BIOCHEMICAL PHARMACOLOGY 76 (2008) 258–267

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Regulation of liver cytochrome P450 by activation of brain dopaminergic system: Physiological and pharmacological implications

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ARTICLE INFO

Article history:
Received 13 March 2008
Accepted 25 April 2008

Keywords:
Brain dopaminergic system
Activation
Liver cytochrome P450
Plasma hormone levels

ABSTRACT

The aim of the present study was to investigate the influence of activation of brain dopaminergic system by different dopaminomimetics on the level and activity of liver cytochrome P450 (CYP) isoforms. Studies into the identification of hormones and cytokines which are known to mediate liver CYP expression were also simultaneously carried out.

Stimulation of dopaminergic receptors in the pituitary, a target for the tuberoinfundibular pathway, by dopamine (a D_1/D_2 receptor agonist) administered intraperitoneally caused a significant increase in the activities and protein levels of CYP2B, CYP2C11 and CYP3A, a substantial increase in the blood plasma level of growth hormone (GH) and a significant decrease in triiodothyronine (T_3) level. Local stimulation of dopaminergic receptors in the nucleus accumbens, a target for the mesolimbic pathway, by apomorphine (a D_1/D_2 receptor agonist), amphetamine (an indirect D_1/D_2 dopaminemimetic) and quinpirole (a D_2 receptor agonist) produced a substantial rise in CYP3A activity and protein level, caused a large increase in corticosterone concentration and a moderate drop in T_3 level. SKF82958 (a D_1 receptor agonist) did not significantly affect the CYP isoforms or hormones studied. In both cases (activation of the tuberoinfundibular or mesolimbic pathway), the activity and the protein level of CYP1A considerably decreased. Plasma levels of thyroxine, testosterone, interleukin-2 and interleukin-6 were not changed after activation of the two pathways.

The obtained results establish the brain dopaminergic system as a physiological centre regulating cytochrome P450 (engaging D_2 receptors and pituitary hormones) and demonstrate new pharmacological aspects of neuroactive drugs that affect this system.

1. Introduction

Cytochrome P450 (CYP) enzymes are members of a superfamily of heme-containing monooxygenases catalyzing the metabolism of endogenous substances (e.g. steroid hormones, neurosteroids, monoaminergic neurotransmitters, arachidonic acid) and the majority of clinically important drugs including neuroactive agents [1,2]. Genes coding for different CYP isoenzymes are regulated by nuclear receptors that are activated by endogenous ligands such as hormones (GH, thyroid hormones, glucocorticoids) and by the immune system (cytokines) [3]. All of them remain under control of the central nervous system (CNS). Up until now there are no literature data, which describe the result of the stimulation of discrete CNS neurotransmission systems/pathways on the expression of CYP isoenzymes in the liver. It is conceivable, however that dopamine released from the tuberoinfundibular pathway in the median eminence (and transported by

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.04.016

hypophysial portal blood to the anterior pituitary lobe) *via* pituitary dopaminergic D_2 receptors may affect CYP expression in the liver by altering the secretion of pituitary hormones. Moreover, certain neuronal projections which originate in the nucleus accumbens septi and end in the hypothalamus (e.g. in the paraventricular nucleus) may be of functional significance for neuroendocrine-regulating mechanisms [4,5]. It has been shown that the corticotropin- and thyrotropin-releasing hormones are principally derived from cells in the paraventricular nucleus of the hypothalamus, while the GH-releasing hormone is mainly liberated from cells in the arcuate nucleus of the hypothalamus [6,7].

In the brain, dopamine as a neurotransmitter activates five subtypes of dopaminergic receptors (D_1–D_5). All of the known dopaminergic receptors belong to the G protein-coupled receptor superfamily. There are distinct similarities between the D_1 and D_5 subtypes and between the D_2, D_3 and D_4 ones, hence these receptors are classified as D_1-like and D_2-like receptor subfamilies [8]. Both types of receptors have wide but heterogeneous distribution in the brain. In rats, the highest D_1-like and D_2-like receptor densities were observed in the caudate-putamen, nucleus accumbens, olfactory tubercle and substantia nigra [9–11]. D_2 receptors are also present in the pituitary (mainly in its anterior lobe), though their density in this structure is relatively lower [12–15]. It was demonstrated that stimulation of dopaminergic receptors elicited an increase in GH and corticosterone secretion, while the levels of thyroid hormones fell. The above effects were observed in humans and rats after systemic administration of different dopaminomimetics, such as apomorphine, amphetamine, L-dopa and bromocriptine [16–18]. On the other hand, it was reported that GH and glucocorticoids positively influenced CYP3A, CYP2B and CYP2C expression, while thyroid hormones and cytokines negatively regulated those isoforms in the liver [19–23].

At a molecular level, different nuclear receptors contribute to the regulation of these CYP isoforms as discovered in induction studies [3,23–26]. The glucocorticoid receptor (GR), pregnane X receptor (PXR) and constitutive androstane receptor (CAR) play an important role in the regulation of *CYP* genes. The GR contributes mainly to CYP3A induction *via* a direct or an indirect molecular mechanism, including a functional cross-talk between GR-, PXR- and CAR-signalling pathways. The CAR appears to be one of the main regulators involved in the transcriptional activation of CYP2B genes, although the PXR, GR and other nuclear receptors may be required for their optimal activation. On the other hand, CYP2C11 is regulated *via* the Janus tyrosine kinase A (JAK2)-signal transducer and activator of transcription 5b (STAT5b) pathway [27].

Our earlier results provided the first direct evidence for the important role of the brain dopaminergic system in the regulation of cytochrome P450 in rat liver. We observed decreases in the activities and protein levels of CYP2B, CYP2C11 (a lesion of the tuberoinfundibular pathway) and CYP3A (a lesion of the tuberoinfundibular or mesolimbic pathway). At the same time, the level and activity of CYP1A were considerably elevated (a lesion of the tuberoinfundibular or mesolimbic pathway). On the other hand, the activity of CYP2A, CYPC6 and CYP2D was not influenced by damage to those dopaminergic pathways, nor did a lesion of the nigrostriatal pathway affected any CYP isoforms studied [28].

