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Activation of 5-HT$_{1A}$ Receptors in the Hypothalamic Paraventricular Nuclei Negatively Regulates Cytochrome P450 Expression and Activity in Rat Liver

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ABSTRACT

Our recent work suggested a negative effect for the serotonergic innervation of the paraventricular nuclei (PVN) of the hypothalamus on growth hormone secretion and growth hormone-dependent expression of CYP2C11. The aim of our present research was to determine the effect of the activation of the 5-hydroxytryptamine [(5-HT) serotonin] 5-HT$_1$ or 5-HT$_2$ receptors in the PVN on the expression and activity of cytochrome P450 in male rat liver. The serotonergic agonists 5-carboxyamidotryptamine [(5-CT), a 5-HT$_1$ receptor-type agonist], 8-hydroxy-2-(di-n-propyloamino)-tetralin [(8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist], sumatriptan (a 5-HT$_{1B/D}$ receptor agonist), and 2,5-dimethoxy-4-iodoamphetamine [(DOI), a 5-HT$_{2A/C}$ receptor agonist] were individually injected into the PVN. The liver cytochrome P450 activity and expression and the levels of serum and pituitary and hypothalamic hormones were measured. 5-CT and 8-OH-DPAT significantly decreased the activity and expression of *CYP2C11* at both the mRNA and protein levels, which was accompanied by an increase in pituitary and hypothalamic somatostatin levels and a decrease in the serum growth hormone concentration. The expression of *CYP3A1/23* also decreased. The serum corticosterone concentration declined after the injection of 8-OH-DPAT. The obtained results indicated that 5-HT$_{1A}$ but not the 5-HT$_{1B/D}$ or 5-HT$_2$ receptors in the PVN are engaged in the negative neuroendocrine regulation of cytochrome P450 via the stimulation of hypothalamic somatostatin secretion and in the decreases in the serum growth hormone and corticosterone concentrations. Since the affected enzymes metabolize steroids and drugs and 5-HT$_{1A}$ receptors are engaged in the action of psychotropic drugs, the results obtained may be of both physiologic and pharmacological meaning.

Introduction

The physiologic regulation of the expression of liver cytochrome P450 (P450) is governed by hormones such as growth hormone (GH), glucocorticoids, thyroid, and sex hormones. By acting on membrane, cytoplasmic, or nuclear receptors, they regulate the transcription of genes coding for P450 isoforms, affecting the expression and activity of particular hormone-dependent P450 isoforms (Waxman and O'Connor, 2006; Waxman and Holloway, 2009; Monostory et al., 2009; Dvorak and Pavek, 2010; Brtko and Dvorak, 2011; Monostory and Dvorak, 2011; Wójcikowski and Daniel, 2011).

Our earlier studies showed the important role of brain monoaminergic systems, such as the dopaminergic (Wójcikowski et al., 2007, 2008; Wójcikowski and Daniel, 2009), noradrenergic (Sadakierska-Chudy et al., 2013; Kot et al., 2015), and serotonergic (Rysz et al., 2015, 2016a) systems in the physiologic neuroendocrine regulation of P450 expression and activity in the liver. They indicated opposite effects of the catecholaminergic and serotonergic systems on the regulation of the expression and activity of CYP2C11, CYP3A, CYP2B, or CYP2A isoforms (positive or negative, respectively), but positive effects of both those systems on CYP1A expression and activity. Moreover, the results obtained to date showed the important but reverse effects of monoaminergic innervations (both catecholaminergic and serotonergic) of the hypothalamic paraventricular nuclei (PVN) and arcuate (ARC) nuclei on the regulation of CYP2C11 and CYP3A isoforms, which were negative in the case of PVN and positive in the case of ARC and involved corresponding changes in GH secretion (Wójcikowski and Daniel, 2009; Bromek et al., 2013; Rysz et al., 2016b).

The main role in the central neuroendocrine regulation of liver P450 is played by the hypothalamus, which contains the PVN-producing corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), and somatostatin (SST), and the ARC, which produces the GH-releasing hormone. These hypothalamic hormones act differently on the secretion of anterior pituitary gland hormones engaged in the regulation of P450 expression. Both the PVN and ARC are innervated by serotonergic projections originating from the frontal raphe serotonin-containing neuronal groups, mainly from the dorsal and median raphe nuclei (Sawchenko et al., 1983; Gruber et al., 1987; Willoughby and Blessing, 1987; Larsen et al., 1996). Serotonin [5-hydroxytryptamine (5-HT)] released in the PVN or ARC stimulates serotonergic receptors located therein and thus may affect the synthesis and release of liberating

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ABBREVIATIONS: ACTH, adrenocorticotropic hormone; ARC, arcuate nucleus; CRH, corticotropin-releasing hormone; CRT, corticosterone; 5-CT, 5-carboxyamidotryptamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; ELISA, enzyme-linked immunosorbent assay; GH, growth hormone; 5-HT, 5-hydroxytryptamine (serotonin); IL, interleukin; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propyloamino)-tetralin; P450, cytochrome P450; PCR, polymerase chain reaction; PVN, paraventricular nuclei; SST, somatostatin; T_3, triiodothyronine; T_4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

(CRH, TRH, or GH-releasing hormone) or inhibiting (SST) hormones. These hormones, in turn, affect pituitary hormone secretion.

It is assumed that the stimulating effect of brain serotonin on GH release can be initiated by either GH-releasing hormone release from the ARC via 5-HT_2 receptors (Murakami et al., 1986; Willoughby et al., 1987; Katz et al., 1996) or the inhibition of SST release from the PVN. The latter effect likely proceeds via 5-HT_1 receptors, as suggested in humans and dogs (Mota et al., 1995; Seletti et al., 1995; Valverde et al., 2000; Pitchot et al., 2004). The peripheral or intracerebral administration of selective serotonergic agonists indicated that the stimulatory effect of serotonin on the synthesis and release of CRH from the PVN is mediated by 5-HT_{1A} and 5-$HT_{2A/2C}$ receptors (Jørgensen, 2007). The stimulation of thyroid hormone release may occur via the inhibition of SST secretion, while the inhibitory action seems to stem from the suppression of TRH release from the hypothalamic PVN by serotonin (Tuomisto and Männistö, 1985; Toivonen et al., 1990).

