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Serotonin receptors of 5-HT_2 type in the hypothalamic arcuate nuclei (ARC) positively regulate liver cytochrome P450 *via* stimulation of the growth hormone-releasing hormone – growth hormone (GHRH-GH) hormonal pathway

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Running title page

a) The running title:

Brain 5-HT_2 receptors positively regulate cytochrome P450

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The number of words in the introduction – 613
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d) ABBREVIATIONS: ARC, arcuate nucleus; PVN, paraventricular nucleus; CNS, central nervous system; CYP, cytochrome P450; HPLC, high performance liquid chromatography; 5-HT, serotonin; GHRH, growth hormone-releasing hormone; GH, growth hormone; CRT, corticosterone; T3, triiodothyronine; T4, thyroxine; TST, testosterone

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ABSTRACT

Our recent study carried out after local injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine into the arcuate nuclei (ARC) of the hypothalamus suggested a positive influence of the serotonergic innervation of the ARC on growth hormone secretion and growth hormone-dependent expression of cytochrome P450. The aim of our present study was to determine the effect of the activation of the 5-HT_1 or 5-HT_2 type receptors in the ARC on the expression and activity of cytochrome P450 in the liver of male rats. The serotonergic agonists 5-CT (5-carboxyamidotryptamine, a 5-HT_1 type receptor agonist) or DOI (2,5-dimethoxy-4-iodoamphetamine, a 5-HT_2 type receptor agonist) were injected into the ARC for 5 days. The activity and expression of cytochrome P450 isoenzymes and the levels of serum and pituitary hormones were estimated. DOI significantly increased the activity and expression (both mRNA and protein levels) of *CYP2C11*, *CYP3A1/23* and *CYP3A2*, which positively correlated with an increase in the pituitary GHRH and serum growth hormone level. The injection of 5-CT into the ARC did not affect the activity of liver P450 enzymes or hormone levels. The obtained results indicate that 5-HT_2, but not the 5-HT_1 type receptors in the ARC, are engaged in the positive neuroendocrine regulation of cytochrome P450, possibly by the stimulation of hypothalamic GHRH release and pituitary GH secretion, and an increase in the serum growth hormone concentration. Further studies are going to identify which of the 5-HT_2 receptor subtypes (5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C}) is responsible for the observed neuroendocrine regulation of cytochrome P450.

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Introduction

Our earlier studies conducted after damage to or stimulation of the brain serotonergic system by intracerebroventricular administration of the serotonergic neurotoxin 5,7-dihydroxytryptamine or 5-hydroxytryptophan (respectively) showed an important role of this brain system in the physiological neuroendocrine regulation of cytochrome P450 expression and activity in the liver (Rysz et al., 2015; Rysz et al., 2016a). Moreover, the results obtained using local intrahypothalamic injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine showed the reverse effect of the serotonergic innervation of the paraventricular (PVN) and arcuate (ARC) nuclei of the hypothalamus on the regulation of the main male P450 enzyme, CYP2C11 in the rat (Rysz et al., 2016b). The observed effect was negative in the case of the PVN and positive in the case of the ARC, and was accompanied by respective changes in growth hormone (GH) secretion. Recent studies carried out after repeated injection of selective 5-HT_1 and 5-HT_2 receptor agonists have revealed that activation of 5-HT_{1A} receptor in the PVN negatively regulates liver cytochrome P450 expression and activity in the rat. The activation of the 5-HT_{1A} receptor in the PVN produced an increase in somatostatin release and a decrease in growth hormone and corticosterone concentration in the blood, which led to a reduction in the hormone-dependent CYP2C11 and CYP3A1/23 expression and activity in the liver (Bromek et al., 2018).

It has been suggested that the stimulating effect of brain serotonin on GH secretion may proceed *via* 5-HT_1 or 5-HT_2 type receptor stimulation which increases growth hormone-releasing hormone (GHRH) release from the ARC (Murakami et al., 1986; Willoughby et al., 1987; Katz et al., 1996). The ARC nuclei receive serotonergic innervation from the frontal raphe groups of serotonergic neurons such as the dorsal and median raphe nuclei (Gruber et al., 1987; Willoughby and Blessing, 1987). By acting on its receptors in the ARC serotonin

may affect the synthesis and release of GHRH which, in turn, stimulates pituitary secretion of GH.

GH is the main physiological regulator of liver CYP2C11 and one of the regulators of other P450 enzymes, such as CYP3A enzymes in rat liver (Waxman and O'Connor, 2006; Waxman and Holloway, 2009; Wójcikowski and Daniel, 2011). Our previous studies showed that the ARC-mediated stimulation of pituitary GH secretion was strongly involved in the regulation of cytochrome P450 by brain monoaminergic neurotransmitters. Damage to one of the monoaminergic innervation pathways of the ARC (dopamine, noradrenaline or serotonin) decreased plasma GH level and GH-stimulated cytochrome P450 expression and activity (Wójcikowski et al., 2007; Wójcikowski and Daniel, 2009; Bromek et al., 2013; Rysz et al., 2016b). However, a particular 5-HT receptor type/subtype of the ARC, which is engaged in the neuroendocrine regulation of liver cytochrome P450 has not been found, as yet.

Considering a possible presence of both 5-HT_1 and 5-HT_2 receptor types in the ARC (Steffens et al., 2008), the aim of this work was to identify the 5-HT receptor types located in the ARC (5-HT_1 or 5-HT_2) that are engaged in the cytochrome P450 regulation in the liver. To achieve this goal we injected selective 5-HT_1 and 5-HT_2 receptor agonists in pharmacologically active doses into the ARC. GHRH produced therein is conveyed by neuronal transport to the median eminence and then *via* portal circulation it is carried forward to the pituitary where it stimulates the secretion of GH (Bluet-Pajot et al, 1998; Wójcikowski and Daniel, 2011). Therefore, the level of pituitary GHRH and serum GH as well as serum concentrations of other hormones that may directly regulate cytochrome P450 (corticosterone, thyroid hormones) were measured. At the same time, hormone-dependent expression and activity of liver P450 enzymes were examined. The obtained results indicate a new neuroendocrine mechanism of liver cytochrome P450 regulation by the brain serotonergic system.

