optical microscope Eclipse E600 (Nikon).

Statistical analysis

Data was analyzed using the Statistica 8.0 computer software. Parametric analysis of the obtained data was performed with the one-way ANOVA. The Mann-Whitney U (for independent samples) or Wilcoxon tests (for related samples) were used if normal distributions could not be assumed. Differences were considered significant if $p < 0.05$.

RESULTS

Hormonal changes

We noticed significantly reduced serum concentrations of 17β-estradiol in analog-treated rats. Concentrations of progesterone were low and unchanged in dalarelin-treated rats, and reduced ($p < 0.05$) in cetrorelix-treated females. In most groups, changes in levels of testosterone were statistically insignificant (Table 2).

In the case of the GH, we observed age-dependent reduction of mean 24-h concentrations between months 5 (group II) and 6 (group III), and its stabilization in subsequent age groups; inhibitory effect in rats treated with dalarelin for 3 months, as well as after cessation of injections; and inductive effect in rats treated with cetrorelix for 2 and 3 months (Table 3).

We investigated the 24-h profile of plasma GH secretion and found that 24-h GH secretion in 5-month-old control female rats (group II) was characterized by increasing hormone concentrations, with maximum at 1:30 p.m. (10.24 ng/ml) and minimum at 6 p.m. (3.75 ng/ml) (Figure 2).

The growth hormone level was stable during night hours and kept at the level of the 24-h mean value (6.55 mg/ml). In the control group of 6-month-old (group III) and 6.5-month-old rats (group III+2), 24-h fluctuations of GH concentration were not distinct.

We found that 2- and/or 3-month treatment of rats with dalarelin caused 24-h changes in serum GH levels statistically insignificant in many time points as compared to appropriate controls (Figure 2). The influence of cetrorelix on the 24-h profile of the GH secretion was more distinct. After a 2-month treatment there was a strong tendency to increase the hormone levels with the daily maxima at 7:30 a.m. (increased by 227%) and at 10:30 a.m. (increased by 383%). The smaller increase was observed

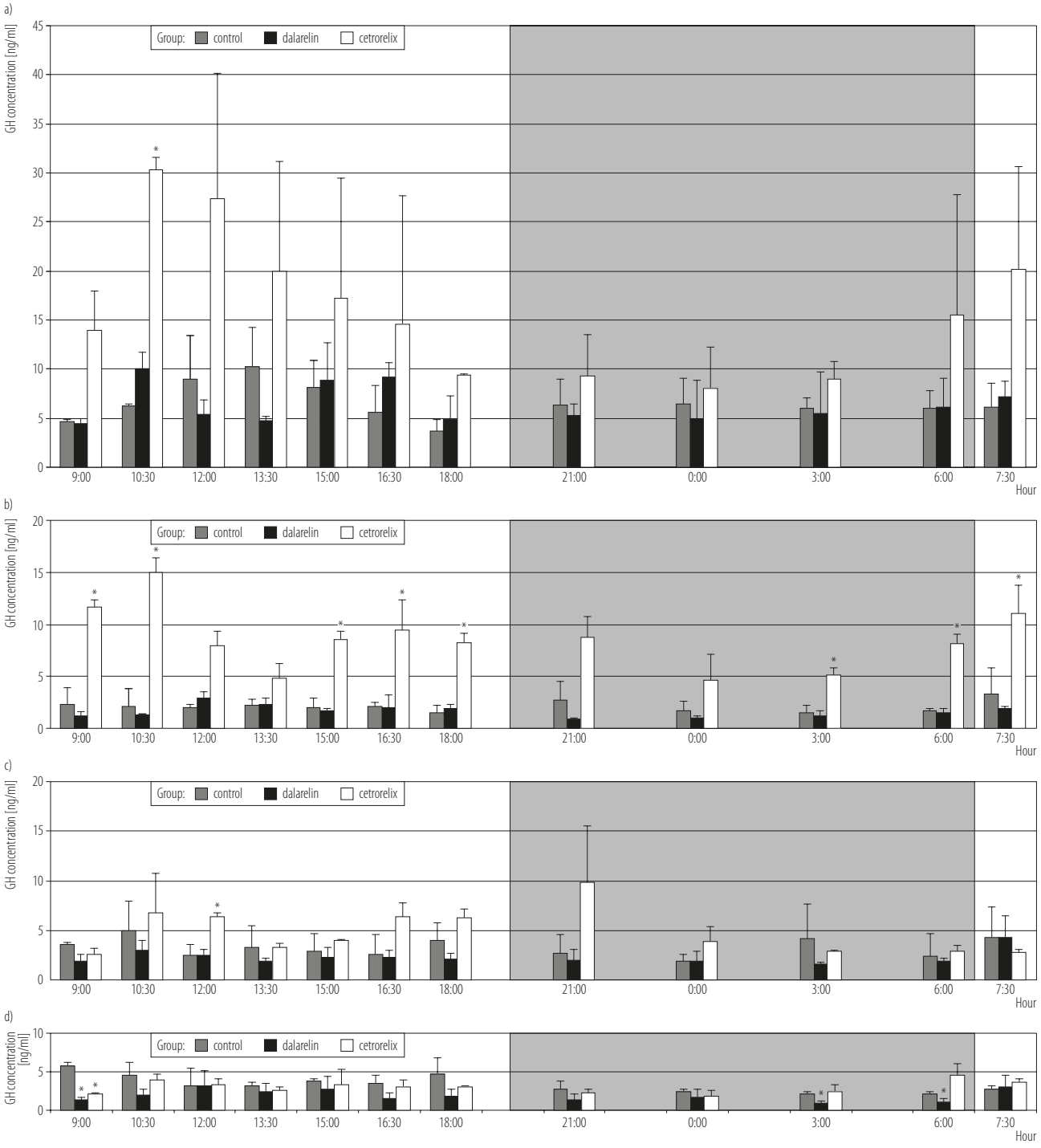
Table 2. Sex hormones concentrations in the blood serum of rats