Our previous study carried out after overall lesion or activation of the brain serotonergic system indicated the engagement of that system in the central neuroendocrine regulation of P450 expression in the liver (Rysz et al., 2015, 2016a). The follow-up study performed after damage to the serotonergic innervation of the hypothalamic nuclei PVN or ARC showed the important role of these brain structures in the aforementioned enzyme regulation (Rysz et al., 2016b). Damage to the PVN led to an increase in serum GH and testosterone concentrations, subsequently leading to an increase in the expression (mRNA and protein level) and activity of CYP2C11, the main P450 isoform in male rat liver (Rysz et al., 2016b). Since both 5-HT_1 and 5-HT_2 receptor types are present in the PVN (Zhang et al., 2004), the aim of our present research was to identify the 5-HT_1 or 5-HT_2 receptor types/subtypes located in the PVN that are involved in P450 expression in the liver. To this end, we administered different selective 5-HT receptor agonists in pharmacologically active doses into the PVN and then examined the levels of serum, pituitary and hypothalamic hormones, and liver P450 function.

Materials and Methods

Animals. Male Wistar Han rats (Charles River Laboratories, Sulzfeld, Germany), weighing 280–300 g, were housed under standard laboratory conditions (12:12 hour light/dark cycle; temperature of 22°C ± 2°C; room humidity of 55% ± 5%). The animals had free access to food and tap water; however, 18 hours before decapitation, they were deprived of food. The experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011), and with approval of the Local Bioethics Commission at the Institute of Pharmacology of the Polish Academy of Sciences (Kraków).

Drugs and Chemicals. The following serotonergic receptor agonists were used: 5-carboxyamidotryptamine (5-CT) maleate, 8-hydroxy-2-(di-*n*-propyloamino)-tetralin (8-OH-DPAT) hydrobromide, and sumatriptan succinate, which were obtained from Sigma (St. Louis, MO); and 2,5-dimethoxy-4-iodoamphetamine (DOI) hydrochloride, which was obtained from Tocris (Bristol, United Kingdom). Caffeine, its metabolites, and RNA-free water were purchased from Sigma. Testosterone and its metabolites were provided by Steraloids (Newport, KY). The polyclonal primary anti-rat CYP2C11 antibody (obtained from Abcam, Cambrige, United Kingdom), anti-rat CYP3A1/23 and CYP3A2 antibodies (obtained from Millipore, Temecula, CA), and polyclonal anti-rat β-actin antibody (obtained from Santa Cruz, Dallas, TX) were applied. The chemiluminescence reagents LumiGlo kit was obtained from KPL (Gaithersburg, MD). The following enzyme-linked immunosorbent assay (ELISA) kits were used: GH and corticosterone (CRT) (obtained from DRG MedTek, Warsaw, Poland); triiodothyronine (T_3) and thyroxine (T_4) (obtained from Cloud-Clone Corp., Katy, Texas); CRH, adrenocorticotropic hormone (ACTH), TRH, and thyroid-stimulating hormone (TSH) (obtained from Elabscience, Bethesda, MD); pituitary SST (obtained from MyBiosource, San Diego, CA); and interleukin (IL)-2 and IL-6 (obtained from R&D Systems, Minneapolis, MN). A mirVana kit, TaqMan assays, and the TaqMan Gene Expression Master Mix (obtained from Life Technologies, Carlsbad, CA), and a Transcriptor High-Fidelity cDNA synthesis kit (obtained from Roche Diagnostics, Indianapolis, IN) were used. Ketamine hydrochloride and xylazine hydrochloride were purchased from Biowet (Puławy, Poland).

Surgery and Intracerebral Injection of the Serotonergic Receptor Agonists 5-CT, 8-OH-DPAT, Sumatriptan, or DOI. The rats were anesthetized with ketamine HCl and xylazine HCl (65 and 5 mg/kg i.p., respectively) and were placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Stainless-steel guide cannulas were implanted bilaterally into the PVN of the hypothalamus. The following coordinates were used: anterior-posterior from the bregma, −1.6; lateral, ± 0.3; and ventral from the surface of the dura, −5.2 (Paxinos and Watson, 2007). Intracerebral injections were made through an inner cannula (ventral, −7.2) 7 days after implanting the guide cannula. The serotonergic agonists 5-CT (a general 5-HT_1 receptor agonist), 8-OH-DPAT (a 5-HT_{1A} receptor agonist), sumatriptan (a 5-$HT_{1B/D}$ receptor agonist), and DOI (a 5-$HT_{2A/2C}$ receptor agonist) were dissolved in a 0.9% NaCl solution and injected into the PVN at a pure substance concentration of 10 μg/μl (0.5 μl infused into one side at a rate of 0.5 μl/min) for 5-CT, 8-OH-DPAT, and sumatriptan or 6 μg/μl (0.5 μl infused into one side at a rate of 0.5 μl/min) for DOI. The agonists were injected as a single dose or for 5 days once a day. Control animals (sham-operated rats, $n = 10$) were subjected to the same procedure as the 5-HT receptor agonist-treated group ($n = 10$), except that they received 0.9% NaCl (0.5 μl) instead of an agonist. The 0.9% NaCl or agonist solution was administered into the PVN using a Hamilton syringe (0.5 μl/min), which was left in place for 5 minutes after injection. The rats were decapitated, and their brains were rapidly removed. The upper and lower parts of the middle hypothalamus (containing the PVN or ARC, respectively) and the pituitary gland were dissected. The placement of the needle was histologically verified as reported previously (Rysz et al., 2016b).

Collection of Brain, Pituitary, Liver, and Serum Samples. At 30 minutes after a single injection or at 3 hours after the last injection from the 5-day administration of a serotonergic agonist, the rats were decapitated. The middle hypothalamus, pituitaries, and livers were removed and stored at –80°C until they were further analyzed. The blood serum was separated by centrifugation and stored at –80°C. Liver microsomes were prepared from individual animals by differential centrifugation as described previously (Kot and Daniel, 2011).

Determination of Cytochrome P450 Isoform Activities in Liver Microsomes. The activity of CYP1A was examined by measuring the rate of caffeine metabolism (C-8-hydroxylation and 3-*N*-demethylation), as described previously (Kot and Daniel, 2008). The activities of CYP2A, CYP2B, CYP2C11, and CYP3A were evaluated by measuring the rate of testosterone 7α-, 16β-, 2α- and 16α-, and 6β-hydroxylation, respectively, as described previously (Haduch et al., 2006, 2008; Wójcikowski et al., 2013).