The aim of the present study was to investigate the influence of activation of dopaminergic receptors in the pituitary (a target for the tuberoinfundibular pathway) and in the nucleus accumbens (a target for the mesolimbic pathway) by different dopaminomimetics on the level and activity of selected liver CYP isoforms. Studies into the identification of hormones and cytokines which possibly mediate the effect of the dopaminergic system on liver CYP expression were also simultaneously carried out. The obtained results establish the brain dopaminergic system as an important physiological centre of cytochrome P450 regulation and indicate the mechanism of that regulation.

2. Materials and methods

2.1. Animals

All the experiments with animals were performed in accordance with the Polish state regulations (Animal Protection Act, DZ.U. 97.111.724, 1997). Male Wistar rats (220–250 g) were used for the study.

2.2. Chemicals

Amphetamine, apomorphine, quinpirole, SKF82958, corticosterone, caffeine, paraxanthine, 1,3,7-trimethyluric acid, NADP, DL-isocitric acid, isocitric dehydrogenase, glucose 6-phosphate, glucose-6-phosphate-dehydrogenase, dopamine hydrochloride, anti-rabbit IgG and anti-goat IgG were provided by Sigma (St. Louis, USA). Testosterone and its metabolites 2α-, 2β-, 6β-, 16α- and 16β-hydroxytestosterone were purchased from Steraloids (Newport, USA). A polyclonal antibody, anti-rat CYP1A1, CYP2B1 and CYP2C11 goat sera and an anti-rat CYP3A2 rabbit serum, as well as rat Supersomes 1A2, 2B1, 2C11 and 3A2 were obtained from Gentest Corp. (Woburn, USA). A LumiGLO chemiluminescent substrate was provided by KPL (Gaithersburg, USA). A rat GH ELISA kit was donated by Linco Research (St. Charles, USA), while T_3 and T_4 ELISA kits were from Alpha Diagnostic International (San Antonio, USA). Rat interleukin 2 (IL-2) and interleukin 6 (IL-6) ELISA kits were obtained from R&D Systems (Minneapolis, USA). All the organic solvents with HPLC purity were supplied by Merck (Darmstadt, Germany).

2.3. Stimulation of dopaminergic receptors in the pituitary and in the nucleus accumbens

Pituitary dopaminergic receptors (a target for the tuberoinfundibular pathway) were stimulated by 4-day intraperitoneal injection of dopamine (a D_1/D_2 receptor agonist) in a dose of 1 mg/kg, dissolved in a 0.9% NaCl [29]. Since dopamine released from the tuberoinfundibular pathway in the median eminence can alter – *via* pituitary dopaminergic receptors – the levels of pituitary hormones, the stimulation of pituitary dopaminergic receptors by intraperitoneally administered dopamine may simulate the activation of the tuberoinfundibular pathway in this respect. Control rats received the same

volume of physiological saline. The animals were injected on 4 consecutive days, and were scarified 24 h after the last injection.

The mesolimbic pathway was activated by stereotactic microinjection of the nonselective D_1/D_2 receptor agonist apomorphine, or the indirect dopaminemimetic, amphetamine (releasing dopamine from dopaminergic neurons and inhibiting dopamine reuptake to dopaminergic neurons) at a level of the right and the left nucleus accumbens septi. Moreover, to determine the receptor type engaged in the regulation of cytochrome P450 expression after activation of the mesolimbic pathway, the selective agonists of dopamine receptors SKF82958 (a D_1 receptor) or quinpirole (a D_2 receptor) were administered locally into the nucleus accumbens. The animals were anesthetized with ketamine (75 mg/kg) and xylasine (10 mg/kg), and were then placed in stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Their skulls were exposed and small holes were drilled for the insertion of steel cannulae. Then the animals received bilateral injections of apomorphine, amphetamine, SKF82958 or quinpirole in a dose of 5 μg, dissolved in 0.5 μl of a 0.9% NaCl at a level of the right and the left nucleus accumbens using a Hamilton syringe [30–32]. The stereotaxic coordinates [33] used for the placement of cannulae in the nucleus accumbens septi were: +1.7 mm anterior from the bregma, +1.2 mm lateral from the midline, and −8.0 mm ventral to the top of brain dura. Control animals were injected likewise with the vehicle (0.5 μl of a 0.9% NaCl). The animals were injected on 4 consecutive days, and were scarified 24 h after the last injection.

2.4. Preparation of liver microsomes

After decapitation of the rats, liver microsomes were prepared by differential centrifugation in 20 mM Tris/KCl buffer (pH 7.4) including washing with 0.15 M KCl, according to a conventional method. Protein concentration in microsomal preparations was assayed according to Lowry et al. [34] using bovine serum albumin as a standard.

2.5. Cytochrome P450 activity assay

The conditions of all the specific reactions used for assessing the CYP isoform activities were optimized *in vitro*. On the basis of the obtained results, caffeine and testosterone metabolism in liver microsomes was studied in respect of the linear dependence of product formation on the time and concentrations of protein and substrate.

The activity of CYP1A was studied by measuring the rate of caffeine 3-*N*-demethylation and caffeine C-8-hydroxylation as described previously [35]. Recent studies conducted by Kot and Daniel [36] demonstrated that caffeine C-8-hydroxylation (72%) was more specifically catalyzed by CYP1A in the rat than was 3-*N*-demethylation (47%) at a therapeutic concentration of caffeine (100 μM). Therefore, we used both those reactions for assessing the CYP1A activity.