Group	Sex hormone (M±SD)		
	17β-estradiol [pg/ml]	progesterone [ng/ml]	testosterone [ng/ml]
K			
I (N = 6)	26.78±5.63	4.96±2.03	0.05±0.01
II (N = 6)	24.53±4.99	6.95±1.81	0.12±0.03
III (N = 6)	28.60±4.28	8.93±4.69	0.19±0.04
III+1 (N = 6)	26.63±2.21	13.45±7.26	0.14±0.03
III+2 (N = 6)	n.d.	n.d.	n.d.
III+4 (N = 6)	23.52±4.73	13.34±5.66	0.13±0.03
D			
I (N = 6)	12.68±1.99*	7.29±1.72	0.13±0.04
II (N = 6)	15.81±3.84*	3.82±1.57	0.07±0.02
III (N = 6)	17.15±5.35*	8.47±1.52	0.12±0.03
III+1 (N = 6)	19.17±5.17	11.87±1.71	0.12±0.01
III+2 (N = 6)	19.67±3.06	6.86±3.64	0.08±0.03
III+4 (N = 6)	23.54±7.86	6.31±5.06	0.08±0.03
C			
I (N = 6)	15.99±2.24*	2.31±0.45*	0.19±0.03*
II (N = 6)	16.65±4.50*	4.47±1.43	0.25±0.10
III (N = 6)	11.32±3.59*	1.66±0.39*	0.16±0.03
III+1 (N = 6)	12.21±2.38*	2.81±1.23*	0.15±0.04
III+2 (N = 6)	19.07±6.13	6.17±1.33	0.17±0.03
III+4 (N = 6)	19.59±7.62	6.93±1.78	0.23±0.09

K – control; D – dalarelin; C – cetrorelix; M – mean; SD – standard deviation; n.d. – not detected.
I – 1 month of treatment; II – 2 months of treatment; III – 3 months of treatment; III+1 – 3 months and 1 week after treatment discontinuation; III+2 – 3 months and 2 weeks after treatment discontinuation; III+4 – 3 months and 4 weeks after treatment discontinuation.
* Statistically significant as compared to appropriate controls (K) ($p < 0.05$).
The measurement was performed between 9 a.m. and 10 a.m. Groups IIID+2 and IIIC+2 were compared with IIIK+1 and IIIK+4 controls. Rats from groups I, II, III and III+4 were 4-, 5-, 6- and 7-month-old, respectively, at the time of decapitation.

between 6 p.m. (by 150%) and 3 a.m. (by 50%) resulting in day and night minimal values at midnight, at the level of 8.02 ng/ml.

In 2 months cetrorelix-administered female rats the highest levels of the GH were observed in the light phase (day) (Figure 2) but the GH secretory pattern was not changed into the male-specific pattern. Similar changes in GH concentrations were observed after 3 months of cetrorelix administration (Figure 2). The growth hormone level increased significantly in most time points (from 121% at 1:30 p.m. to 622% at 10:30 a.m.). Moreover, 3 maxima (at 7:30 a.m., 10:30 a.m., 4:30 p.m.) as well as 2 minima (at 1:30 p.m. and midnight) were found. The increase in the number of peaks was accompanied by the decrease in the GH concentration values in the blood. In group IIIC, GH levels were lower as compared to the younger group IIC.



* Statistically significant as compared to appropriate controls (K) ($p < 0.05$).
Animals were kept under a 12-h/12-h cycle of either light/dark (7 a.m. – 7 p.m., respectively). Gray shading indicates the dark phase.

Fig. 2. Twenty-four-hour pattern of growth hormone (GH) concentration in the plasma of rats administered placebo, dalarelin and cetrorelix for: a) 2 months of GnRG analog administration, b) 3 months of GnRH analog administration, c) 1 week after treatment discontinuation, d) 2 weeks after treatment discontinuation

Table 3. Mean 24-h growth hormone (GH) concentrations in the plasma of rats

Group	GH concentration [ng/ml] (M±SD)
K	
II (N = 6)	6.55±1.80
III (N = 6)	2.09±0.53**
III+1 (N = 6)	3.27±0.95**
III+2 (N = 6)	3.38±1.12**
D	
II (N = 6)	6.37±1.96
III (N = 6)	1.65±0.59*
III+1 (N = 6)	2.30±0.73*
III+2 (N = 6)	1.94±0.77*
C	
II (N = 6)	16.24±7.23*
III (N = 6)	8.62±3.02*
III+1 (N = 6)	4.81±2.26
III+2 (N = 6)	3.02±0.80

Abbreviations as in Table 2.
* Statistically significant as compared to appropriate controls (K) ($p < 0.05$).
** Statistically significant as compared to the control group IIK ($p < 0.05$).
Each value is a mean of 12 measurements at 9.00 a.m., 10.30 a.m., noon, 1.30 p.m., 3.00 p.m., 4.30 p.m., 6.00 p.m., 9.00 p.m., midnight, 3.00 a.m., 6.00 a.m., and 7.30 a.m.

Nevertheless, most of the differences between the maximal and minimal values were statistically significant. The tendency for the GH concentration values to decrease was maintained after discontinuation of cetrorelix administration, and it resulted in the equilibration of GH concentrations at the level of control values in almost all time points.

Cytochrome mRNA, protein expression and immunolocalization

"Male" CYP isoforms

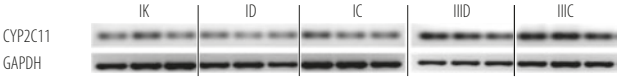
In control females, male-specific CYP2C11 mRNA expression was detectable between month 4 (group I) and month 7 (group III+4) of age. During 3 months of dalarelin and cetrorelix administration, statistically insignificant changes of this expression were noted (Figure 3, Table 4). The expression of CYP2C11 protein in the liver of control and analog treated female rats was weakly detectable (data not shown).

Low and individually variable male-specific CYP3A2 mRNA expression was detected in the livers of females administered dalarelin (Figure 4).

Table 4. Relative male CYP2C11 messenger ribonucleic acid (mRNA) expression in the liver of rats[1]

Group	Relative CYP2C11 mRNA expression
D	
I (N = 6)	1.25
II (N = 6)	1.58
III (N = 6)	1.48
III+1 (N = 6)	1.01
III+2 (N = 6)	1.01
III+4 (N = 6)	1.30
C	
I (N = 6)	0.85
II (N = 6)	1.06
III (N = 6)	0.93
III+1 (N = 6)	0.86
III+2 (N = 6)	0.69
III+4 (N = 6)	0.98

Abbreviations as in Table 1 and 2.
[1] Examined mRNA was normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA level for individual livers. The values are presented as relative expressions to appropriate controls (K = 1.00).