An Analysis of the Cytochrome P450 Proteins in Liver Microsomes. The protein levels of CYP2C11, CYP3A1/23, and CYP3A2 in the liver microsomes (10 μg of proteins) of control and serotonergic agonist-treated rats were estimated by western immunoblot analysis (a semiquantitative method), as previously described (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 used. Rat cDNA-expressed CYP2C11 (5 μg), CYP3A1/23 (1 μg), and CYP3A2 (1 μg) (Supersomes, Gentest, Woburn, MA) were used as standards. The intensity of the bands on a nitrocellulose membrane was quantified with a luminescent image analyzer and the data were normalized to protein loading based on the β-actin levels (Rysz et al. 2016a,b).

An Analysis of the Serum, Pituitary and Hypothalamic Hormones, and Serum Cytokines. Hormonal effects were measured 30 minutes after a single injection and 3 hours after the repeated administration of one of the serotonergic agonists. The levels of serum and pituitary or hypothalamic (PVN) hormones were measured using the following ELISA kits: GH kits (DRG, MedTek); CRT, T_3, and T_4 kits (Cloud-Clone Corp); CRH, ACTH, TRH, and TSH kits (Elabscience). The pituitary and hypothalamic (ARC) SST levels were measured using an ELISA kit from MyBiosource. The serum concentrations of cytokines were analyzed using the IL-2 and IL-6 kits (R&D Systems). Absorbance was measured using a microplate reader (Rysz et al. 2016a,b).

Isolation of Liver RNA and Quantitative Real-Time Polymerase Chain Reaction (PCR) Measurements. RNA was isolated from the liver, and quantitative real-time PCR was performed as described previously (Rysz et al., 2016b). Briefly, frozen liver tissue was homogenized, total RNA was extracted

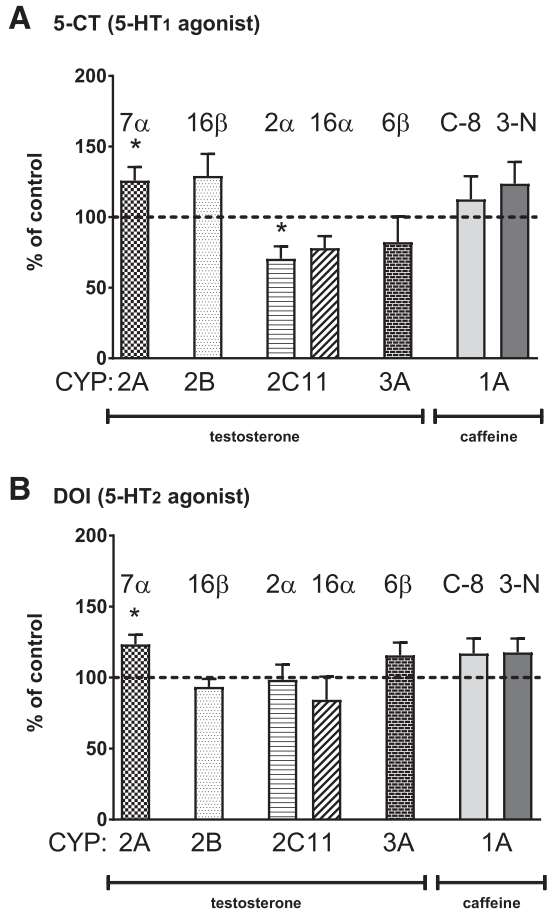

Fig. 1. The effect of the repeated injection of the 5-HT_1 receptor agonist 5-CT (A) and the 5-HT_2 receptor agonist DOI (B) into the PVN (5 or 3 μg/side, respectively) on different P450 isoform activities, measured as the rates of P450 isoform-specific reactions in rat liver microsomes: caffeine 8-hydroxylation and 3-*N*-demethylation (CYP1A) and testosterone 7α- (CYP2A), 16β- (CYP2B), 2α- and 16α- (CYP2C11), and 6β- (CYP3A) hydroxylation. All values are the mean ± S.E.M. (n = 8–10). Statistical significance was assessed by one-way analysis of variance followed by Duncan's test and is indicated as $^*P < 0.05$ compared with the control. The control values (picomoles per milligram protein per minute) are as follows: (A and B) 16.8 ± 2.3 (caffeine 8-hydroxylation); 3.5 ± 0.4 (caffeine 3-*N*-demethylation); and 86.4 ± 10.2, 22.1 ± 1.5, 540.7 ± 73.9, 484.5 ± 71.1, and 535.9 ± 50.9 (testosterone 7α-, 16β-, 2α-, 16α-, and 6β-hydroxylation, respectively).