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Materials and Methods

Animals. Male Wistar Han rats weighing 280–300 g (Charles River Laboratories, Sulzfeld, Germany) were housed under standard laboratory conditions (a 12 h light/12 h dark cycle; a temperature of 22 ± 2ºC; a room humidity of 55 ± 5%). The animals had free access tap water and standard laboratory food; however, 18 h before decapitation, they were deprived of food. All experiments were carried out in accordance with the 86/609/EEC Directive and received approval of the Local Bioethics Commission at the Institute of Pharmacology of the Polish Academy of Sciences in Kraków.

Drugs and chemicals. The 5-HT_1 receptor agonist 5-carboxyamidotryptamine maleate (5-CT) was obtained from Sigma (St. Louis, MO, USA) and the 5-HT_2 receptor agonist 2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) from Tocris (Bristol, UK). Testosterone and its specific hydroxy-metabolites were provided by Steraloids (Newport, KY, USA). The following antibodies were used: the polyclonal primary anti-rat CYP2C11 antibody from Abcam (Cambrige, UK), the anti-rat CYP3A1/23 and CYP3A2 antibodies from Millipore (Temecula, USA) and the polyclonal anti-rat β-actin antibody from Santa Cruz (Dallas, TX, USA). The chemiluminescence reagents LumiGlo kit came from KPL (Gaithersburg, MD, USA). The ELISA kits for pituitary GHRH (Elabscience, Bethesda, MD, USA), for growth hormone and corticosterone (Demeditec, Kiel, Germany), for T_3 and T_4 (Cloud-Clone Corp., Katy, Texas, USA) were used. A mirVana kit, TaqMan assays and the TaqMan Gene Expression Master Mix from Life Technologies (Carlsbad, CA, USA), and a Transcriptor High-Fidelity cDNA synthesis kit from Roche Diagnostics (Indianapolis, IN,

USA) were used for RNA isolation and mRNA estimation. Ketamine hydrochloride and xylazine hydrochloride were produced by Biowet (Puławy, Poland).

Surgery and local injection of 5-CT or DOI into the ARC. The rats were anesthetized with ketamine HCl (65 mg/kg i.p.) and xylazine HCl (5 mg/kg i.p.) and then placed in the Kopf stereotaxic apparatus (Tujunga, CA, USA). Stainless-steel guide cannulas were implanted bilaterally into the arcuate nuclei (ARC) of the hypothalamus. The following coordinates were applied (Paxinos and Watson, 2007): AP –2.6 (anterior-posterior from the bregma), L ±0.4 (lateral) and V –7.4 (ventral from the surface of the dura). Local injections into the ARC were made through an inner cannula (V –9.4) 7 days after implanting the guide cannula. 5-CT (a general 5-HT_1 receptor agonist) or DOI (a general 5-HT_2 receptor agonist) were dissolved in 0.9% NaCl and injected into the ARC at a concentration of 10 μg/μl (0.5 μl infused at a rate of 0.5 μl/min) or 6 μg/μl (0.5 μl infused at a rate of 0.5 μl/min), respectively, once a day for five days. The pharmacological doses of the serotonergic agonists were selected on the basis of literature data (Mamede Rosa and Prado, 1997; Willins and Meltzer, 1997; Fletcher and Korth, 1999; Fletcher et al, 2002; Currie et al., 2010; de Paula et al, 2012). Control rats were subjected to the same procedure as were the 5-HT receptor agonist-treated animals (stainless-steel guide cannulas were implanted bilaterally into the ARC), except that they received a vehicle (0.9% NaCl) instead of one of the agonists. The vehicle, 0.5 μl, or an agonist solution was administered into the ARC using a Hamilton syringe (at a flow rate of 0.5 μl/min), which was left in place for 5 min after injection. Two hours after the last injection the rats were sacrificed by decapitation. The position of the needle in the hypothalamus was histologically controlled in a preliminary experiment with ten rats (using a color marker to visualize the needle track).

Collection of pituitary, liver and serum samples. At 2 h after the last injection of the five-day administration of the serotonergic agonists 5-CT or DOI, the animals were

decapitated. The pituitaries and livers were isolated and stored at –80ºC until they were further processed. The blood serum was separated and liver microsomes were prepared from individual animals by differential centrifugation as described previously (Kot and Daniel, 2008). The obtained sera and microsomes were stored at –80°C.

Determination of P450 enzyme activities in liver microsomes. The activities of P450 enzymes in liver microsomes were measured by conducting specific metabolic reactions under linear conditions, as described previously. The activity of CYP1A was examined by measuring the rate of caffeine C-8-hydroxylation and 3-N-demethylation (Kot and Daniel, 2008). The activities of CYP2A, CYP2B, CYP2C11 and CYP3A were estimated by measuring the rate of testosterone hydroxylation in specific positions: 7α-, 16β-, 2α- and 16α-, and 2β- and 6β-hydroxylation, respectively (Haduch et al., 2006; Haduch et al., 2008; Wójcikowski et al., 2013). The substrates and the metabolites formed were analyzed using HPLC with UV detection, as described previously.

Estimation of the cytochrome P450 protein levels in liver microsomes. The levels of CYP2C11, CYP3A1/23 and CYP3A2 in the liver microsomes (10 μg of proteins) of control and 5-HT receptor agonist-treated rats were estimated by Western immunoblot analysis, as previously described (Rysz et al., 2015; Rysz et al., 2016a,b). Polyclonal rabbit anti-rat CYP2C11, CYP3A1/23 or CYP3A2 antibodies and a secondary antibody (a species-specific horseradish peroxidase-conjugated anti-IgG) were applied (1:2000 dilution of both primary and secondary antibodies). After incubation with a primary antibody (15 h at 8°C), the blots were incubated with a secondary antibody (2 h at room temperature). Rat cDNA-expressed CYP2C11 (5 μg), CYP3A1/23 and CYP3A2 (1 μg) (Supersomes, Gentest) were used as standards. The bands intensity on a nitrocellulose membrane was quantified with a Luminescent Image Analyzer. The data were normalized to protein loading based on the β-actin levels (Rysz et al., 2015; Rysz et al., 2016a,b).