The activities of CYP2A, CYP2B, CYP2C11 and CYP3A were assessed in liver microsomes by measuring the rate of testosterone hydroxylation in the following positions: 7α (CYP2A), 16β (CYP2B), 2α and 16α (CYP2C11), 2β and 6β (CYP3A), as described previously [37].

2.6. Western blot analysis

The levels of CYP1A, CYP2B, CYP2C11 and CYP3A proteins in the liver microsomes of control and experimental rats were estimated by a Western blot analysis. SDS-PAGE and an immunoblot assay were performed using the methodology provided by Gentest, USA. Microsomal protein, 5–10 μg, was separated on a 0.75 mm thick 4% (w/v) sodium dodecyl sulfate-polyacrylamide) stacking gel and on a 12% (w/v) resolving gel using the Miniprotean II electrophoresis system (Bio-Rad, Hemmel Hempstead, UK; 130 V, 65 min). The protein was electroblotted onto a nitrocellulose membrane (100 V, 100 min) and blocked overnight with a 5% dried non-fat milk in PBS (phosphate-buffered saline, pH 7). The following primary antibodies were used: a polyclonal goat anti-rat antibody raised against CYP1A1, which also recognized the CYP1A2 form; a polyclonal goat anti-rat antibody raised against CYP2B1, which also recognized the CYP2B2 form; a polyclonal rabbit anti-rat antibody raised against CYP2C11, which recognized to a lesser degree the CYP2C6 and CYP2C13 forms, and a polyclonal rabbit anti-rat antibody raised against CYP3A2, which also recognized the CYP3A1 form. After incubation with the primary antibody, the blots were incubated with a secondary antibody, i.e. an appropriate species-specific horseradish peroxidase-conjugated anti-IgG. Rat CYP1A2, CYP2B1, CYP2C11 and CYP3A2 Supersomes (cDNA-expressed rat isoenzymes) were used as standards. Immunoreactivity was assessed using an enhanced LumiGLO chemiluminescent substrate. The intensities of the bands corresponding to the enzyme protein on the nitrocellulose membrane were measured with the Luminescent Image analyzer LAS-1000 using an Image Reader LAS-1000 and an Image Gauge 3.11 programs (Fuji Film, Japan).

2.7. Determination of hormone and cytokine levels in the blood plasma

After decapitation, the rats' trunk blood was collected in tubes moistened with a 30% solution of sodium citrate. Blood samples were centrifuged at $2000 \times g$ for 30 min, and then plasma samples were stored at −20 °C. Plasma GH, T_3, T_4, IL-2 and IL-6 levels were measured using commercial rat GH, T_3, T_4, IL-2 and IL-6 ELISA kits, respectively, according to the manufacturer's instructions. Optical density was measured using a Multiskan Spectrum UV microplate reader (Thermolab System, Vantaa, Finland). Concentrations of corticosterone and testosterone were assessed in blood plasma by the slightly modified HPLC method of Wong [38]. The above hormones were extracted with 6 ml of hexane from 1 ml of blood plasma. The residue obtained after evaporation of the plasma extracts was dissolved in 100 μl of the mobile phase described below. An aliquot (20 μl) was injected into the HPLC system (LaChrom, Merck-Hitachi) equipped with an UV detector, an L-7100 pump and a D-7000 System Manager. The analytical column (Econosphere, C-18, 250 mm × 4.6 mm, 5 μm) was from Alltech (Carnforth, England). The mobile phase consisted of acetonitrile, water and glacial acetic acid in the ratio 35:64.5:0.5 (v/v/v). The flow rate was 1 ml/min (0–13 min), followed by 1.8 ml/min (13.1–24 min). The column temperature was ambient. Absorbance was measured at a wavelength of 254 nm.

2.8. Statistical analysis

Statistical significance was assessed using an analysis of variance, followed by Dunnett's test. All the obtained values are the mean ± S.E.M. from 6 to 8 animals.

3. Results

3.1. The influence of activation of brain dopaminergic pathways on cytochrome P450 activities in the liver

After 4-day stimulation of pituitary dopaminergic receptors by dopamine (simulating activation of the tuberoinfundibular pathway), the activities of CYP2B, CYP2C11 and CYP3A were significantly increased (up to 143, 145 and 170% of the control value, respectively), while the activity of CYP1A was slightly lowered (to 75% of the control) (Fig. 1). Four-day stimulation of dopaminergic receptors in the nucleus accumbens (activation of the mesolimbic pathway) by apomorphine, amphetamine and quinpirole significantly elevated CYP3A activity (up to 159, 169 and 180% of the control, respectively), while CYP1A activity was considerably decreased (to 69, 72 and 66% of the control, respectively) (Fig. 2A, B and D). On the other hand, CYP2A activity was not changed after activation of either dopaminergic pathway, nor did SKF82958 significantly influence the activity of any CYP isoform studied (Figs. 1 and 2A–D).