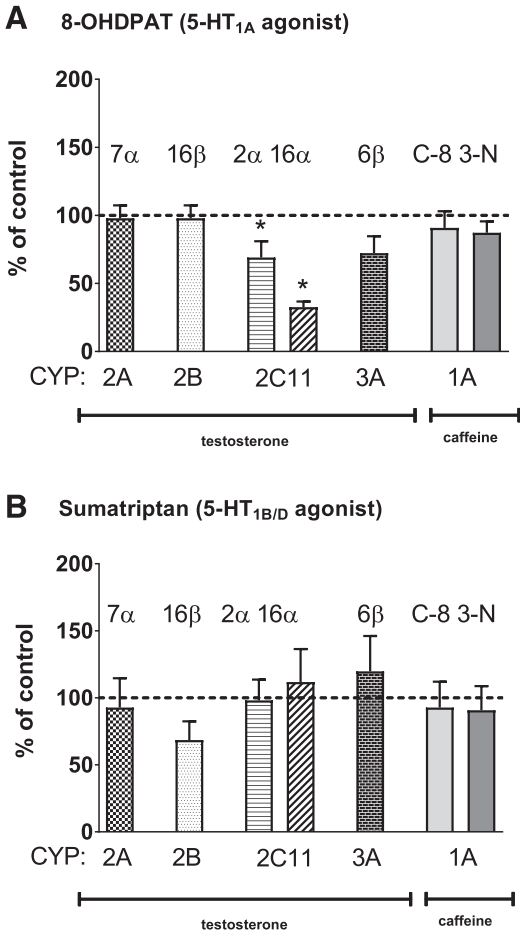

Fig. 2. The effect of the repeated injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT (A) and the 5-$HT_{1B/D}$ receptor agonist sumatriptan (B) into the PVN (5 μg/side) on different P450 isoform activities, measured as the rates of P450 isoform-specific reactions in rat liver microsomes: caffeine 3-*N*-demethylation and 8-hydroxylation (CYP1A) and testosterone 7α- (CYP2A), 16β- (CYP2B), 2α- and 16α- (CYP2C11), and 6β- (CYP3A) hydroxylation. All values are the mean ± S.E.M. (n = 8–10). Statistical significance was assessed by Student's t-test and is indicated as $^*P < 0.05$, compared with the control. The control values (picomoles per milligram protein per minute) are as follows: (A) 31.9 ± 3.3 (caffeine 8-hydroxylation); 6.2 ± 0.6 (caffeine 3-*N*-demethylation); and 85.6 ± 9.7, 17.5 ± 1.7, 294.6 ± 23.8, 233.4 ± 23.1, and 611.9 ± 49.2 (testosterone 7α-, 16β-, 2α-, 16α-, and 6β-hydroxylation, respectively); (B) 19.2 ± 2.1 (caffeine 8-hydroxylation); 6.4 ± 0.4 (caffeine 3-*N*-demethylation); and 81.7 ± 16.3, 10.4 ± 2.3, 220.2 ± 70.0, 417.3 ± 33.2, and 366.6 ± 98.4 (testosterone 7α-, 16β-, 2α-, 16α-, and 6β-hydroxylation, respectively).

using a mirVana isolation kit, and then the first-strand cDNA products were generated using a Transcriptor High Fidelity cDNA Synthesis Kit. The expression of the genes encoding the P450 isoforms CYP2C11, CYP3A1/23, and CYP3A2 (*CYP2C11*, *CYP3A1/23*, and *CYP3A2)* and the reference genes *HPRT1* and *GAPDH* were detected by real-time PCR using commercially available TaqMan Gene Expression Master Mix and species-specific TaqMan-type probes and primers. The gene names for the tested P450 genes and two reference genes with identification numbers of the TaqMan primers used in the study 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) as described previously (Rysz et al., 2016b). The PCR reactions of *CYP2C11*, *CYP3A1/23*, and *CYP3A2* and reference genes were run in duplicate. The level of P450 transcripts was normalized to *HPRT1* and *GAPDH* expression, and the relative quantification was obtained by a comparative $2^{-\Delta\Delta Ct}$ method. The amount of the investigated transcript was expressed as the fold change in the expression level relative to the calibrator (i.e., the average ΔCt of the control group).

Statistical Analysis of the Data. The obtained values are shown as the mean ± S.E.M. of 8–10 rats. Changes in the levels of hormones, interleukins, P450 activity, protein, and mRNA levels were evaluated for statistical significance using one-way analysis of variance followed by Duncan's post hoc test, or by two-tailed Student's t test. The results were considered as statistically significant when $P < 0.05$.

Results

Studies into liver P450 activity and expression were conducted after the administration of pharmacological doses of the selected serotonergic agonists into the PVN. The effective doses of the agonists were chosen on the basis of the literature data showing characteristic biochemical and/or behavioral effects of the intracerebrally administered 5-CT (Mamede Rosa and Prado, 1997; Fletcher and Korth, 1999), DOI (Willins and Meltzer, 1997; Fletcher et al., 2002; Currie et al., 2010; de Paula et al., 2012), 8-OH-DPAT (Fletcher et al., 2002; de Souza Villa et al., 2008; Clissold et al., 2013), or sumatriptan (Piñeyro and Blier, 1996; Hopwood and Stamford, 2001; Johnson et al., 2001; Guo and Rainnie, 2010).

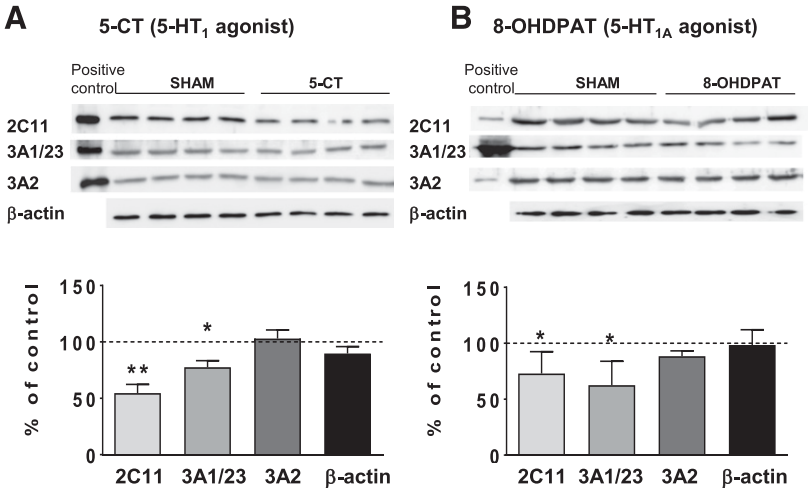

Fig. 3. The effect of the repeated injection of the 5-HT$_1$ receptor agonist 5-CT (A and B) and the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (C and D) into the PVN (5 μg/side) on the protein levels of CYP2C11, CYP3A1/23, and CYP3A2 isoforms in rat liver microsomes. Microsomal proteins (10 μg) were subjected to western immunoblot analysis. The presented results are typical of four (for the control and for 5-CT or 8-OH-DPAT) separate rats per treatment (A and C). The data are expressed as the mean ± S.E.M. (n = 8) (B and D). Statistical significance was assessed by Student's t-test and is indicated as $^*P < 0.05$ and $^{**}P < 0.01$, compared with the control.

The Effect of the Injection of 5-HT$_1$ and 5-HT$_2$ Receptor Agonists into the PVN on Cytochrome P450 Activity in the Liver. In the liver microsomes of rats receiving an injection of 5-CT (a nonselective 5-HT$_1$ receptor agonist) into the PVN (5 μg/side) for 5 days, the activity of CYP2C11 was measured as the rate of the 2α- and 16α-hydroxylation of testosterone, which was decreased (to 71% and 78% of the control, respectively) at 3 hours after the last injection (Fig. 1A). The activity of CYP2A evaluated as the rate of the 7α-hydroxylation of testosterone significantly increased (to 126% of the control). The activities of CYP2B and 3A (assessed as the rate of the 16β- or 6β-hydroxylation of testosterone, respectively), as well as CYP1A (measured as the rate of caffeine C-8-hydroxylation and 3-*N*-demethylation), did not significantly change. The repeated injection of DOI (a nonselective 5-HT$_2$ receptor agonist) into the PVN (3 μg/side) also elevated the activity of CYP2A (to 123% of the control), but it did not affect the activity of CYP2C11 or other isoenzyme subfamilies studied (Fig. 1B).