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Determination of the pituitary GHRH and serum hormones. Hormonal effects were measured 2 h after the last dose of one of the serotonergic agonists. The levels of pituitary GHRH and serum hormones were measured using the following ELISA kits: GHRH kit (Elabscience); growth hormone and corticosterone kits (Demeditec); T_3 and T_4 kits (Cloud-Clone Corp.). Pituitaries were processed according to the manufacturer's instruction. Briefly, pituitaries were homogenized in PBS (phosphate buffer saline, pH=7.0; 1:20 w/v). The homogenates were stored at –20°C for 24 h and then centrifuged for 5 min at 5000g. The obtained supernatants were transferred to clean Eppendorf vials for ELISA. Absorbance was measured using a microplate reader (Rysz et al., 2015; Rysz et al., 2016a,b).

Isolation of liver RNA, cDNA synthesis and quantitative real-time polymerase chain reaction (qRT-PCR) measurements. Livers were homogenized, and total RNA was extracted using a mirVana isolation kit (Rysz et al., 2016a). The first-strand cDNA products were generated using a Transcriptor High Fidelity cDNA Synthesis Kit. The expression of the genes coding for the studied P450 enzymes (*CYP2C11, CYP3A1/23,* and *CYP3A2)* and of the reference genes *hypoxanthine phosphoribosyltransferase 1 (HPRT1)* and *glyceraldehyde-3-phosphate dehydrogenase (GAPDH*) was detected by real-time PCR using TaqMan Gene Expression Master Mix and species-specific TaqMan type probes and primers as described previously (Bromek et al., 2018). The gene names and identification numbers of the TaqMan primers applied in the analysis are as follows: *CYP2C11* (Rn01502203_m1), *CYP3A1/23 (*Rn03062228_m1*), CYP3A2 (*Rn00756461_m1*), HPRT1* (Rn01527840_m1), and *GAPDH* (Rn01462662_g1). Real-time PCR runs were performed using the Bio-Rad CFX96 PCR system (Bio-Rad, Hercules, CA, USA). The PCR reactions were run in duplicate. The level of the *CYP* gene's transcripts was normalized to the *HPRT1* and *GAPDH* genes' expression, and the relative quantification was calculated using a comparative delta-delta Ct method ($2^{-\Delta\Delta Ct}$).

Statistical analysis of the data. The obtained data were statistically assessed using a one-way analysis of variance (ANOVA) followed by Duncan's post hoc test or by a two-tailed Student's *t*-test. The results were regarded as statistically significant when $p < 0.05$.

Results

The expression and activity of liver cytochrome P450 were studied after the injection of pharmacological doses of the selected 5-HT_1 and 5-HT_2 receptor agonists into the ARC (bilaterally). The applied doses were shown to produce specific biochemical and/or behavioral effects after intracerebral administration of 5-CT (Mamede Rosa and Prado, 1997; Fletcher and Korth, 1999) or DOI (Willins and Meltzer, 1997; Currie et al., 2010; de Paula et al, 2012).

The effect of the local injection of 5-HT_1 and 5-HT_2 receptor agonists into the ARC on the activity of cytochrome P450 in liver microsomes. The repeated injection of 5-CT (a 5-HT_1 type receptor agonist) into the ARC did not affect the activity of any P450 enzyme studied in liver microsomes (Fig. 1 A). However, in rats injected with DOI (a 5-HT_2 type receptor agonist) into the ARC (3 μg/side) for five days, the activities of CYP2C11 and CYP3A significantly increased (Fig. 1 B). The CYP2C11 activity, estimated as a rate of the 2α- and 16α-hydroxylation of testosterone, was enhanced to 164% and 207% of the control, respectively, at 2 h after the last injection. At the same time, the activity of CYP3A, assessed as the rate of the 2β- or 6β-hydroxylation of testosterone, was elevated to 140% and 150% of the control, respectively. The activities of CYP2A (estimated as the rate of the 7α-hydroxylation of testosterone), CYP2B (evaluated as the rate of the 16β-hydroxylation of testosterone) or CYP1A (presented as the rate of caffeine C-8-hydroxylation and 3-N-demethylation) were significantly altered.

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The effect of the local injection of the 5-HT_2 receptor agonist DOI into the ARC on the expression of liver cytochrome P450. Looking for the molecular mechanisms of the 5-HT_2 receptor agonist-produced increases in the activity of CYP2C11 and CYP3A, the cytochrome P450 expression was investigated in the liver after the repeated administration of DOI into the ARC. The observed increases in the activity of CYP2C11 and CYP3A correlated positively with the enzyme expression level of the respective genes observed at 2 h after the last injection of the 5-HT_2 receptor agonist DOI (Fig. 2, Table 1). DOI significantly increased the CYP2C11 protein level, CYP3A1/23 and CYP3A2 to 170%, 187% and 170% of the control, respectively (Fig. 2). Accordingly, the *CYP2C11, CYP3A1/23,* and *CYP3A2* mRNA levels significantly increased after the injection of DOI (Table 1). The repeated injection of the 5-HT_1 agonist 5-CT into the ARC did not affect the protein level of CYP2C11, CYP3A1/23 and CYP3A2 (Supplemental Data, Fig. 1S).

The effect of the local injection of the 5-HT_2 receptor agonist DOI into the ARC on the level of pituitary GHRH and serum hormones. With the aim to find the central neuroendocrine mechanisms of the 5-HT_2 receptor agonist DOI-evoked increases in the *CYP2C11, CYP3A1/23* and *CYP3A2* expression in the liver, the levels of the ARC-produced hormone GHRH and pituitary-synthesized GH were measured in the pituitary and blood serum, respectively. Moreover, the concentrations of serum corticosterone (CRT) and thyroid hormones (T_3 and T_4) that, like GH, may directly regulate cytochrome P450 expression were also analyzed (Fig. 3). The ELISA tests revealed that a repeated administration of the 5-HT_2 receptor agonist DOI produced an increase in the GHRH level in the pituitary (to 137% of the control) and in the serum concentration of GH (to 330% of the control) at 2 h after the last dose of five-day injection of the agonist (Fig. B). In contrast, the injection of the 5-HT_1 receptor agonist 5-CT did not increase pituitary GHRH or serum GH level. The serum

concentrations of corticosterone or thyroid hormones (T_3, T_4) were not affected by 5-CT or DOI.