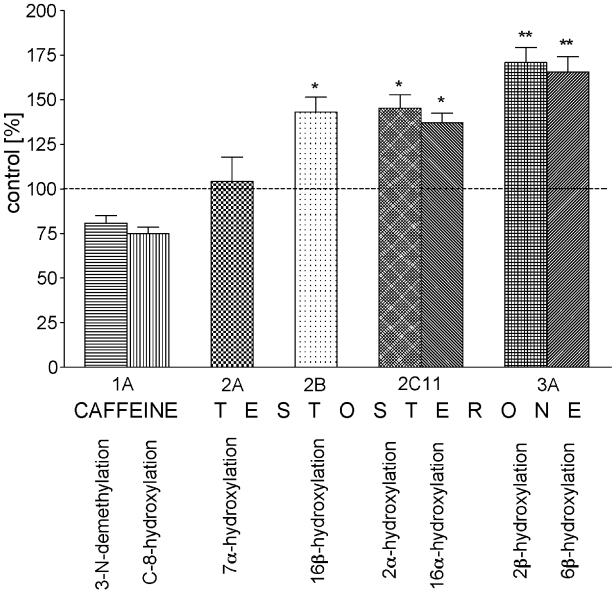

Fig. 1 – The effect of stimulation of pituitary dopaminergic receptors by dopamine on different CYP isoform activities measured as rates of the specific reactions of caffeine (CYP1A) and testosterone (CYP2A, CYP2B, CYP2C11, CYP3A) in rat liver microsomes. All values are the mean ± S.E.M. (n = 6–8 animals). Statistical significance was assessed by Dunnett's test and indicated with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control. Absolute control values were 4.7 ± 0.5, 28 ± 2, 184 ± 10, 12.4 ± 1.3, 640 ± 92, 634 ± 71, 48 ± 4 and 621 ± 33 pmol/mg protein/min for paraxanthine (caffeine 3-N-demethylation), 1,3,7-trimethyluric acid (caffeine C-8-hydroxylation), 7α-, 16β-, 2α-, 16α-, 2β- and 6β-hydroxytestosterone, respectively.

3.2. The influence of activation of brain dopaminergic pathways on cytochrome P450 protein levels in the liver

The changes observed in CYP protein levels after activation of dopaminergic pathways corresponded well with those related to the enzyme activities. As shown in Fig. 3B–D, after 4-day stimulation of pituitary dopaminergic receptors by dopamine, the protein levels of CYP2B, CYP2C11 and CYP3A were significantly elevated (up to 132, 127 and 156% of the control value, respectively), while that of CYP1A tended to decrease (to 83% of the control) (Fig. 3A). Four-day activation of the mesolimbic pathway by apomorphine, amphetamine and quinpirole visibly increased CYP3A protein level (up to 136, 143 and 163% of the control, respectively) (Fig. 4A, B and D). At the same time, the protein level of CYP1A was significantly reduced (to 79% of the control value by apomorphine, to 79% by amphetamine, and to 74% by quinpirole) (Fig. 4A, B and D). SKF82958 did not significantly influence CYP1A and CYP3A protein levels (Fig. 4C).

3.3. The influence of activation of brain dopaminergic pathways on the plasma levels of hormones and cytokines

Stimulation of pituitary dopaminergic receptors caused a substantial increase in GH level (up to 184% of the control) and a significant decrease in T_3 level (to 67% of the control) in rat blood plasma (Fig. 5). On the other hand, activation of the mesolimbic pathway by apomorphine, amphetamine or quinpirole produced a substantial increase in corticosterone concentration (up to 160, 170 and 186% of the control, respectively) and a moderate drop in T_3 level (to 70% of the control), evoked by quinpirole (Fig. 6A, B and D). Furthermore, activation of the mesolimbic pathway by SKF82958 did not significantly affect the levels of the hormones or cytokines studied (Fig. 6C). Nor were the plasma levels of T_4, testosterone, IL-2 and IL-6 changed after stimulation of pituitary dopaminergic receptors and the mesolimbic pathway (Figs. 5 and 6A–D).

4. Discussion

The present results based on the activation of brain dopaminergic pathways has provided some evidence which suggests that the brain dopaminergic system contributes to the regulation of liver CYP1A, CYP2B, CYP2C11 and CYP3A. It also indicates the mechanism of such regulation *via* dopaminergic D_2 receptors and different hormones (GH and T_3 in the case of the tuberoinfundibular pathway, or corticosterone and T_3 in the case of the mesolimbic pathway).

The above conclusions are based on the following arguments. First, the activation of dopaminergic system was correctly conducted. Since local stimulation of the tuberoinfundibular pathway is technically impossible to perform, pituitary dopaminergic receptors (a target for the tuberoinfundibular pathway) were activated by peripheral injection of dopamine (a D_1/D_2 receptor agonist), which simulated