The 5-day injection of 8-OH-DPAT (a selective 5-HT$_{1A}$ receptor agonist) into the PVN (5 μg/side) significantly diminished the activity of CYP2C11 (which performs the 2α- and 16α-hydroxylation of testosterone) at 3 hours after the last injection, but it did not significantly affect the activities of CYP1A, 2A, 2B, or 3A (Fig. 2A). In contrast, the repeated injection of sumatriptan (a 5-HT$_{1B/D}$ receptor agonist) into the PVN (5 μg/side) did not influence the activity of CYP2C11 or other isoenzyme subfamilies tested (Fig. 2B). However, both 5-CT and 8-OH-DPAT tended to decrease the activity of CYP3A measured as the 6β-hydroxylation of testosterone (Figs. 1A and 2A).

The Effect of the Injection of 5-HT$_1$ Receptor Agonists into the PVN on Cytochrome P450 Expression in the Liver. Searching for the molecular mechanisms of the 5-HT$_1$/5-HT$_{1A}$ receptor agonist-evoked decreases in the activity of CYP2C11, the enzyme expression was investigated in liver tissue after the repeated injection of the agonists into the PVN. The observed decreases in the activity of CYP2C11 correlated positively with the enzyme expression level observed at 3 hours after the last injection of the 5-HT$_1$ receptor agonist 5-CT or the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (Fig. 3; Table 1). 5-CT significantly reduced the level of the CYP2C11 protein to 54% of the control (Fig. 3A), while 8-OH-DPAT diminished the enzyme protein level to 73% of the control (Fig. 3B). Accordingly, the level of *CYP2C11* mRNA significantly decreased after the injection of 5-CT or 8-OH-DPAT (Table 1). At the same time, the CYP3A1/23 protein level decreased (to 77% and 62.5% of the control, respectively), and the level of *CYP3A1/23* mRNA diminished in parallel after the repeated injection of 5-CT or 8-OH-DPAT (Fig. 3; Table 1).

The Effect of the Injection of 5-HT$_1$ Receptor Agonists into the PVN on the Levels of Serum, Pituitary and Hypothalamic Hormones, and Serum Cytokines. In searching for the central neuroendocrine mechanisms of the 5-HT$_1$/5-HT$_{1A}$ receptor agonist-evoked decreases in *CYP2C11* and *CYP3A1* expression, the concentrations of the hormones engaged in the regulation of liver P450 were

TABLE 1

The effect of the repeated injection of the 5-HT$_1$ receptor agonist 5-CT and the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT into the PVN (5 μg/side) on the mRNA expression level of the *CYP2C11*, *CYP3A1/23*, and *CYP3A2* genes in rat liver

The results are expressed as the fold change in relation to two housekeeping genes, HPRT1 *and* GAPDH. *All of the values are the mean fold change (±S.E.M.) calculated by the comparative* $2^{-\Delta\Delta Ct}$ *method for the control* (n = 8–10) *and agonist-treated* (n = 8–10) *groups. Statistical significance was assessed by Student's* t-*test and is indicated as* $^*P < 0.05$, $^{**}P < 0.01$, *and* $^{***}P < 0.001$, *compared with the control. Changes that were statistically significant are shown in bold.*

Gene Name	*HPRT1* Gene		*GAPDH* Gene	
	Fold Change	Statistical Significance	Fold Change	Statistical Significance
5-CT injection				
CYP2C11	**1.78** ↓ (±0.44)	***P* < 0.05**	**1.81** ↓ (±0.27)	***P* < 0.001**
CYP3A1/23	**1.96** ↓ (±0.56)	***P* < 0.05**	**1.61** ↓ (±0.41)	***P* < 0.05**
CYP3A2	1.28 ↓ (±1.21)	*P* = 0.081	1.60 ↓ (±1.17)	*P* = 0.053
8-OH-DPAT injection				
CYP2C11	**1.35** ↓ (±0.21)	***P* < 0.05**	**1.54** ↓ (±0.23)	***P* < 0.01**
CYP3A1/23	**1.25** ↓ (±0.15)	***P* < 0.05**	**1.54** ↓ (±0.22)	***P* < 0.05**
CYP3A2	0.92 (±0.067)	*P* = 0.39	1.19 ↓ (±0.24)	*P* = 0.09

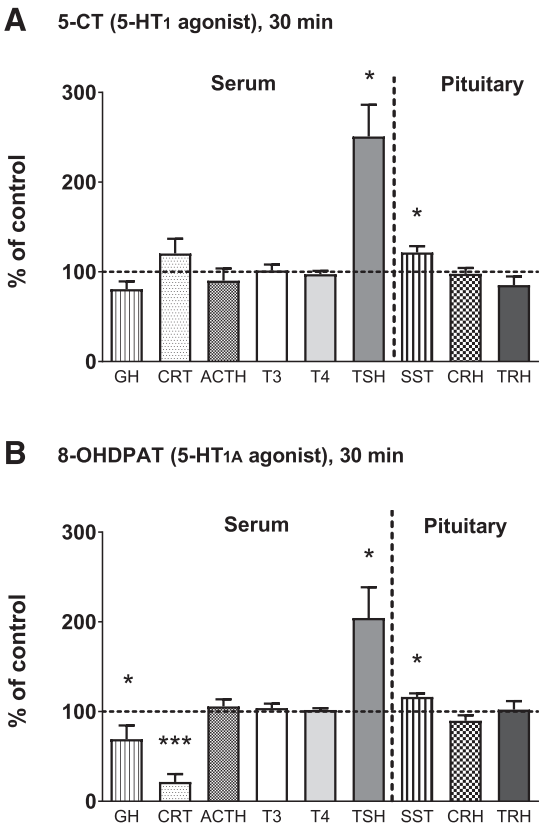

Fig. 4. The effect of a single injection of the 5-HT_1 receptor agonist 5-CT (A) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (B) into the PVN (5 μg/side) on the serum hormones and pituitary SST, CRH, and TRH levels. All values are the mean ± S.E.M. (n = 8–10). Statistical significance was assessed by one-way analysis of variance followed by Duncan's test and is indicated as $^*P < 0.05$ and $^{***}P < 0.001$, compared with the control. The absolute control values for 5-CT and 8-OH-DPAT were 13.5 ± 0.6 and 77.8 ± 12.7 ng/ml; 187.8 ± 11.2 pg/ml; 5.7 ± 0.25, 68.1 ± 2.9, and 2.9 ± 0.2 ng/ml; 11.7 ± 0.7 pg/mg; 0.97 ± 0.058 ng/mg; and 6.3 ± 0.5 pg/mg for serum GH, CRT, ACTH, T_3, T_4, TSH, SST, CRH, and TRH in the pituitary, respectively.