Discussion

Our previous study carried out after local damage to the serotonergic innervation of the paraventricular (PVN) or arcuate (ARC) nuclei showed an important role of these hypothalamic areas in the regulation of liver cytochrome P450 *via* a neuroendocrine mechanism including growth hormone (Rysz et al., 2016 b). Our recent study, carried out after intracerebral injection of type 5-HT_1 or 5-HT_2 receptor agonists into the PVN, allowed for identification of the serotonergic receptor subtype engaged in that neuroendocrine regulation (Bromek et al., 2018). The obtained results indicated that the serotonergic 5-HT_{1A} receptors present in the PVN were involved in the central neuroendocrine regulation of liver cytochrome P450. The activation of the 5-HT_{1A} receptor in the PVN produced an increase in somatostatin release and a decrease in growth hormone and corticosterone concentration in the blood, which led to a reduction in the hormone-dependent CYP2C11 and CYP3A1/23 expression and activity.

Our present study shows that in contrast to the PVN, in the ARC 5-HT_2, but not 5-HT_1 type receptors are involved in the neuroendocrine regulation of cytochrome P450. Moreover, the regulation of cytochrome P450 by 5-HT_2 receptors of the ARC, which proceeds by GHRH-GH hormonal pathway, is positive as compared to the negative regulation of the enzyme by 5-HT_1 receptors of the PVN. The applied intracerebral dose of DOI (3 μg/side) was pharmacologically effective not only in our experiment, since it was shown to elicit also other biochemical or behavioral responses in rodents (e.g. head-twitch responses) which were

inhibited by 5-HT_2 receptor antagonists (Willins and Meltzer, 1997; Currie et al., 2010; de Paula et al, 2012). This corroborates our finding of the engagement of 5-HT_2 receptors in the ARC in the regulation of liver cytochrome P450 expression.

Rats receiving an injection of the non-selective 5-HT_2 type receptor agonist DOI into the ARC for five days showed the increased activity of the principal P450 enzymes in the liver of male rats, i.e. CYP2C11 and CYP3A at 2 h after the last injection. The two reactions, namely the 2α- and 16α-hydroxylation of testosterone are characteristic of CYP2C11 while 2β- or 6β-hydroxylation reactions of testosterone are specific for both CYP3A enzymes (CYP3A1/23 and CYP3A2). CYP3A1/23 is expressed constitutively to a lesser degree (Gibson et al., 2002). In contrast, the repeated injection of the non-selective 5-HT_1 type receptor agonist 5-CT into the ARC did not affect the activity of CYP2C11 or CYP3A.

The increased activity of the above three P450 enzymes, produced by the repeated injection of the 5-HT_2 receptor agonist DOI into the ARC, correlated positively with the enzyme expression level (mRNA and protein) in the liver. DOI significantly enhanced the mRNA levels of *CYP2C11, CYP3A1/23* and *CYP3A2* genes and their respective protein products in the liver. This indicates that the activation of 5-HT_2 type receptor in the ARC stimulates the synthesis of the liver CYP2C11, CYP3A1/23 and CYP3A2 protein at the transcriptional level, and this effect is opposite to that observed previously after the bilateral damage to the serotonergic innervation of the ARC (Rysz et al., 2016b).

Liver cytochrome P450 is regulated hormonally by pituitary-produced growth hormone (GH), adrenal corticosterone (CRT) and thyroid hormones (T_3 and T_4). In our experiment, the 5-HT_2 receptor agonist-evoked increases in cytochrome P450 expression and activity were accompanied by elevation of the pituitary GHRH and serum GH with no change in serum concentrations of corticosterone or thyroid hormones. Growth hormone is the main

physiological factor stimulating the expression of the *CYP2C11* gene in male rats due to its pulsatile secretion by the anterior pituitary gland (Waxman and O'Connor, 2006; Waxman and Holloway, 2009). A good positive correlation between the serum GH concentration and the protein level and activity of CYP2C11 in the liver was found in male rats (Kot et al., 2015). Moreover, apart from corticosterone, the main CYP3A regulator (Gibson et al., 2002; Dvorak et al., 2010), GH can also positively regulate the CYP3A subfamily enzymes (Waxman et al., 1995; Waxman and Holloway, 2009). A positive correlation between the GH concentration and the CYP3A activity was observed in the liver of male rats (Kot et al., 2015). Thus, the observed increase in the expression of liver *CYP2C11, CYP3A1/23* and *CYP3A2* may be connected with the enhanced GH concentration in blood serum. An alteration of the temporal pattern of serum GH (a shift from pulsatile to continuous secretion) would possibly produce a decrease, rather than an increase in the expression and activity of these enzymes in our experiment (Waxman and O'Connor, 2006).

In conclusion, the injection of the selected 5-HT_1 and 5-HT_2 type receptor agonists at pharmacologically active doses into the arcuate nuclei (ARC) of the hypothalamus, followed by the estimation of pituitary GHRH, serum hormone levels and liver cytochrome P450 expression, showed a new brain serotonergic mechanism engaged in the neuroendocrine regulation of liver cytochrome P450 (Fig. 4). The collected data indicate that the serotonergic receptors of 5-HT_2 type, but not of 5-HT_1 type present in the ARC, are involved in the central neuroendocrine regulation of cytochrome P450 in the liver. The activation of the 5-HT_2 receptor in the ARC produces an increase in GHRH release and pituitary growth hormone secretion, which possibly leads to an elevation in the hormone-dependent *CYP2C11, CYP3A1/23 and CYP3A2* genes' expression and activity in the liver. Since 5-HT_2 receptors are engaged in the mechanisms of pharmacological action of CNS drugs (e.g. atypical neuroleptics, antidepressants or anti-obesity drugs), treatment with these drugs may affect the

physiological regulation of hormone-dependent P450 enzymes that take part in the metabolism of endogenous substrates and other co-administered drugs (Meltzer and Massey, 2011; Quesseveur et al., 2012; Rosenzweig-Lipson et al., 2012; Jensen et al., 2010; Nigro et al., 2013). However, further studies are necessary to identify which of the 5-HT_2 receptor subtypes (5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C}) present in the ARC is responsible for the observed central neuroendocrine regulation of cytochrome P450 expression in the liver.