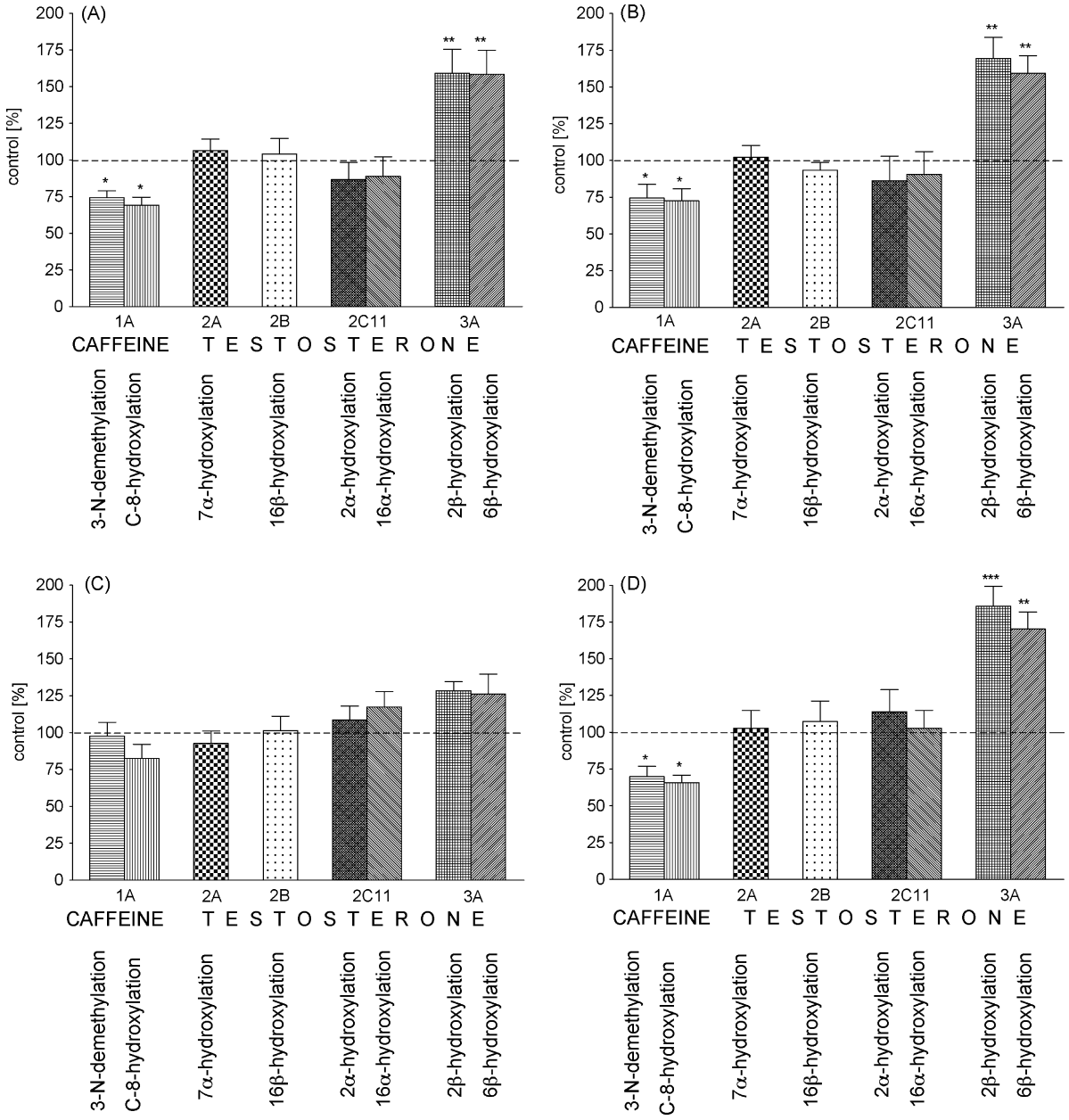

Fig. 2 – The effect of activation of the brain mesolimbic pathway by apomorphine (A), amphetamine (B), SKF82958 (C) and quinpirole (D) on different CYP isoform activities measured as rates of the specific reactions of caffeine (CYP1A) and testosterone (CYP2A, CYP2B, CYP2C11 and CYP3A) in rat liver microsomes. All values are the mean ± S.E.M. (n = 6–8 animals). Statistical significance was assessed by Dunnett's test and indicated with $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the control. Absolute control values were 4.3 ± 0.5, 24 ± 2, 177 ± 12, 15.1 ± 1.2, 746 ± 144, 682 ± 121, 49 ± 10 and 525 ± 117 pmol/mg protein/min for paraxanthine (caffeine 3-N-demethylation), 1,3,7-trimethyluric acid (caffeine C-8-hydroxylation), 7α-, 16β-, 2α-, 16α-, 2β- and 6β-hydroxytestosterone, respectively.

activation of the tuberoinfundibular pathway. Although dopamine does not cross the blood–brain barrier, it penetrates into the median eminence and neural lobe of the pituitary, which are devoid of the latter barrier and are connected with the anterior lobe of the pituitary *via* blood vessels [39]. Accordingly, the activation of pituitary dopaminergic receptors by systemically administered dopamine caused a substantial increase in GH level and a significant decrease in T_3 concentration in blood plasma. The mesolimbic pathway was activated by bilateral local administration of different dopaminomimetics, such as apomorphine (a non-selective D_1/D_2 receptor agonist), amphetamine (an indirect D_1/D_2 dopaminemimetic), SKF82958 (a D_1 receptor agonist) or quinpirole (a D_2 receptor agonist), into the nucleus accumbens to achieve selective stimulation of solely this dopaminergic pathway, and to assess the type of dopaminergic receptors engaged in the regulation of liver CYP. After stereotactic administration of dopamine agonists into the nucleus accumbens, the

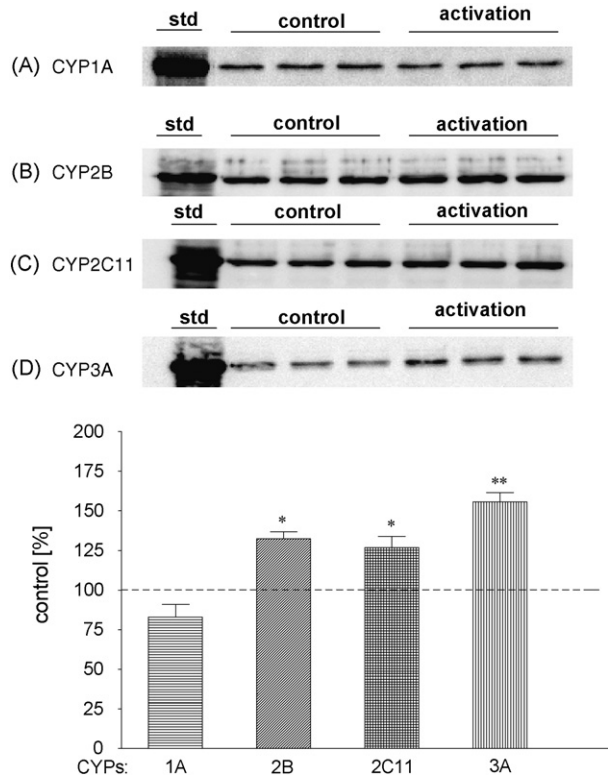

Fig. 3 – The effect of stimulation of pituitary dopaminergic receptors by dopamine on CYP isoform protein levels in rat liver microsomes (n = 6–8). 5 μg (panels C, D) or 10 μg (panels A, B) of microsomal protein was subjected to the Western analysis, and the immunoblot was probed with a polyclonal goat anti-rat antibody raised against CYP1A1, which also recognized the CYP1A2 form; a polyclonal goat anti-rat antibody raised against CYP2B1, which also recognized the CYP2B2 form; a polyclonal rabbit anti-rat antibody raised against CYP2C11, which recognized to a lesser degree the CYP2C6 and CYP2C13 forms, or a polyclonal rabbit anti-rat antibody raised against CYP3A2, which also recognized CYP3A1 form. The presented results are typical of three separate animals per treatment. Rat CYP1A2, CYP2B1, CYP2C11 and CYP3A2 Supersomes (cDNA-expressed rat isoforms) were used as standards. The histogram is the quantification of the corresponding band intensities from the tested isoforms. All values are the mean ± SEM (n = 6). Statistical significance was assessed by Dunnett's test and indicated with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control.