Abbreviations as in Table 1, 2 and 4.
Representative samples taken from individual rats are presented.
Numerical data is shown in Table 4.

Fig. 3. Male CYP2C11 mRNA expression in the liver of female rats (specific polymerase chain reaction (PCR) products)

Cytochrome P450 3A2 mRNA expression was practically undetectable in the control groups, and experimental groups after dalarelin administration was stopped. There was no evidence of CYP3A2 mRNA expression in the liver of rats receiving cetrorelix. It was in accordance with the lack of male-specific CYP3A2 protein presence in both experimental groups in the Western blot analysis (data not presented).

"Female" CYP isoforms

Most of the changes in female-specific CYP2C12 mRNA expression observed during 3 months of dalarelin and cetrorelix administration were not statistically significant. Contrary to unchanged CYP2C12 mRNA expression, dalarelin decreased CYP2C12 protein expression by 52–60% ($p < 0.05$) after month 1 and 2 of administration and by 17% – after month 3 (Table 5, Figure 5).

In turn, the influence of cetrorelix on CYP2C12 mRNA and protein expression was slightly inductive during the period of exposition ($p < 0.05$ for group IIC).

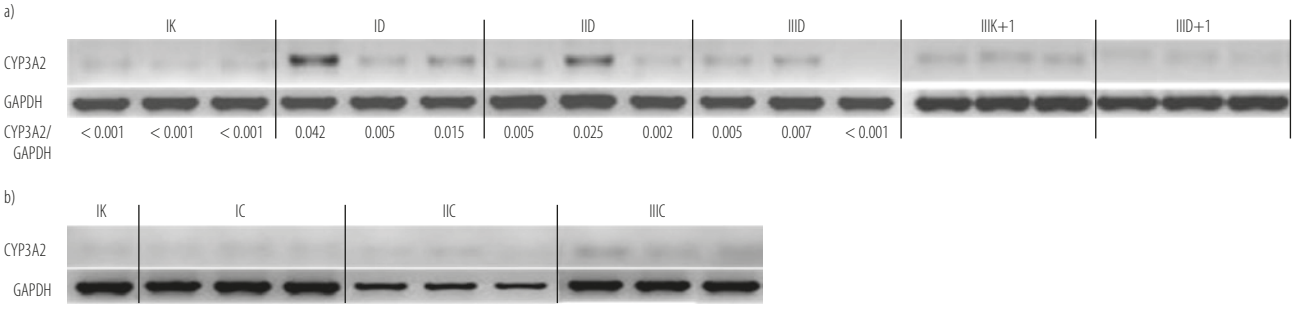
In control and analog-treated rats, positive immunohistochemical reaction for CYP2C12 was localized in hepatocytes of zone 3 of the liver acini (Figure 5). Positive immunohistochemical reaction for CYP2C12 after dalarelin injections was weaker as compared to controls.

Table 5. Relative female cytochrome CYP2C12 and CYP3A9 mRNA expression in the liver of rats[1]

Group	Relative mRNA expression	
	CYP2C12	CYP3A9
D		
I (N = 6)	1.47	0.89
II (N = 6)	1.11	0.62
III (N = 6)	1.43	0.80
III+1 (N = 6)	0.97	0.98
III+2 (N = 6)	0.94	0.91
III+4 (N = 6)	1.15	0.94
C		
I (N = 6)	0.82	1.60*
II (N = 6)	2.49*	1.58*
III (N = 6)	1.20	1.94*
III+1 (N = 6)	0.80	1.16
III+2 (N = 6)	0.79	1.30
III+4 (N = 6)	0.88	1.11

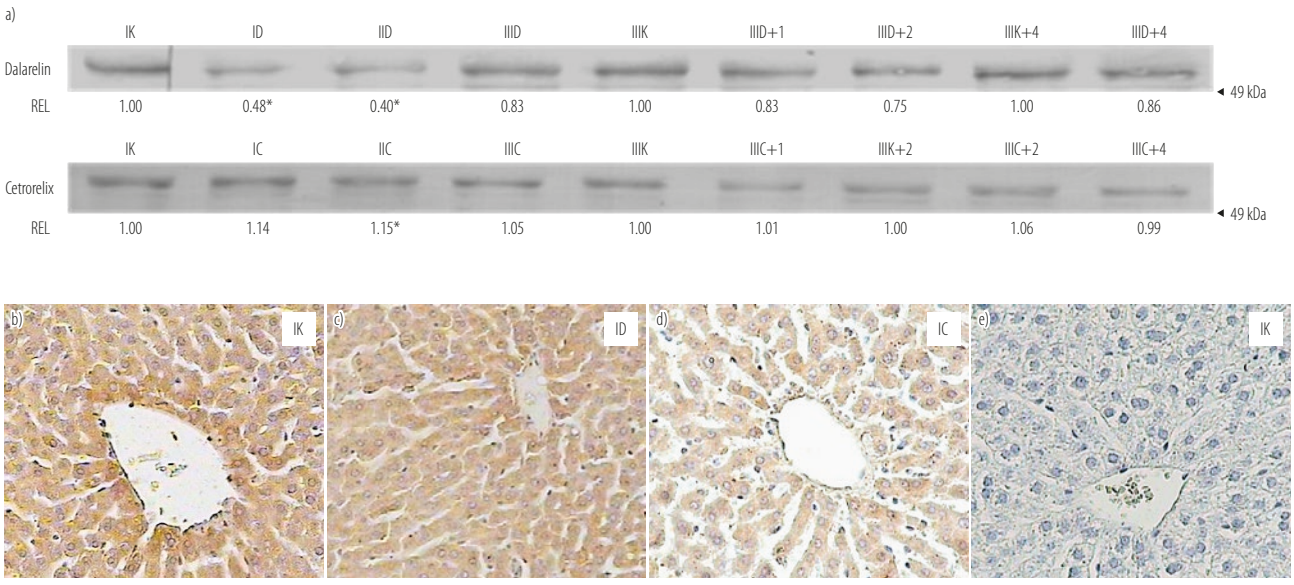
Abbreviations as in Table 1, 2 and 4.