Authorship Contributions:

Participated in research design: Daniel
Conducted experiment: Bromek, Rysz, Haduch
Performed data analysis: Bromek, Rysz, Haduch, Daniel
Wrote or contributed to the writing of the manuscript: Daniel, Bromek, Rysz

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Footnotes

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Legend for Figures

Fig. 1. The effect of the repeated injection of the 5-HT_1 receptor agonist 5-CT (**A**) and the 5-HT_2 receptor agonist DOI (**B**) into the ARC (5 or 3 μg/side, respectively) on different CYP isoenzyme activities, measured as the rates of P450 enzyme-specific reactions in rat liver microsomes: testosterone 7α- (CYP2A), 16β- (CYP2B), 2α- and 16α- (CYP2C11), and 2β- and 6β- (CYP3A) hydroxylation and caffeine 8-hydroxylation and 3-N-demethylation (CYP1A). All values are shown as the mean ± S.E.M. (n=8-10). Statistical significance was assessed by a one-way ANOVA followed by Duncan's test and indicated as *$p < 0.05$, **$p < 0.01$, compared to the control. The control values (pmol/mg protein/min) are as follows: A, B – 154.8 ± 10.4, 219.1 ± 35.0, 431.4 ± 36.9, 315.1 ± 61.4, 253.6 ± 23.9 and 810.3 ± 97.5 (testosterone 7α-, 16β-, 2α-, 16α-, 2β- and 6β-hydroxylation, respectively), and 2.1 ± 0.1 (caffeine 8-hydroxylation), 16.0 ± 1.2 (caffeine 3-N-demethylation). ARC – the arcuate nucleus of the hypothalamus.

Fig. 2. The effect of the repeated injection of the 5-HT_2 receptor agonist DOI into the ARC (3 μg/side) on the protein level of CYP2C11, CYP3A1/23 and CYP3A2 enzymes in rat liver microsomes. Microsomal proteins, 10 μg, were subjected to the Western immunoblot analysis. The presented results are representative blots from four (for the control and for DOI) separate rats per treatment (**A**). The data are expressed as the mean ± S.E.M. (n=6-8) (**B**). Statistical significance was assessed by Student's *t*-test and indicated as *$p < 0.05$, compared to the control.

Fig. 3. The effect of the repeated injections of the 5-HT_1 receptor agonist 5-CT (**A**) and the 5-HT_2 receptor agonist DOI (**B**) into the ARC (5 or 3 μg/side, respectively) on the pituitary

GHRH level and serum hormone concentrations. All values are presented as the mean ± S.E.M. (n=6-9). Statistical significance was assessed by a one-way ANOVA followed by Duncan's test and indicated as *$p < 0.05$ compared to the control. The absolute control values for 5-CT and DOI were 2.9 ± 1.1 ng/ml, 140.8 ± 19.5 ng/ml, 6.7 ± 0.15 ng/ml, 73.5 ± 1.8 ng/ml and 25.6 ± 1.1 pg/mg of the tissue for serum growth hormone (GH), corticosterone (CRT), triiodothyronine (T_3), thyroxine (T_4) and growth hormone-releasing hormone (GHRH) in the pituitary, respectively.

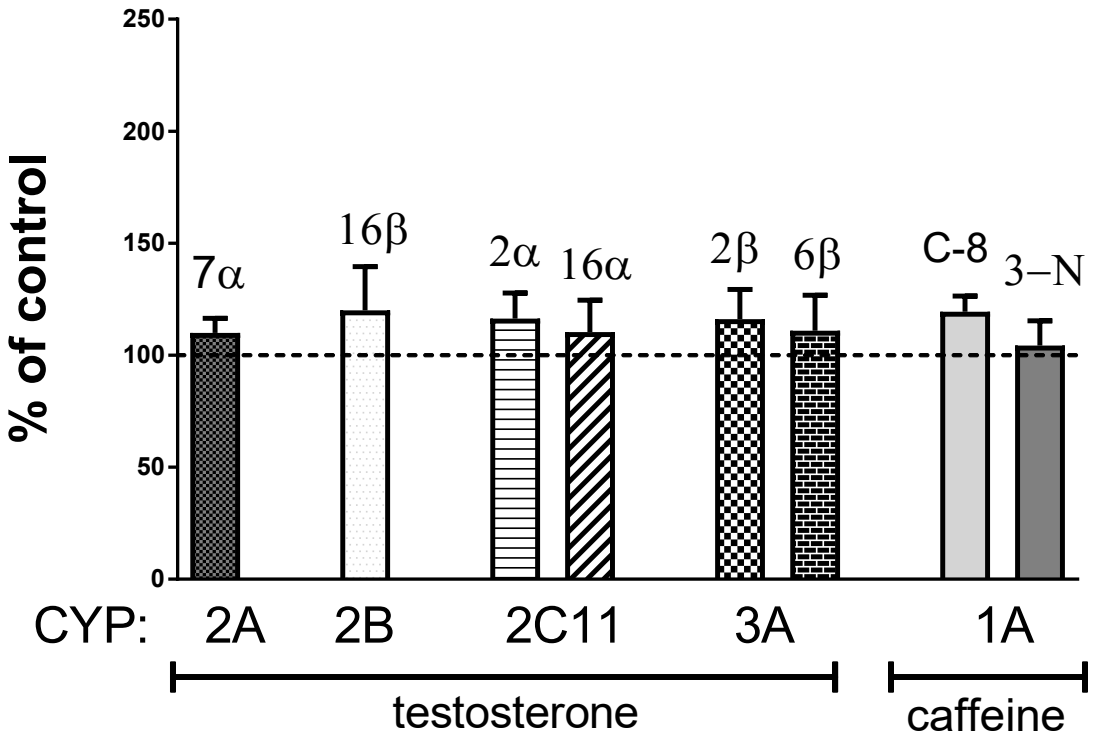
Fig. 4. Neuroendocrine regulation of liver cytochrome P450 by serotonergic receptor of 5-HT_2 type located in the ARC (the scheme depicts the results presented in this work). The activation of the 5-HT_2 type receptor in the ARC of the hypothalamus stimulates GHRH release and thus pituitary GH secretion, which may lead to increased expression and activity of the GH-dependent CYP2C11, CYP3A1/23 and CYP3A2 enzymes in the liver. ARC, arcuate nucleus; GHRH, growth hormone-releasing hormone; GH, growth hormone.

Table 1. The effect of the repeated injection of the 5-HT_2 receptor agonist DOI into the ARC (3 μg/side) on the mRNA expression level of the *CYP2C11, CYP3A1/23* and *CYP3A2* genes in rat liver.