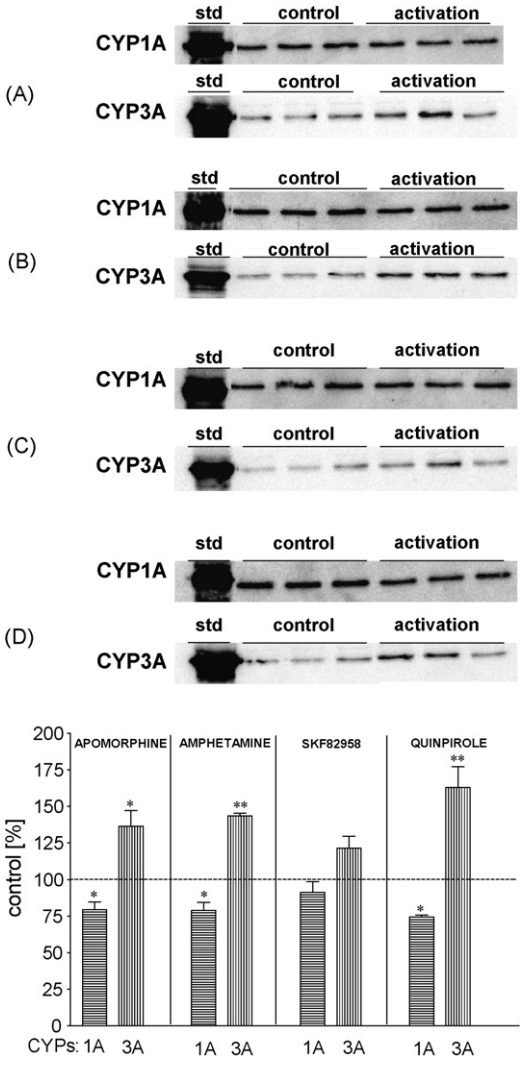

Fig. 4 – The effect of activation of the brain mesolimbic pathway by apomorphine (A), amphetamine (B), SKF82958 (C) or quinpirole (D) on CYP1A and CYP3A protein levels in rat liver microsomes (n = 6–8). The presented results are typical of three separate animals per treatment. The histogram is the quantification of corresponding band intensities from the tested isoforms. All values are the mean ± S.E.M. (n = 6). Statistical significance was assessed by Dunnett's test and indicated with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control. For further information, see Fig. 3.

syndrome of stereotyped behaviour was observed (an increase in animal locomotion, sniffing and climbing after apomorphine, amphetamine and quinpirole administration; enhancement of grooming behaviour in rats after SKF82958 application). Since the above stereotypies are connected with stimulation of D_2 and D_1 receptors, respectively [40–42], it is obvious that in the present study activation of that dopaminergic pathway was carried out correctly and reached the level of functional significance. Moreover, activation of the mesolimbic pathway by apomorphine, amphetamine or quinpirole led to a large increase in corticosterone concentration and to a moderate drop in T_3 level.

Second, activation of pituitary dopaminergic receptors by dopamine produced significant increases in the activities and the protein levels of CYP2B, CYP2C11 and CYP3A. Since male liver expresses much more CYP3A2 protein than CYP3A1 one, it seems justifiable to conclude that in our study the anti-CYP3A1/2 antibody primarily detected CYP3A2. At the same time, the activity and the protein level of CYP1A were considerably decreased. After activation of the mesolimbic

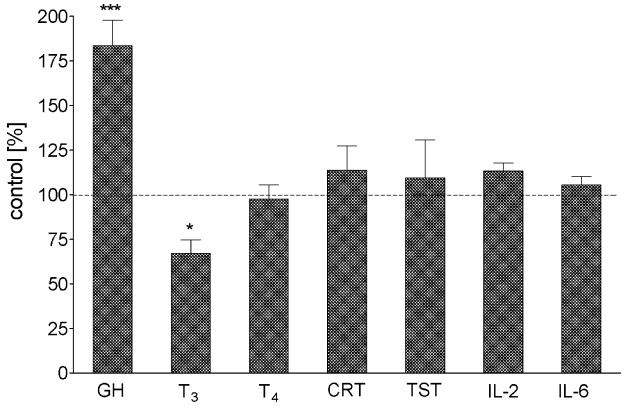

Fig. 5 – The effect of stimulation of pituitary dopaminergic receptors by dopamine on hormone and cytokine concentrations in rat blood plasma. All values are the mean ± S.E.M. (n = 6–8 animals). Statistical significance was assessed by Dunnett's test and indicated with $^{*}p < 0.05$, $^{***}p < 0.001$ compared to the control. Absolute control values were 5.8 ± 1.2 ng/ml, 199 ± 37 pg/ml, 7.7 ± 0.7 ng/ml, 5.3 ± 1 ng/ml, 4.4 ± 1 ng/ml, 27 ± 4 pg/ml and 56 ± 12 pg/ml for growth hormone (GH), triiodothyronine (T_3), thyroxine (T_4), corticosterone (CRT), testosterone (TST), interleukin-2 (IL-2) and interleukin-6 (IL-6), respectively.

pathway by apomorphine, amphetamine or quinpirole, the activity and the protein level of CYP3A were substantially elevated, while the activity and the protein level of CYP1A were significantly reduced. SKF82958 (a D_1 receptor agonist) did not significantly affect the CYP isoforms, hormones or cytokines studied.