During the period of treatment, dalarelin did not cause changes in female-specific CYP3A9 mRNA expression (Table 5). Administration of dalarelin caused the decrease in CYP3A9 protein expression after 1, 2 and 3 months by 13%, 42% and 71% ($p < 0.05$),



Abbreviations as in Table 2 and 4 and Figure 3.
Cytochrome P450 mRNA was normalized with GAPDH mRNA level for individual livers. Values for detectable CYP3A2 expressions ($p > 0.001$) are shown.

Fig. 4. Cytochrome 3A2 mRNA expression in the liver of female rats (specific PCR products): a) dalarelin group, b) cetrorelix group



Abbreviations as in Table 2.

Representative samples taken from individual rats are presented. Relative expression level (REL) vs. K = 1.00. Relative expression level values are means obtained from 3 independent rats. The position of molecular weight marker 49 kDa (Bio Rad) is indicated. Samples of liver microsomes contained 10 μg of microsomal protein per lane. Zone 3 of the liver acinus surrounding the central vein is shown.

Fig. 5. Cytochrome 2C12 a) protein expression in liver microsomes of rats; b) protein immunolocalization (magnification: 200×) in control group, c) dalarelin group, d) cetrorelix group, e) negative control (section without primary antibody)

respectively. Cetrorelix significantly ($p < 0.05$) induced CYP3A9 mRNA expression after month 1 (by 60%), month 2 (by 58%) and month 3 (by 94%) of administration. All these changes were reversible after treatment discontinuation. At the same time, cetrorelix significantly induced the CYP3A9 protein expression (even more than 4.5 times, $p < 0.05$). All these changes were reversible 2 weeks after analog administration had been stopped (Figure 6).

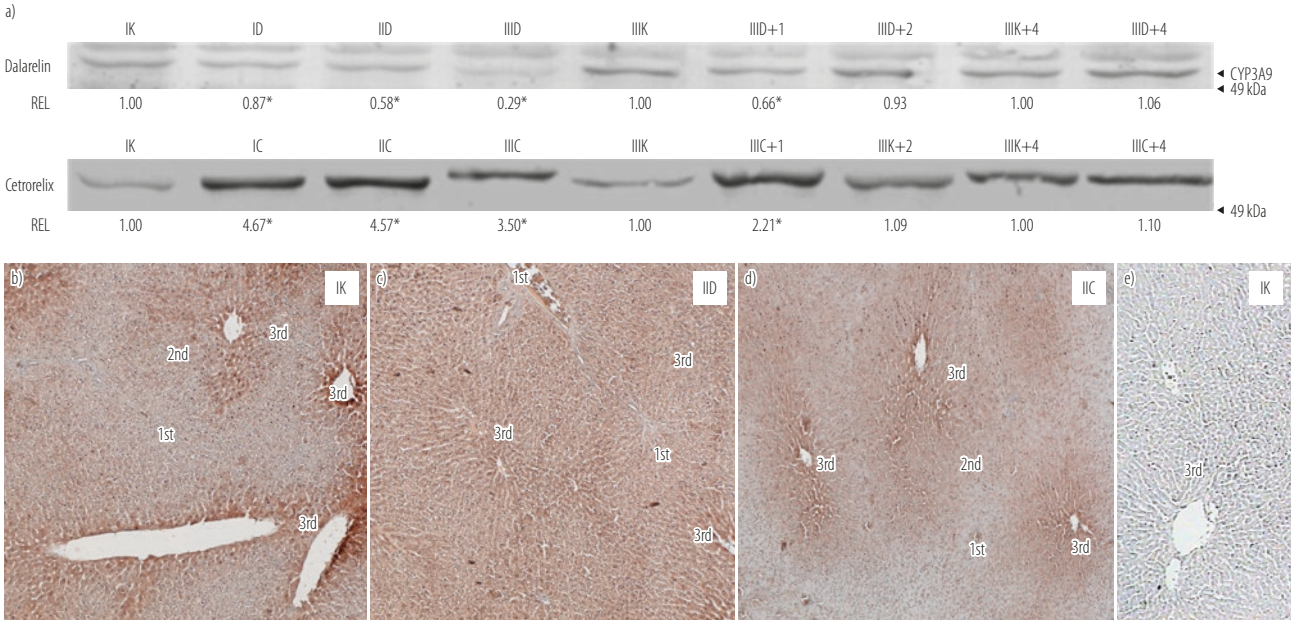
Zone 3 of the liver acinus was the most important localization of CYP3A9 in rats from both, control and experimental groups (Figure 6). In general, CYP3A9 expression reversibly decreased after dalarelin administration but it was visible in all 3 zones of the hepatic acini. After cetrorelix administration, numerous strongly CYP3A9-positive cells were visible mostly in zone 3 and 2, and (to a lesser degree) in zone 1. These changes were reversible after antagonist treatment discontinuation.

Sex-independent CYP isoform

Dalarelin significantly elevated sex-independent CYP3A1 mRNA expression (by 35%) after month 1 of drug administration (Table 6). Dalarelin-induced CYP3A1 protein expression (by 10–11%) was observed in groups IID and IIID, and (by 17%) in group IIID+2 ($p < 0.05$).

Cetrorelix significantly induced CYP3A1 mRNA expression after month 1 (by 238%) and month 2 (by 96%) of administration ($p < 0.05$). The inductive but not statistically significant influence of cetrorelix was also observed after month 3 (by 64%) and during the period after the last injection. At the same time, cetrorelix decreased CYP3A1 protein expression in group IIIC (by 53%, $p < 0.05$) and IIIC+1 (by 28%, $p < 0.05$) (Figure 7). These changes were quickly reversible.

Zone 3 of the liver acinus was the most important localization of CYP3A1 in rats from both, control and experimental groups (Figure 7). A positive reaction for CYP3A1 was



1st, 2nd, 3rd – zones of the liver acinus. Other abbreviations as in Table 2.
Representative samples taken from individual rats are presented. Relative expression level (REL) values are means obtained from 3 independent rats. Positions of molecular weight marker 49 kDa (Bio Rad) and CYP3A9 band are indicated. Samples of liver microsomes contained 10 μg of microsomal protein per lane. Cytochrome 3A9 protein was detectable in the cytoplasm of hepatocytes of both, control and analog-treated females.