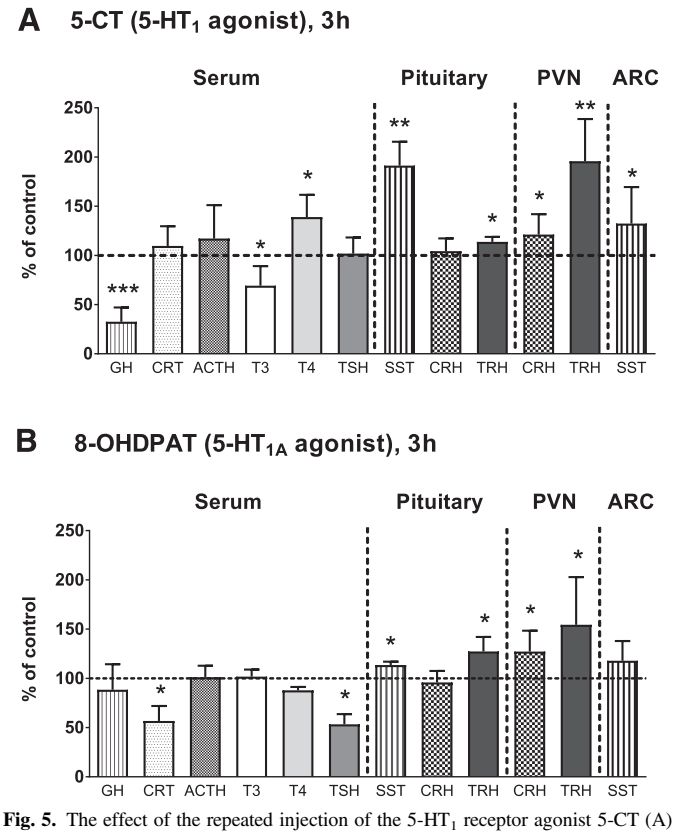

Fig. 5. The effect of the repeated injection of the 5-HT_1 receptor agonist 5-CT (A) and the 5-HT_{1A} receptor agonist 8-OH-DPAT (B) into the PVN (5 μg/side) on the serum, pituitary, and hypothalamic hormone levels. All values are the mean ± S.E.M. (n = 8–10). Statistical significance was assessed by Student's t-test and is indicated as $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, compared with the control. The absolute control values for 5-CT were 12.45 ± 0.67 and 153.53 ± 21.95 ng/ml; 114.71 ± 19.70 pg/ml; 3.05 ± 0.23, 162 ± 16.85, and 3.57 ± 0.14 ng/ml; 33.90 ± 8.51 pg/mg; 2.59 ± 0.155 ng/mg; and 22.78 ± 0.95, 42.63 ± 3.08, 4.00 ± 0.77, and 6.29 ± 0.68 pg/mg for serum GH, CRT, ACTH, T_3, T_4, TSH, SST, CRH, and TRH in the pituitary; CRH and TRH in the PVN; and SST in the ARC, respectively. The absolute control values for 8-OH-DPAT were 3.76 ± 0.93, 244.21 ± 42.64, 206.87 ± 4.34, 6.31 ± 1.65, 316.18 ± 7.81, and 1.48 ± 0.22 ng/ml; 31.35 ± 1.68 pg/mg; 1.14 ± 0.15 ng/mg; 8.48 ± 0.90 and 128.89 ± 9.96 pg/mg; 4.67 ± 0.86 ng/mg; and 5.76 ± 0.75 pg/mg for serum GH, CRT, ACTH, T_3, T_4, TSH, SST, CRH, and TRH in the pituitary; CRH and TRH in the PVN; and SST in the ARC, respectively.

measured in the blood serum, pituitary, or hypothalamus. The level of serum GH, CRT, and thyroid hormones (T_3 and T_4) that directly regulate P450 expression were measured. Simultaneously, the pituitary-produced hormones, ACTH and TSH, were measured in the serum. Moreover, the PVN-produced hormones, the inhibiting hormone SST, and the releasing hormones CRH and TRH, were measured in the pituitary or hypothalamus (Figs. 4 and 5). The measurements were performed 30 minutes after a single injection (a screening test for acute hormonal effects of the agonists) or 3 hours after the repeated injection of the agonist into the PVN, i.e., at the time when the hormonal effects of the 5-HT_1 agonists on P450 expression and activity were translated and investigated biochemically.

The ELISA results revealed that a single injection of the 5-HT_1/5-HT_{1A} receptor agonists 5-CT and 8-OH-DPAT produced a decrease in the serum concentration of GH (to 81% and 69% of the control, respectively) and an increase in the SST level in the pituitary (to 122% and 116% of the control, respectively) at 30 minutes (Fig. 4, A and B). At 3 hours after the 5-day injection of the agonists, these hormonal effects were even far more pronounced in the case of 5-CT (GH decreased to 30%, pituitary SST increased to 190%, and ARC SST increased to 132% of the control) (Fig. 5A), but they were hardly perceived for 8-OH-DPAT (Fig. 5B).

Moreover, the serum CRT concentration significantly decreased to 22% of the control at 30 minutes after a single injection of 8-OH-DPAT (Fig. 4B) but not for 5-CT (Fig. 4A). Such a decrease, although less pronounced (57% of the control), was maintained at 3 hours after the 5-day injection of 8-OH-DPAT (Fig. 5B). At the same time, the serum concentration of ACTH and the pituitary level of CRH were not changed (neither at 30 minutes nor at 3 hours), while the hypothalamic level of CRH in the PVN area was enhanced at 3 hours after repeated injection of 5-CT or 8-OH-DPAT (to 121% and 127% of the control, respectively) (Fig. 5B).