Gene name	Fold-change relative to HPRT1 gene	Statistical significance	Fold-change relative to GAPDH gene	Statistical significance
CYP2C11	1.71 ↑ (±0.18)	$p < 0.05$	1.60 ↑ (±0.16)	$p < 0.05$
CYP3A1/23	2.60 ↑ (±0.87)	$p = 0.228$	2.76 ↑ (±0.50)	$p < 0.05$
CYP3A2	4.06 ↑ (±0.91)	$p < 0.05$	4.49 ↑ (±1.19)	$p < 0.01$

The results are expressed as the fold-change in relation to 2 housekeeping genes, i.e. *hypoxanthine phosphoribosyltransferase 1 (HPRT1)* and *glyceraldehyde-3-phosphate dehydrogenase (GAPDH).* All the values are shown as the mean fold-change (± S.E.M.) calculated by the comparative delta-delta Ct method ($2^{-\Delta\Delta Ct}$) for the control (n=6-8) and agonist-treated (n=6-7) groups. Statistical significance was assessed by Student's *t*-test and indicated as $p < 0.05$ and $p < 0.01$, compared to the control.

A. 5-CT (5-HT$_1$ agonist)



B. DOI (5-HT$_2$ agonist)

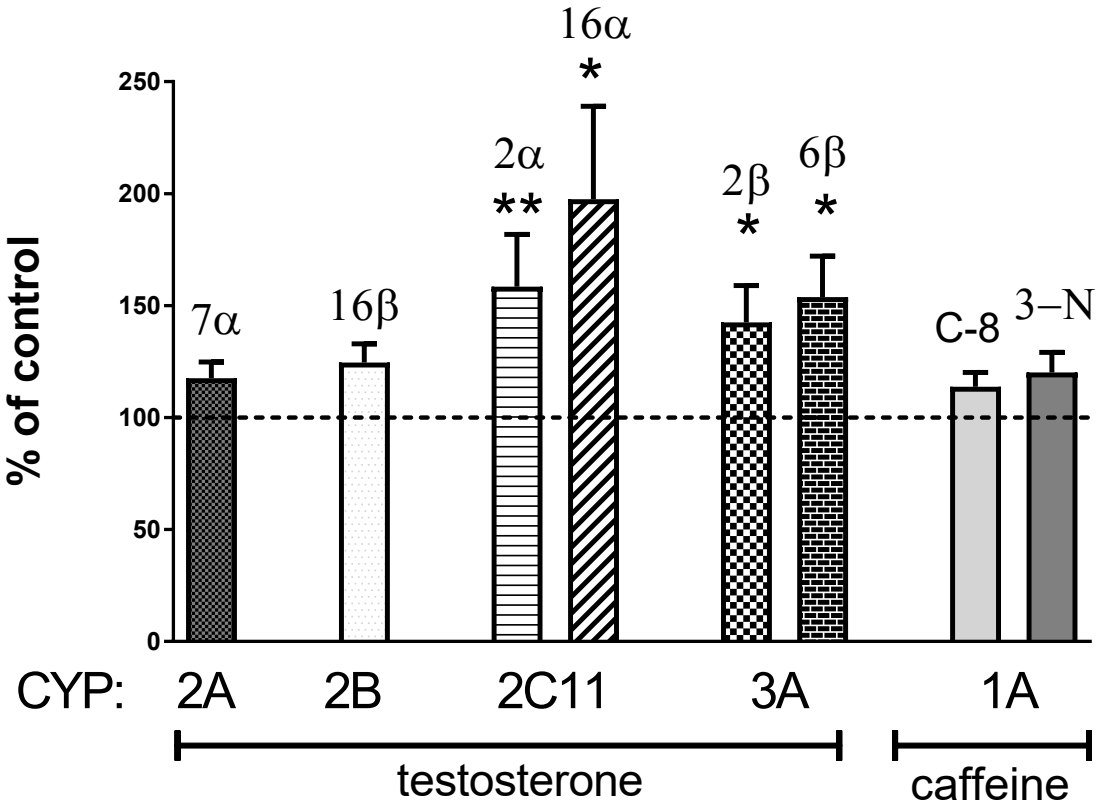
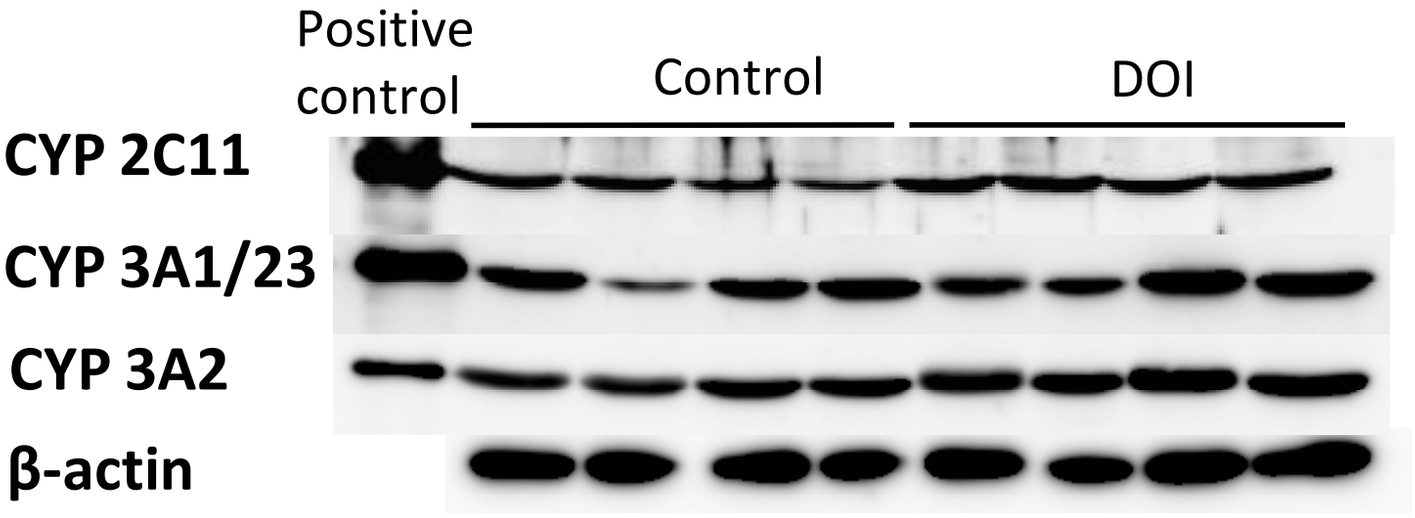