Fig. 6. Cytochrome 3A9 a) protein expression in liver microsomes of rats; b) protein immunolocalization (magnification: 40×) in control group, c) dalarelin group, d) cetrorelix group, e) negative control (section without primary antibody)

Table 6. Relative sex-independent CYP3A1 mRNA expression in the liver of rats[1]

Group	Relative CYP3A1 mRNA expression
D	
I (N = 6)	1.35*
II (N = 6)	0.74
III (N = 6)	1.07
III+1 (N = 6)	0.93
III+2 (N = 6)	0.88
III+4 (N = 6)	0.73
C	
I (N = 6)	3.38*
II (N = 6)	1.96*
III (N = 6)	1.64
III+1 (N = 6)	1.90
III+2 (N = 6)	0.98
III+4 (N = 6)	1.47

Abbreviations as in Table 1, 2 and 4.

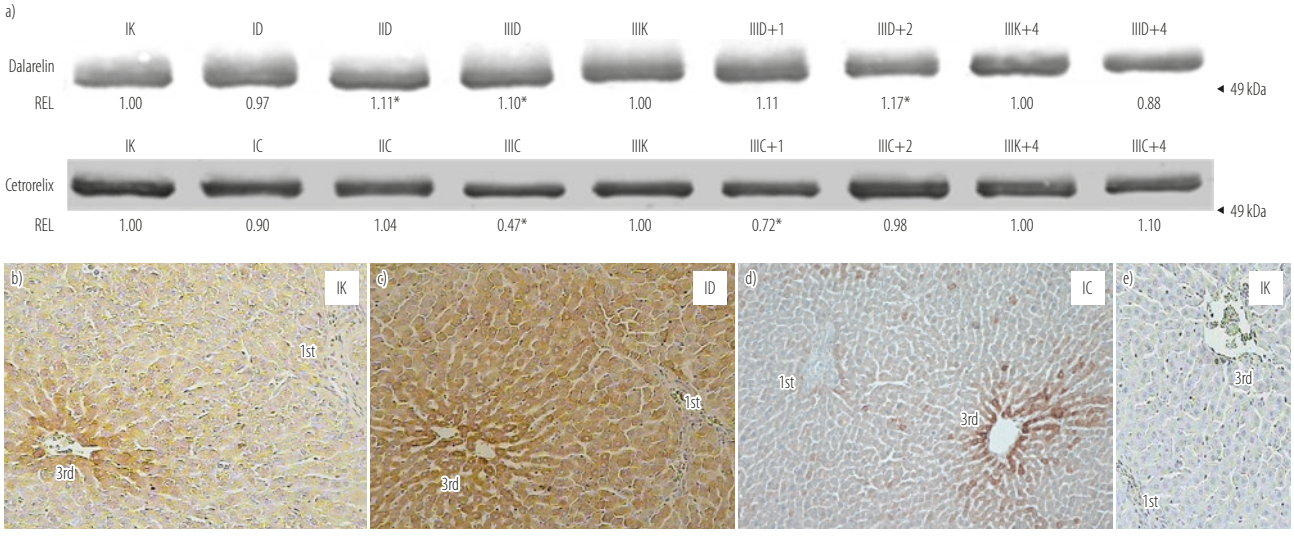
extended after 1 month of dalarelin administration. It was observed not only in zone 3 but also in zones 1 and 2 of the liver acini. Hepatic localization of CYP3A1 remained unchanged after cetrorelix administration and was restricted mostly to zone 3.

Transcriptional factors mRNA expression and PXR immunolocalization

Dalarelin significantly increased PXR mRNA expression only after the 1st month of administration (by 40%, $p < 0.05$) (Table 7).

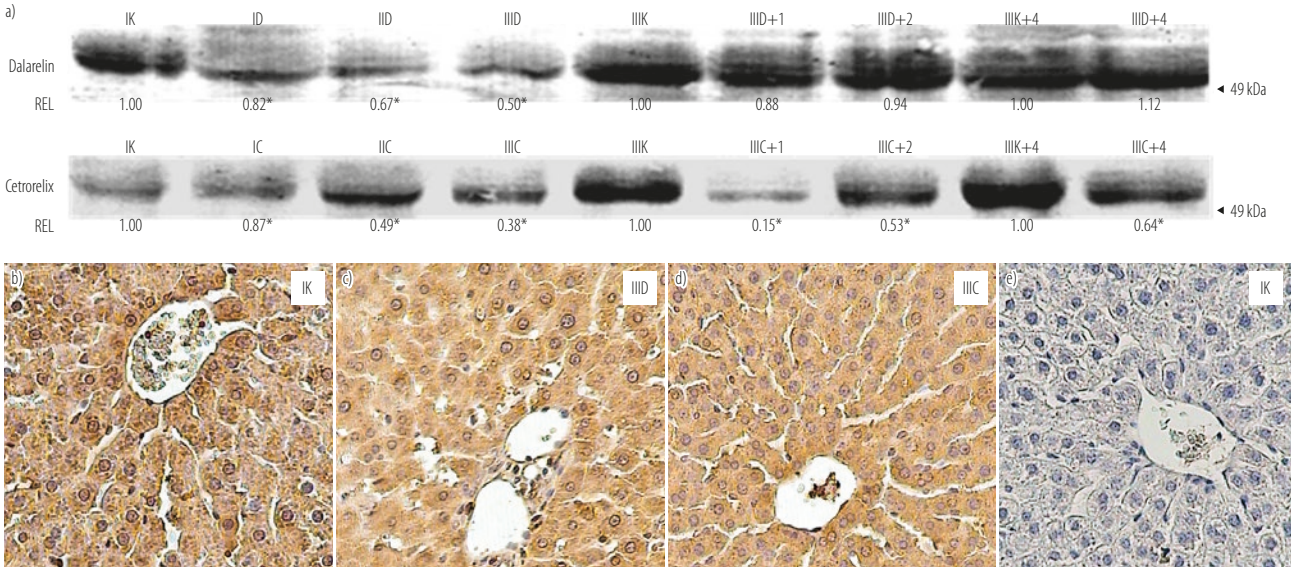
After 1, 2 and 3 months of dalarelin administration, nuclear PXR protein expression decreased by 18%, 33% and 50% ($p < 0.05$), respectively (Figure 8).