In addition, the serum concentration of TSH was augmented at 30 minutes after a single injection of 5-CT or 8-OH-DPAT (to 251% and 205% of the control, respectively), while the level of pituitary TRH and the concentrations of the thyroid hormones T_3 and T_4 were not changed (Fig. 4, A and B). The level of serum TSH returned to the control value in the case of 5-CT but was reduced in the case of 8-OH-DPAT (to 50% of the control) at 3 hours after the 5-day injection of the agonists (Fig. 5, A and B). At the same time, the serum concentration of T_4 was elevated (to 139% of the control), and that of T_3 was diminished (to 69% of the control) after the repeated injection of 5-CT (Fig. 5A). However, no change in thyroid hormone concentrations (T_3 or T_4) was observed after the repeated injection of 8-OH-DPAT (Fig. 5B). However, the TRH levels in the hypothalamic PVN area and pituitary were significantly

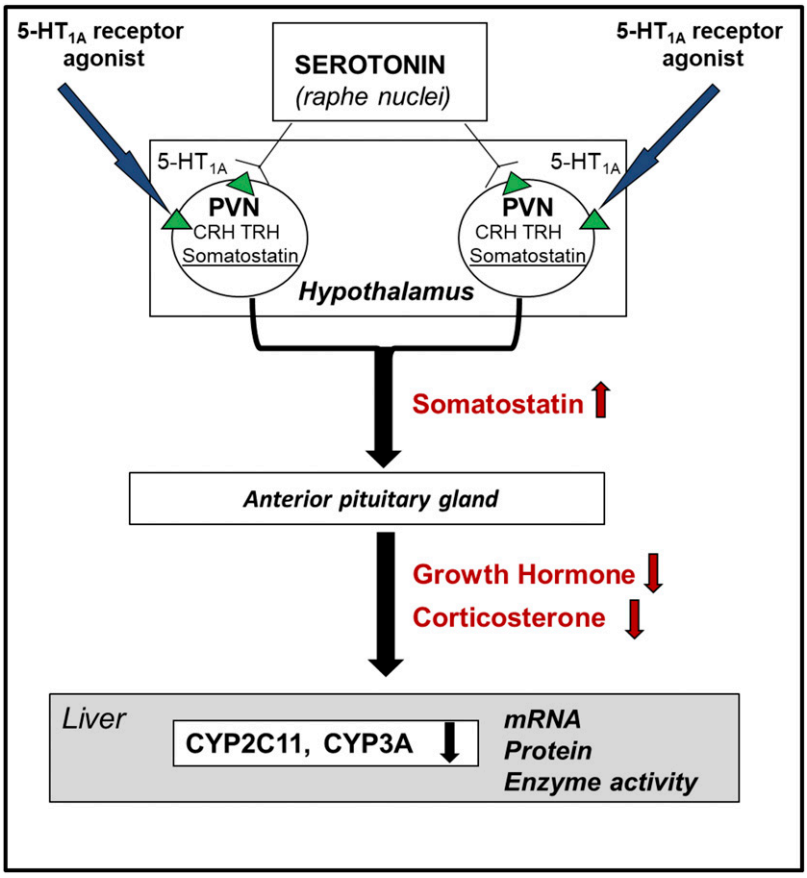

Fig. 6. The central neuroendocrine regulation of liver P450 via 5-HT$_{1A}$ receptor located in the PVN (the scheme is based on the results presented in the article). The activation of the 5-HT$_{1A}$ receptor in the PVN of the hypothalamus increases SST secretion and decreases GH and CRT concentration in the blood, which leads to a reduction in hormone-dependent CYP2C11 and CYP3A1/23 expression and activity in the liver.

increased at 3 hours after the repeated injection of 5-CT or 8-OH-DPAT (to 196% and 154% of the control for PVN and to 112% and 127% for the pituitary, respectively). The serum concentrations of IL-2 and IL-6 were not significantly changed by the single or repeated administration of 5-CT or 8-OH-DPAT into the PVN (data not shown).

Discussion

The results of our previous study suggested that the serotonergic innervation of the PVN of the hypothalamus negatively regulates P450 (CYP2C11) via a neuroendocrine mechanism involving GH (Rysz et al., 2016b). Our present study, carried out after injection of type 5-HT$_1$ and 5-HT$_2$ serotonergic receptor agonists into the PVN, allowed us to identify the receptor subtype engaged in that regulation. It has been revealed that serotonergic 5-HT$_{1A}$ receptors, but not 5-HT$_{1B/D}$ subtype or 5-HT$_2$ type receptors, present therein are engaged in the central neuroendocrine regulation of liver P450 by the brain serotonergic system. The regulation of P450 by 5-HT$_{1A}$ receptors of the PVN is negative, which corresponds well with the observed positive effect of PVN lesion on enzyme expression (Rysz et al., 2016b). It is also noteworthy that the effect of 5-HT$_{1A}$ receptors of the PVN on liver P450 was observed after administration of a pharmacologically effective dose of the agonist 8-OH-DPAT into the PVN (5 μg/side). The applied dose was shown to induce a characteristic set of behavioral responses that were blocked by pretreatment with the 5-HT$_{1A}$ receptor antagonist pMPPF given into the PVN (de Souza Villa et al., 2008). The latter finding strongly testifies to the engagement of brain 5-HT$_{1A}$ receptors of the PVN in the regulation of liver P450 expression.

Rats receiving an injection of the nonselective 5-HT$_1$ receptor–type agonist 5-CT into the PVN for 5 days showed decreased activity of the principal rat male P450 isoform CYP2C11 in the liver at 3 hours after the last injection. In contrast, the repeated injection of the nonselective 5-HT$_2$ receptor–type agonist DOI into the PVN did not affect the activity of CYP2C11. Further studies into the 5-HT$_1$ receptor subtype responsible for the aforementioned effect of 5-CT showed that the 5-day injection of the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT into the PVN significantly diminished the activity of CYP2C11 at 3 hours after the last injection, while the repeated injection of the 5-HT$_{1B/D}$ receptor agonist sumatriptan did not affect the CYP2C11 activity. The aforementioned results show that the 5-HT$_{1A}$ serotonergic receptor subtype of PVN is engaged in the negative regulation of the activity of liver CYP2C11. The observed increase in the CYP2A activity after 5-CT injection does not seem to be connected with 5-HT$_{1A}$ or 5-HT$_{1B/D}$ receptor activation since such a change does not appear after the administration of 8-OH-DPAT or sumatriptan, but may stem from the affinity of 5-CT for other 5-HT receptors present in the hypothalamus (Hoyer et al., 1994; Wesołowska, 2002).

The decreased activity of CYP2C11, produced by the repeated injection of the 5-HT$_1$ receptor agonist 5-CT or the 5 -HT$_{1A}$ receptor agonist 8-OH-DPAT into the PVN, correlated positively with the enzyme expression level (mRNA and protein) in the liver. This indicates that the activation of 5-HT$_{1A}$ serotonergic receptor subtype in the PVN inhibits the synthesis of the CYP2C11 protein in the liver at the transcriptional level, the effect being opposite to that observed previously after the lesion of the serotonergic innervation of the PVN (Rysz et al., 2016b). Moreover, the CYP3A1/23 expression (mRNA and protein levels) also decreased, which was consistent with the observed tendency to decrease the CYP3A activity measured as the rate of 6β-hydroxylation of testosterone. The reaction is characteristic for both CYP3A1/23 and CYP3A2 isoforms, the first one being expressed constitutively to a lesser degree (Gibson et al., 2002).