The results obtained in experiments with stimulation of the dopaminergic system reflect well our previous findings based on the damage caused to that system, the latter showing reverse effects, i.e. down-regulation of CYP2B and CYP2C11 (a lesion of the tuberoinfundibular pathway), as well as CYP3A down-regulation and CYP1A up-regulation (lesions of the tuberoinfundibular or mesolimbic pathways) [28]. Hence, the corresponding outcome of the activation and lesion of the brain dopaminergic system constitute a complete pharmacological proof of the important role of this system in CYP regulation in the liver. Moreover, these results are in line with the literature data showing the positive influence of dopamine on GH and corticosterone and its negative effect on the secretion of thyroid hormones, as well as the positive influence of GH and corticosterone, but the negative effect of thyroid hormones on CYP2B, CYP2C11 and CYP3A regulation [16–23]. It has been demonstrated that thyroid hormones rather than GH play a dominant role in the regulation of CYP2B, whereas CYP3A may be regulated to a similar degree by different hormones (GH, thyroid hormones, glucocorticoids) [20–22]. On the other hand, the expression of ''male-specific''

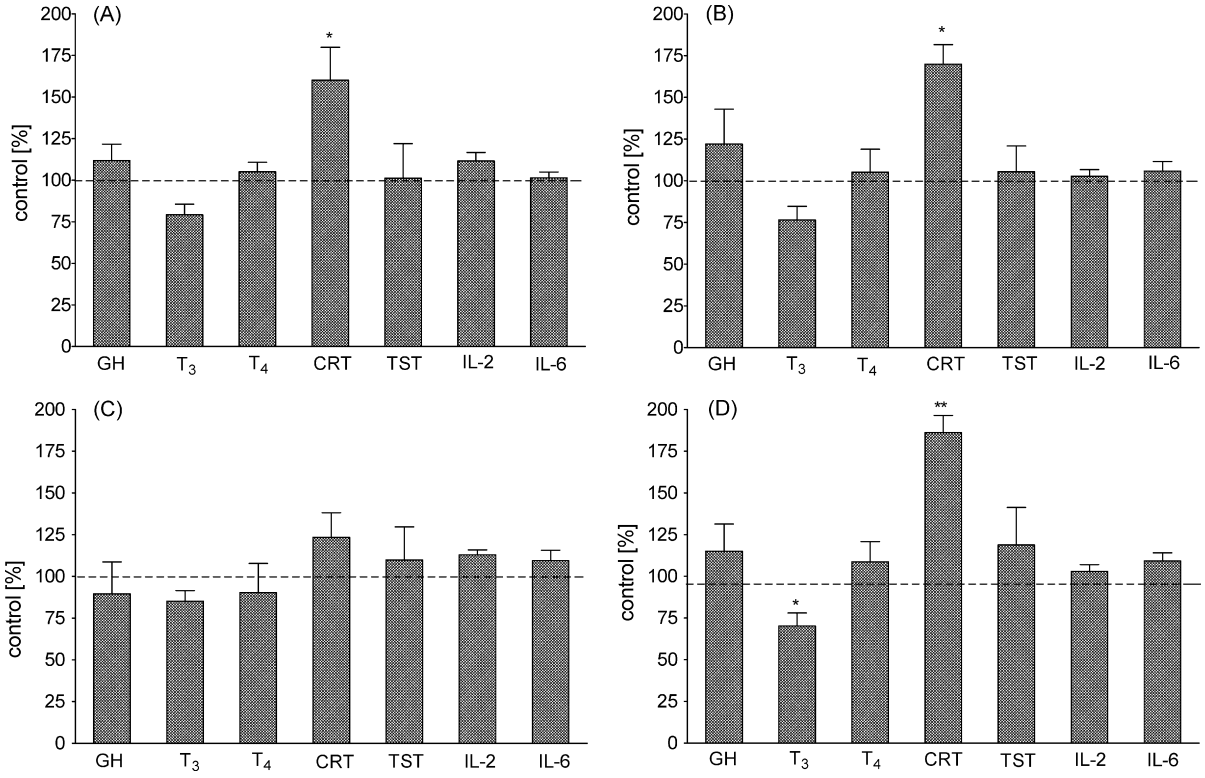

Fig. 6 – The effect of activation of the brain mesolimbic pathway by apomorphine (A), amphetamine (B), SKF82958 (C) or quinpirole (D) on hormone and cytokine concentrations in rat blood plasma. All values are the mean ± S.E.M. (n = 6–8 animals). Statistical significance was assessed by Dunnett's test and indicated with $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the control. Absolute control values were 6.1 ± 0.7 ng/ml, 220 ± 37 pg/ml, 7.6 ± 0.7 ng/ml, 5.8 ± 0.9 ng/ml, 3.9 ± 0.8 ng/ml, 30 ± 7 pg/ml and 57 ± 10 pg/ml for growth hormone (GH), triiodothyronine (T_3), thyroxine (T_4), corticosterone (CRT), testosterone (TST), interleukin-2 (IL-2) and interleukin-6 (IL-6), respectively.

CYP2C11 depends on pulsatile GH secretion [43,44]. Accordingly, in our experiment, only activation of pituitary dopaminergic receptors (a target for the tuberoinfundibular pathway) affecting GH concentration caused changes in the level and activity of CYP2C11.