These changes were quickly reversible. Cetrorelix did not change PXR mRNA expression, with the exception of lower expression in III+1 group. Significant ($p < 0.05$)



Abbreviations as in Table 2.
Representative samples taken from individual rats are presented. Relative expression level (REL) values are means obtained from 3 independent rats. Positions of molecular weight marker 49 kDa (Bio Rad) are indicated. Samples of liver microsomes contained 10 μg of microsomal protein per lane. Cytochrome 3A1 protein was detectable in the cytoplasm of hepatocytes of both, control and analog-treated females.

Fig. 7. Cytochrome 3A1 a) protein expression in liver microsomes of rats; b) protein immunolocalization (magnification: 100×) in control group, c) dalarelin group, d) cetrorelix group, e) negative control (section without primary antibody)



Abbreviations as in Table 2.
Representative samples taken from individual rats are presented. Relative expression level (REL) values are means obtained from 3 independent rats. Positions of molecular weight marker 49 kDa (Bio Rad) are indicated. Samples of nuclear proteins contained 10 μg of protein per lane. Central veins with surrounding hepatocytes of 3rd zone of the liver acinus are presented.

Fig. 8. Pregnane X receptor a) protein expression in liver nuclear fraction of rats; b) protein immunolocalization (magnification: 200×) in control group, c) dalarelin group, d) cetrorelix group, e) negative control (section without primary antibody)

Table 7. Relative pregnane X receptor (PXR) mRNA expression in the liver of rats[1]

Group	Relative PXR mRNA expression
D	
I (N = 6)	1.40*
II (N = 6)	1.12
III (N = 6)	1.18
III+1 (N = 6)	1.20
III+2 (N = 6)	1.45
III+4 (N = 6)	1.05
C	
I (N = 6)	0.93
II (N = 6)	0.97
III (N = 6)	0.90
III+1 (N = 6)	0.63*
III+2 (N = 6)	0.91
III+4 (N = 6)	0.94

Abbreviations as in Table 2 and 4.

Table 8. Relative transcription factors mRNA expression in the liver of rats[1]

Group	Relative mRNA expression		
	STAT5b	HNF4α	HNF6
D			
I (N = 6)	0.40*	0.66	0.44
II (N = 6)	0.68	0.83	0.75
III (N = 6)	0.54*	1.19	1.21
III+1 (N = 6)	0.86	1.22	1.05
III+2 (N = 6)	0.81	0.92	0.94
III+4 (N = 6)	1.38	2.09	1.96
C			
I (N = 6)	1.05	1.13	0.68
II (N = 6)	1.02	1.74*	1.22
III (N = 6)	0.93	2.28*	2.83*
III+1 (N = 6)	0.87	3.27*	3.14*
III+2 (N = 6)	0.57	2.57*	2.74
III+4 (N = 6)	0.64	3.01*	4.27*

Abbreviations as in Table 1, 2 and 4.

decrease in PXR protein expression (from 13% to 51%) during administration of cetrorelix was also observed, however these changes were persistent for 4 weeks.

In the control and experimental groups, PXR protein expression was distinct in zone 3 of the liver acinus, in the cytoplasm, and in some cases in the nuclei of hepatocytes localized around the central veins (Figure 8). A noticeable but reversible decrease in immunoreactivity of hepatocytes was observed in the group administered dalarelin for 3 months. A similar decrease but more pronounced and lasting longer was observed after 2 months of cetrorelix administration. In both cases, positive immunological reaction was found mainly in the cytoplasm of hepatocytes.

Dalarelin caused the decrease in STAT5b mRNA expression after month 1 (by 60%), month 2 (by 32%) and month 3 (by 46%) of administration, reversible after the period of treatment. In turn, cetrorelix did not cause statistically significant changes in STAT5b mRNA expression during treatment (Table 8).

Dalarelin administration did not cause statistically significant changes in HNF4α mRNA expression (Table 8). In contrast to dalarelin, administration of cetrorelix caused a significant ($p < 0.05$) increase in the HNF4α mRNA expression which persisted for up to 4 weeks after administration. The expression increased from 74% to 227%.

Characteristics of changes in HNF6 mRNA expression were very similar to those observed in the case of HNF4α. Dalarelin administration caused a statistically insignificant decrease in HNF6 mRNA expression in groups ID (by 56%) and IID (by 25%). Administration of cetrorelix caused a significant increase in HNF6 mRNA expression after 2 and 3 months, persisting for up to 4 weeks after analog administration. The expression increased from 22% (in group IIC) to 327% (in group IIIC+4). Most of these changes were statistically significant (Table 8).

All the results of this study are summarized in Table 9.

Table 9. Summary of the study – general tendencies in gonadoliberin (GnRH) agonist (dalarelin)- and GnRH antagonist (cetrorelix)-treated groups compared with proestrus rats

Evaluated parameter	GnRH agonist (dalarelin)	GnRH antagonist (cetrorelix)
Sex hormone concentration		
17β-estradiol	↓*	↓*
progesterone	n.s.	↓*
testosterone	n.s.	n.s.
Growth hormone		
24-h concentration (M)	↓*	↑*
24-h secretion pattern	n.s.	changed (↑*)
Male-specific CYPs expression		
CYP2C11		
mRNA	n.s.	n.s.
protein	n.d.	n.d.
CYP3A2		
mRNA	detectable (↑)	n.d.
protein	n.d.	n.d.
Female-specific CYPs expression		
CYP2C12		
mRNA	n.s.	n.s. (↑* group II)
protein	↓*	n.s. (↑* group II)
CYP3A9		
mRNA	n.s.	↑*
protein	↓*	↑↑*
Sex-independent CYP3A1 expression		
mRNA	n.s. (↑* group I)	↑* (group I and II)
protein	↑*	↓* (group III and III+1)
Transcription factor expression		
PXR		
mRNA	n.s. (↑* group I)	n.s.
protein	↓*	↓*
STAT5b mRNA	↓* (group I and III)	n.s.
HNF4α mRNA	n.s.	↑*
HNF6 mRNA	n.s.	↑*

↑ – most expressions increased; ↓ – most expressions decreased; n.s. – most changes statistically insignificant; n.d. – expression not detectable.
* Most changes statistically significant.
Other abbreviations as in Table 1, 2 and 4.