Fig. 1


Positive control
Control
DOI
CYP 2C11
CYP 3A1/23
CYP 3A2
β-actin

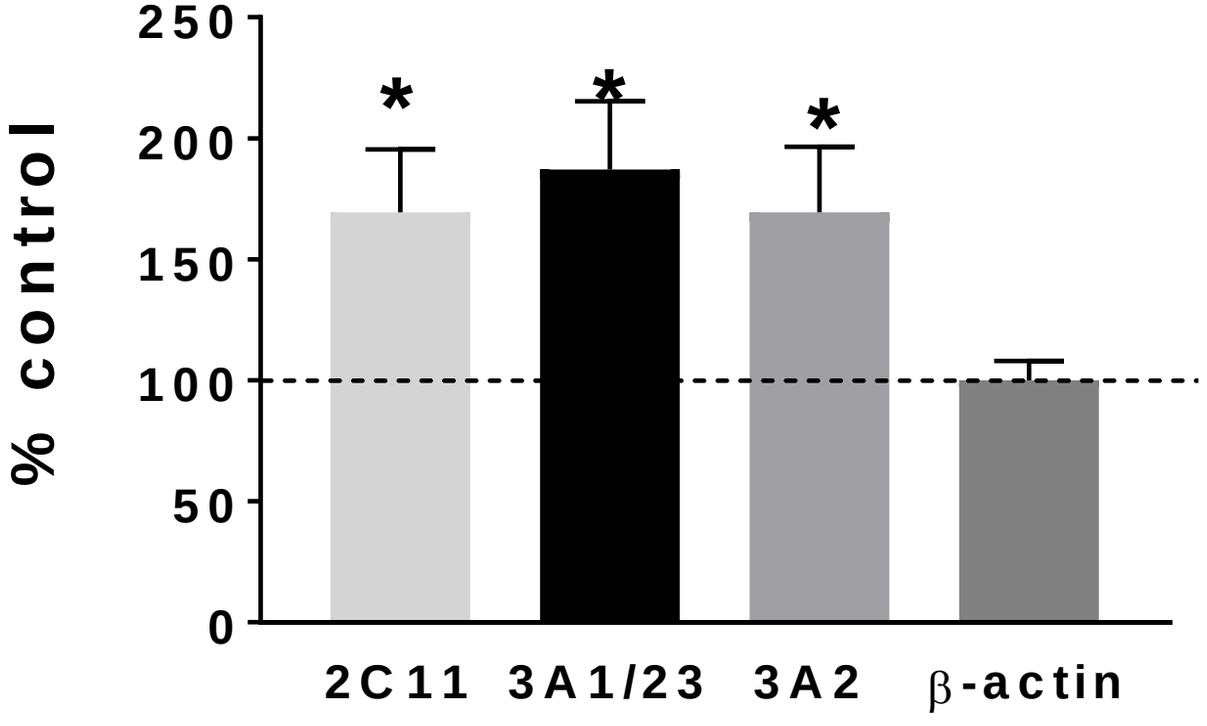
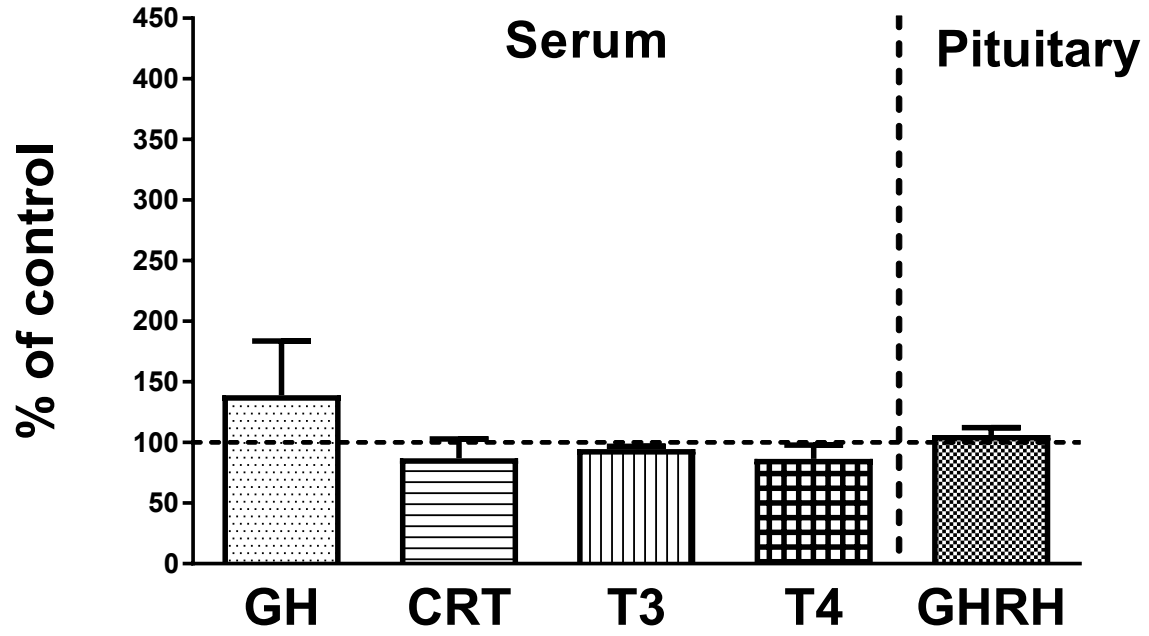