The observed differences in CYP regulation by dopaminergic receptors of the pituitary and the nucleus accumbens (a target for the mesolimbic pathway) may be due to the fact that the activation of dopaminergic receptors in the pituitary stimulates the secretion of GH and inhibits that of thyroid-stimulating hormone (TSH), while the activation of these receptors in the nucleus accumbens, *via* its projection to the hypothalamus affects the secretion of the corticotropin- and thyrotropin-releasing hormones (which are principally derived from cells in the paraventricular nucleus of the hypothalamus) regulating release of respective pituitary hormones [4–7]. Thus, our results suggest that at a molecular level the tuberoinfundibular pathway affects liver CYP2B, CYP2C11 and CYP3A by involving GH and the Janus tyrosine kinase A (JAK2)-signal transducer and activator of transcription 5b (STAT5b) pathway, while the mesolimbic pathway influences CYP3A by engaging corticosterone and a functional cross-talk between the GR-, PXR- and CAR-signalling pathways. Moreover, both these systems seem to employ T_3 and the thyroid receptor (TR) in the above-described regulation of CYP isoforms. It is noteworthy that the effects derived from the tuberoinfundibular and mesolimbic pathways may overlap after *in vivo* administration of dopaminergic agents.

As mentioned above, dopaminomimetics directly and positively regulate the pituitary secretion of GH and the adrenal release of corticosterone. However, the secretion of GH may also be indirectly regulated by corticosterone. Glucocorticoids enhance GHRH receptor synthesis and stimulate GH gene transcription at low physiological concentrations, while at higher levels (stress-induced, or in Cushing's syndrome) they suppress GH secretion by enhancing hypothalamic somatostatin release [45–47]. It has been shown that GHRH receptor mRNA levels are significantly decreased and GH secretion is inhibited in adrenalectomized male rats [48]. Thus, when both the mesolimbic and the tuberoinfunduibular pathways are stimulated at the same time, the increased level of corticosterone (160–180% of the control) should rather potentiate than attenuate the GH-induced CYP up-regulation (CYP2B, CYP2C11 and CYP3A). Moreover, in adrenal-deficient rats, the corticosterone-dependent regulation (both direct and indirect) of CYP does not work and dopaminomimetics can up-regulate CYP only *via* GH-dependent pathway (CYP2B, CYP3A, and in particular CYP2C11, the expression of the latter enzyme being strictly GH-dependent).

In the present study we observed a decrease in CYP1A activity and protein level after stimulation of dopaminergic receptors of the pituitary and the nucleus accumbens. The latter results are also in line with our earlier findings showing CYP1A up-regulation after damage caused to the tuberoinfundibular and the mesolimbic pathways [28]. The obtained effects are difficult to explain, since CYP1A1 and CYP1A2 genes are basically regulated by the cytosolic aryl-hydrocarbon receptor (AhR) which is activated by a variety of xenobiotics, including dioxins and polycyclic aromatic hydrocarbons [3]. However, recent studies by Sérée et al. [49] provided convincing evidence for a new human CYP1A1 regulation pathway involving the peroxisome proliferator-activated receptor α (PPAR-α). Its dimer with the RXR receptor (the PPAR-α/RXR-α heterodimer) is positively modulated by thyroid hormones *via* the thyroid hormone receptor α *in vivo* [50], as discussed in our previous paper [28]. Since CYP1A1 level is very low in rat liver, and in view of the fact that CYP1A1 and CYP1A2 share a high degree of amino acid homology and have similar gene structure [51], the observed decreases in CYP1A level and activity after activation of the dopaminergic pathways seem to be mainly related to changes in CYP1A2 expression.

Interestingly, our present study indicated that in the case of the mesolimbic pathway, mainly dopaminergic D_2 receptors participated in the regulation of liver CYP. Despite the fact that both types of dopaminergic receptors (D_1 and D_2) are densely located in the nucleus accumbens [9–11], activation of the mesolimbic pathway solely by apomorhine (a non-selective D_1/D_2 agonist), amphetamine (an indirect D_1/D_2 dopamine-mimetic) or quinpirole (a selective D_2 agonist) affected liver CYP expression. In contrast, SKF82958 (a selective D_1 agonist) did not significantly influence any CYP isoforms tested. Although stimulation of pituitary dopaminergic receptors was performed by peripheral administration of dopamine (a non-selective D_1/D_2 agonist), it seems that in that case the same receptor (D_2) was engaged in the regulation of liver CYP isoforms, as it also prevailed in the pituitary [12–15].

The obtained results indicate that drugs affecting brain dopaminergic D_2 receptors may change CYP activity in the liver. Accordingly, neuroleptics which are selective (sulpiride, remoxipride) or non-selective (phenothiazines) D_2 receptor antagonists down-regulated liver CYP2C11 and CYP3A expressions after systemic administration to rats [52,53]. Hence, *via* stimulation (e.g. bromocriptine) or inhibition (e.g. neuroleptics) of the neuroendocrine pathways dopaminergic agents may regulate liver CYP, thus affecting the metabolism of endogenous substances (e.g. steroids) and clinically important drugs (e.g. psychotropics), which in turn modifies the final pharmacological effect.

In conclusion, the presented results establish the brain dopaminergic system as an important center regulating liver cytochrome P450. This regulation proceeds *via* dopaminergic D_2 receptors of the pituitary (activated by the tuberoinfundibular pathway) and D_2 receptors of the nucleus accumbens (activated by the mesolimbic pathway). These receptors stimulate *secretion* of anterior pituitary hormones which directly (GH) or indirectly (ACTH → corticosterone, TSH → T_3) activate nuclear receptors controlling CYP genes. It can be assumed that CYP2B, CYP2C11, CYP3A and CYP1A are regulated by the tuberoinfundibular pathway *via* GH and/or T_3 (involving JAK2/STAT5b and/or TR), while the mesolimbic pathway influences CYP3A and CYP1A *via* corticosterone and/or T_3 (engaging GR-, PXR- and CAR-signalling pathways and/or TR). The above findings are not only of physiological, but also pharmacological importance, since they show new aspects of action of neuroactive drugs affecting the brain dopaminergic system. Importantly, these results may also be applicable to neurological or psychiatric diseases involving the central dopaminergic system.

Acknowledgements

The study was supported by grant no. 2 P05F 013 27 from the Ministry of Science and Higher Education, Warszawa, Poland. Thanks are due to Ms. Justyna Staryńska for her excellent technical assistance.

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