DISCUSSION

As described earlier, characteristics of 24-h GH secretions are known to be different in adult females and males [3]. Pulsatile nature of the GH secretion is strongly marked in male rats. Twenty-four-hour GH secretion is more stable in females. Previous studies have shown that mean plasma GH level in young (5 months), middle-aged (11 months) and old (25–29 months) female SD rats decreases with age [32]. Mean plasma GH levels measured with radioimmunoassay were 99.1 ng/ml, 56.3 ng/ml and 49.7 ng/ml, respectively. The average amplitude of GH pulses was as follows: 130.4 ng/ml in young, 87 ng/ml in middle-aged and 69.3 ng/ml in old rats.

Here, we have demonstrated that in aging female rats the GH secretion is reduced and declines before month 11 of life (between months 5 and 6). This observation was confirmed by Dhir et al. [33] who compared the mean plasma GH secretion in 3-month-old and 1-year-old female rats. Mean plasma GH concentrations were 44 ng/ml and 29 ng/ml, respectively, and the average amplitudes of GH peaks were 105 ng/ml and 85 ng/ml, respectively.

Our approach to the studies on the mechanism of GH-dependent CYP450 expression was to reflect clinical therapies, in which the hypothalamic-pituitary-gonadal axis was suppressed by long-term administration of GnRH analogs. In this study we expected pharmacological pituitary desensitization ("chemical gonadectomy," "pharmacological castration") by GnRH analogs in adult female rats to affect not only sex hormones but also GH blood levels. Desensitization may indirectly modify the GH total concentration and its 24-h secretion profile due to the possible relations between estrogens and GH action [23,24].

We noticed low concentrations of ovarian sex hormones in GnRH analog-treated rats as compared to control (proestrus) concentrations, and an opposite and statistically significant effect of GnRH agonist and antagonist on the GH blood level. Long-term administration of cetrorelix, GnRH antagonist, elevates GH blood concentrations and causes changes in the 24-h pattern of the GH secretion in female SD rats, from stabilized to characterized by 3 peaks during a 24-h period (at 7:30 a.m, 10:30 a.m. and 4:30 p.m.). Long-term administration of dalarelin, a GnRH agonist, does not cause significant changes in the circadian pattern of the GH secretion. However, it results in the reduction of mean 24-h GH concentrations. These changes persisted after discontinuation of analog administration. Our findings are consistent with the results of the study using GnRH agonist – triptorelin [24]. The authors found that triptorelin decreased baseline GH levels in female rats ($p < 0.01$) but to a lesser extent than ovariectomy.

Our observations have indicated that the mechanisms of regulation of circadian GH secretion profiles by GnRH agonist and antagonist are extremely different. They probably depend not only on sex hormone levels (because of inhibited secretion) but also other factors which are activated during the process of desensitization, different for agonist and antagonist action. It is why we have observed slight changes in sex-independent CYP3A1 protein expression: after administration of dalarelin, CYP-3A1 expression increased slightly whereas the effect cetrorelix was opposite. However, changes in the 24-h pattern of the GH secretion and in the mean 24-h GH concentrations may be crucial for the GH-dependent and sex-specific expression of hepatic CYP450, especially in cases when concentrations of sex hormones are very low or undetectable [8,32]. It is known that as little as 3% of the normal concentration of the GH found in female rats is sufficient to decrease CYP3A2 and CYP-2C11 expression in males, and to cause feminization of the hepatic CYP450 [9].

Concurrently with different effects of the agonist and antagonist on plasma GH levels and changes in the 24-h GH secretory amplitude under the influence of cetrorelix, we observed different expressions of

male- and female-specific CYP450 isoforms. It allowed us to effectively identify the effects caused by each analog at the level of molecular mechanism regulating GH-dependent CYP450 expression. These changes facilitate identification of the transcription factors responsible for the GH-dependent CYP450 expression.

It should be noted that hormonal changes caused by dalarelin and cetrorelix resulted in 24-h hormonal patterns which were not male-specific. Putting together the results on male-specific CYP450 expressions, we found CYP2C11 mRNA but not CYP2C11 protein expression, in both intact and analog-treated rat liver. It should be noted that CYP2C11 mRNA expression was also detected in other studies, in the liver, spleen, thymus and bone marrow of intact female rats [34]. The expression of male-specific CYP3A2 isoform was also detected but only at low and individually variable level in the livers of females administered dalarelin for 1, 2 and 3 months.

The studies on the expression of female-specific isoforms have shown that although CYP2C12 mRNA and CYP3A9 mRNA expression after 3 months of dalarelin administration was statistically unchanged, appropriate protein expressions were significantly reduced during the exposure period. Similar assumptions were made by the group of Banerjee [35,36] who investigated some clinical protocols in male and female rats by application of somatostatin analog, octreotide. In octreotide-treated female SD rats the GH profiles were characterized by almost 25% reduction in plasma levels, including both pulse and interpulse components but still contained female-like secretory profile. Female-specific CYP450 expression levels were dramatically down-regulated, which could not be explained as a result of alterations in the feminine circulating GH profile.

In turn, in our study the expression of female-specific isoforms, especially CYP3A9, was significantly increased under the influence of GnRH antagonist – cetrorelix. In general, both at mRNA and protein levels, the effect of analogs on CYP3A9 isoform is much more distinct as compared to CYP2C12.

The number of publications dealing with the effects of GnRH analogs administration on CYP450 is highly limited. It was found that buserelin (GnRH agonist) administration to rat males resulted in the decrease (in a dose-dependent manner) in the serum level of testosterone, and CYP450-dependent activity of aminopyrine N-demethylase, 7-propoxycoumarin O-depropylase and testosterone 2α-hydroxylases and 16α-hydroxylases but not aniline p-hydroxylase [37]. Another GnRH agonist, leuprolide, exerted a direct inhibitory action on gonadotropin-induced follicular development by decreasing temporal expression of thecal CYP450 17α-hydroxylase, androgen production, and as a consequence, reduction of estrogen concentrations in developing follicles [38]. Immunohistochemical studies have indicated that leuprolide induces steroidogenic acute regulatory protein (StAR) expression mainly in the theca cells of primary and secondary follicles, and significantly increases levels of progesterone and decreases androsterone in the blood serum, respectively.

Our study has shown differentiated effects of dalarelin and cetrorelix on the expression of selected transcription factors, namely: STAT5b, HNF4α and HNF6. Signal transducer and activator of transcription 5b is thought to be the main transcription mediator of sex-dependent effects of the GH in the liver, due to its stimulatory effects on male-specific genes and inhibitory effects on female-specific genes. In the liver, its activity depends on plasma GH pulses [3].