Liver P450 is regulated hormonally by pituitary-produced GH, adrenal CRT, and thyroid hormones (T_3 and T_4). Growth hormone is a chief factor stimulating the expression of the rat male *CYP2C11* gene as a result of its pulsatile secretion by the anterior pituitary gland (Waxman et al., 1995; Waxman and O'Connor, 2006; Waxman and Holloway, 2009). Corticosterone can also regulate the CYP2C11 isoform. At its lower concentration CRT stimulates, and at its higher (stress) concentration it inhibits, the expression of CYP2C11 by increasing the level of hypothalamic SST and thus inhibiting GH release (Strobl and Thomas, 1994; Devesa et al., 1995; Iber et al., 1997; Mazziotti and Giustina, 2013). However, CRT is foremost one of the main regulators of CYP3A subfamily isoforms (Gibson et al., 2002). On the other hand, thyroid hormones are known to inhibit the expression of different P450 isoforms (Yamazoe et al., 1989; Murayama et al., 1991; Liddle et al., 1998). In our experiment, the 5-HT_1/5-HT_{1A} receptor agonist-evoked decreases in P450 activity and expression were accompanied by changes in the serum concentrations of GH, CRT, and thyroid hormones, as well as in the levels of pituitary-produced TSH and the PVN-produced inhibiting hormones SST, CRH, and TRH.

Thus, both the 5-HT_1 receptor agonist 5-CT and the 5-HT_{1A} receptor agonist 8-OH-DPAT produced a significant decrease in the serum concentration of GH simultaneously with the increase in the level of pituitary SST at 30 minutes and/or 3 hours after a single or repeated injection into the PVN (a screening test for acute hormonal effects of the agonists). The observed opposite changes in the concentrations of the two hormones are physiologically consistent since SST is known to inhibit the secretion of GH at the level of the pituitary gland and ARC nucleus (Liposits et al., 1988; Tannenbaum, 1991; Fodor et al., 1994; Bluet-Pajot et al., 1998; McMahon et al., 2001). It can be suggested that the activation of 5-HT_{1A} presynaptic receptors on GABA neurons inhibits the release of the major inhibitory neurotransmitter GABA and $GABA_A$ receptor-mediated signaling in SST neurons, exerting its stimulatory effect on SST secretion (Rage et al., 1992, 1994; Herbison and Augood, 1994; Murray et al., 1999; Lee et al., 2008), and in turn leading to inhibitory effects on GH secretion and GH-dependent P450 expression. The observed effects agree with the results of some pharmacological experiments showing that the intravenous administration of 8-OH-DPAT to rats decreased plasma GH concentration (Aulakh et al., 1988). Moreover, 8-OH-DPAT significantly decreased the serum CRT concentration at 30 minutes and 3 hours after a single or repeated injection. At 3 hours after the repeated administration of the agonists, which is the time when the hormonal effects of the 5-HT_1 agonists affected P450 expression and activity, these hormonal effects were even more pronounced in the case of 5-CT (GH and SST) but were attenuated in the case of 8-OH-DPAT (GH, SST, and CRT). The latter effects may be due to a faster elimination rate of 8-OH-DPAT than 5-CT from the PVN and/or desensitization of the 5-HT_{1A} receptor produced by the repeated injection of the selective agonist 8-OH-DPAT (Raap and Van de Kar, 1999). However, both 30-minute and 3-hour hormonal responses may contribute to the effect of 8-OH-DPAT on P450 expression observed at 3 hours after injection. Therefore, it seems that the diminished expression of CYP2C11 and CYP3A1/23 was produced by the decrease in the secretion of GH and CRT since these hormonal effects were observed at 30 minutes and/or 3 hours after single or repeated 5-HT_{1A} receptor activation in the PVN. The observed decrease in the CRT level after the selective activation of the 5-HT_{1A} receptor by 8-OH-DPAT could be masked in the case of the nonselective activation of 5-HT_1 receptors by 5-CT because of the simultaneous stimulation of the 5-HT_{1A} receptor and other 5-HT receptor subtypes (Hoyer et al., 1994; Wesołowska, 2002).

Some dynamic, time-dependent changes in the function of the hypothalamic-pituitary-thyroidal axis observed after the injection of 5-CT or 8-OH-DPAT into the PVN (i.e., changes in the TRH, TSH, or T_3 and T_4 levels) might also have certain consequences for P450. Changes in the serum concentration of thyroid hormones (T_4 elevation and T_3 reduction) after repeated 5-CT injection might attenuate the effect of the hypothalamic-pituitary-thyroidal axis on CYP3A expression since T_3, which negatively regulates CYP3A, is a much stronger agonist for the thyroid receptor than T_4 (Yamazoe et al., 1989; Liddle et al., 1998). However, the observed changes in the serum concentration of thyroid hormones may not be connected with the activation of the 5-HT_{1A} subtype receptor since they do not occur after the injection of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT. The serum concentrations of IL-2 and IL-6, which negatively regulate P450 expression, were not changed in our experiment, which justifies the proposed neuroendocrine interpretation of the obtained results.

In conclusion, the intracerebral injection of different specific 5-HT_1 and 5-HT_2 receptor agonists at pharmacologically active doses into the hypothalamic PVN in vivo, followed by the measurement of hypothalamic, pituitary, and peripheral hormone levels and liver P450 expression, revealed a new brain serotonergic mechanism engaged in the neuroendocrine regulation of P450. The obtained results indicate that the serotonergic 5-HT_{1A} receptors, but not the 5-$HT_{1B/D}$ subtype or 5-HT_2 type receptors present in the PVN, are involved in the central neuroendocrine regulation of P450 in the liver. The activation of the 5-HT_{1A} receptor in the PVN produces an increase in SST secretion and a decrease in GH and CRT concentration in the blood, which leads to a reduction in hormone-dependent CYP2C11 and CYP3A1/23 expression and activity in the liver (Fig. 6). Since 5-HT_{1A} receptors are involved in the pharmacological action of antidepressant, antianxiety, and neuroleptic drugs, treatment with these psychotropics may negatively regulate hormone-dependent P450 isoforms in the liver via brain neuroendocrine mechanisms.

Authorship Contributions

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

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