250
200
150
100
50
0
% control
*
*
*
2C11
3A1/23
3A2
β-actin

Fig. 2

A. 5-CT (5-HT1 agonist)



B. DOI (5-HT2 agonist)

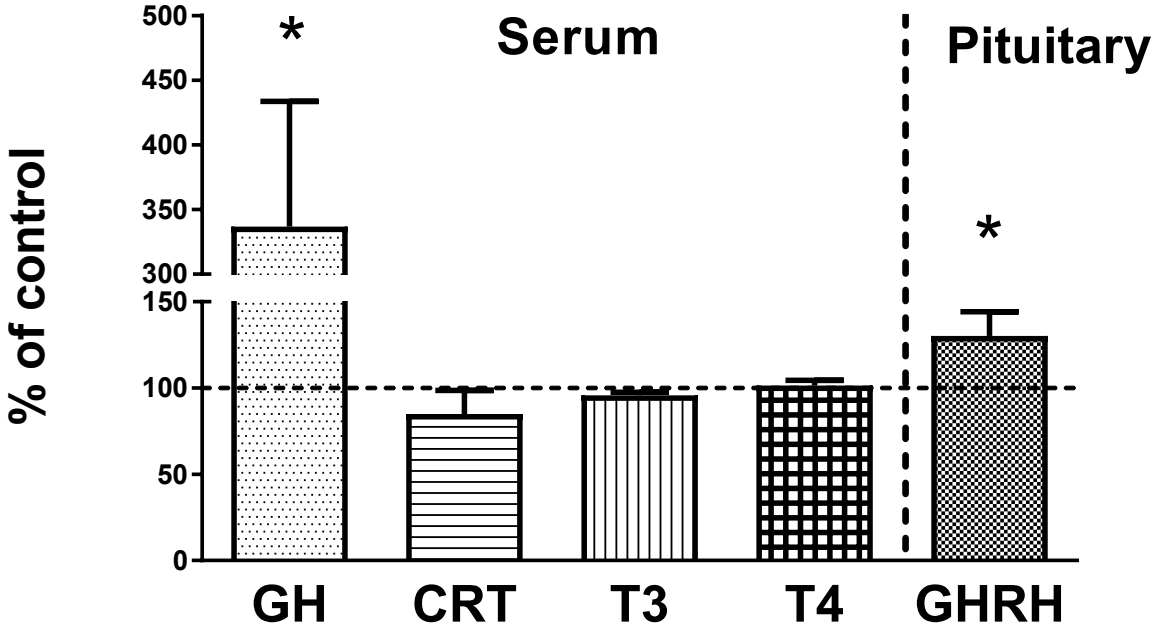


Fig. 3

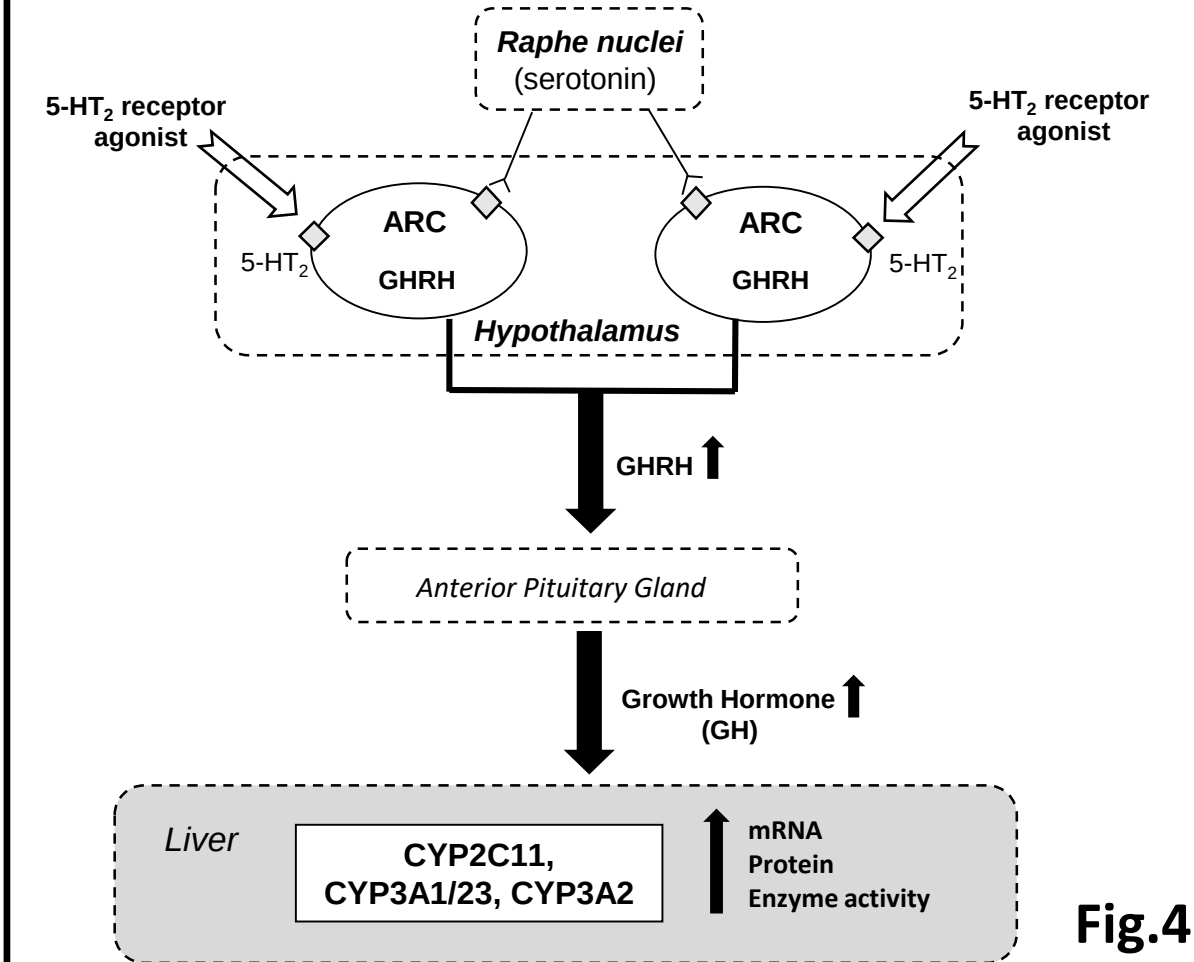

Raphe nuclei
(serotonin)
5-HT2 receptor agonist
5-HT2 receptor agonist
ARC
GHRH
5-HT2
ARC
GHRH
5-HT2
Hypothalamus
GHRH
Anterior Pituitary Gland
Growth Hormone (GH)
Liver
CYP2C11, CYP3A1/23, CYP3A2
mRNA
Protein
Enzyme activity

Fig.4