In our study, dalarelin, unlike cetrorelix caused a reversible decrease in the expression of STAT5b mRNA. However, higher or lower STAT5 activity is not critical for the GH-regulated hepatic sex-dependent gene expression. The specific pattern of activity is crucially intermittent in males and continuous in females. Nevertheless, the decreased GH blood concentrations, stabilization of the 24-h GH secretory profile, as well as

inhibition of STAT5b transcription factor by dalarelin did not promote induction of female-specific: CYP2C12 and CYP3A9 proteins. On the other hand, male-specific isoforms were induced at the same time.

Zhang et al. [39] found a strong correlation between sex-dependent STAT5 binding and sex-biased target gene expression. It was associated, among others, with several sex-dependent epigenetic modifications and sex-differentially enriched motifs for HNF6/CDP (CCAAT enhancer displacement protein) family factors. Moreover, these authors observed that lipid metabolism and CYP450-dependent reactions are top functional categories enriched in STAT5 and BCL6 (B-cell lymphoma 6) transcriptional repressor common target genes. Binding sites for BCL6 overlap those of STAT5, which shows male-biased expression in the liver, and is down-regulated by the female plasma GH profile.

Although STAT5b is the most important among STAT proteins, in some cases STAT5b has to cooperate with STAT5a. For example, both proteins may activate CYP2C12 gene expression by binding sequences which are unavailable in males [40].

In addition to STAT5b, liver-enriched transcription factors HNF4α and HNF6 are also crucial for the GH- and sex-dependent liver gene expression. Hepatocyte nuclear factor 6 is considered to be an early (during 1st 24-h) GH response gene being a direct target for the GH action in the liver, the expression of which is induced by this hormone [13]. The question whether these factors cooperate directly with STAT5 (STAT5b and STAT5a) to regulate hepatic sex differences remains to be elucidated [39]. The study of Conforto and Waxman, revealed that HNF4α was one of the main transcriptional regulators involved in the developmental changes that led to global acquisition of rodent liver sex-specificity by 8 week of age [41]. Hepatocyte nuclear factor 4α was found to be a regulator of several animal (CYP2A, CYP2C, CYP3A) and human CYP450 (CYP2C9, CYP2D6, CYP3A4) expressions, in the case of which it binds with the GT-TAAT sequence [11,16]. Transcription factors, such as HNF-4α, are essential for a proper induction of CYP450 genes with the participation of PXR (human CYP2C9 and CYP3A4) and constitutive androstane receptors (CAR) (CYP2C9) [42]. Pregnane X receptor agonists, such as androgens, dexamethasone, μ-benzene hexachloride (μ-BHC), and some other endocrine disruptors may induce CYP2C11 mRNA in males and females [43]. Our study has shown that both, dalarelin and cetrorelix, significantly decreased PXR protein expression after 1, 2 and 3 months. Cetrorelix-induced changes were persistent even for 4 weeks after the administration.

Hepatocyte nuclear factor 4α is generally known as the positive regulator of "male" CYP2C11 expression whereas HNF6 is known as the positive regulator of "female" CYP2C12, and negative of "male" CYP2A2 expression. Hepatocyte nuclear factor 6 and HNF3β are 2–3-times more expressed in female rat liver [44]. The loss of female-specific GH secretion pattern, following hypophysectomy, resulted in the decrease of HNF6 and HNF6-dependent HNF3β activity, which was reflected by strong activation of male-specific CYP2A2 transcription and inhibition of CYP2C12 expression [45]. On the other hand, HNF6/Onecut 1 probably cooperates with STAT5 in positive regulation of some male-biased genes [39].

We have noticed that the influence of GnRH agonist and antagonist is very similar in the case of HNF4α and HNF6 but different comparing to STAT5b, which seems to be crucial for the regulation of the parity of analog/GH-dependent female/male CYP450 expression. The changes in the expression of HNF4α and HNF6 caused by cetrorelix (a significant increase in both HNF4α and HNF6 mRNA expression) were much more significant and persistent as compared to dalarelin. The changes caused by cetrorelix were accompanied by an increased expression of the female-specific isoforms and unchanged

expression of male-specific isoform CYP2C11 and CYP3A2 as well as by changes in the circadian GH secretory pattern and higher mean 24-h values.

CONCLUSIONS

The expression of male- and female-specific CYP2C and CYP3A cytochromes during a long-term administration of low dose of GnRH analogs is regulated by different, analog-dependent mechanisms which may concern different 24-h patterns and levels of the GH secretion, and lowered sex hormone concentrations.

Long-term administration of cetrorelix, GnRH receptor antagonist, elevates the GH blood concentrations and causes changes in the 24-h pattern of the GH secretion in female rats, from stabilized to characterized by high peaks during a light phase. Long-term administration of dalarelin, GnRH agonist, does not cause significant changes in the circadian pattern of the GH secretion. However, it results in the reduction of mean 24-h GH concentrations. Changes in the circadian pattern/mean level of the GH secretion induced by cetrorelix may cause an increased expression of female-specific isoforms, especially – CYP3A9, and a decreased expression in the CYP3A1 protein. These hormonal changes do not affect male CYP2C11 and CYP3A2 protein expressions.

Increased expression of female-specific isoforms is accompanied by an increased expression of the transcription factors: HNF4α and HNF6, which may indicate their significant role in cetrorelix/GH-dependent regulation of the expression of female-specific CYP450 isoforms.

Decreased GH blood concentrations and stabilization of the 24-h GH secretory profile by dalarelin may promote inhibition of female-specific: CYP2C12 and CYP3A9 proteins as well as STAT5b transcription factor. Induction of male-specific isoform CYP2C11 and CYP3A2 at higher doses of dalarelin cannot be excluded.

Inhibition of the PXR protein expression caused by GnRH analogs indicates that PXR may not be directly related to the changes in the analog/GH-dependent parity of female/male CYP2C and CYP3A expressions in female rats.

Changes in the expression of CYP2C and CYP3A isoforms are probably not a simple result of an increase/decrease in sex positive/negative transcriptional regulators but rather of complex crosstalk between endogenous hormones and GnRH analogs.

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