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Activation of brain serotonergic system by repeated intracerebral administration of 5-hydroxytryptophan (5-HTP) decreases the expression and activity of liver cytochrome P450

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ABSTRACT

Our recent studies suggest that brain serotonergic system may be involved in the neuroendocrine regulation of cytochrome P450 expression. Intracerebral injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine affected serum hormone concentration and increased the expression and activity of the hormone-dependent isoforms CYP1A1/2, CYP2C11 and CYP3A1. Therefore, the aim of the present study was to investigate the effect of stimulation of brain serotonergic system on cytochrome P450 expression in the liver. The serotonin precursor 5-hydroxytryptophan (5-HTP) was injected for 5 days to the lateral ventricles of rat brain. Afterwards, the brain concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid 5-HIAA, serum hormone levels and liver cytochrome P450 expression and activity were measured. 5-HTP potently increased the concentration of serotonin and its metabolite 5-HIAA in all the brain structures studied including the hypothalamus. The brain concentrations of noradrenaline or dopamine and its metabolites were not changed in that structure. At the same time, a significant decrease in the serum concentration of the growth hormone and an increase in that of thyroxine were observed. In the liver, the activity of CYP1A, CYP2A, CYP2B, CYP2C11 and CYP3A was diminished, which positively correlated with a decrease in the respective CYP protein levels and a reduction in the mRNA levels of *CYP1A2*, *CYP2A2*, *CYP2C11*, *CYP3A1* and *CYP3A2*. The obtained results provide evidence to prove that brain serotonergic system negatively regulates liver cytochrome P450 expression via endocrine system and suggest mechanisms by which this enzyme may be regulated by drugs with a serotonergic profile such as antidepressants.

1. Introduction

Cytochrome P450 isoforms metabolize a number of vital endogenous substrates (e.g. steroids, vitamins, retinoids, arachidonic acid, neurotransmitters) and clinically important drugs of different therapeutic applications including psychotropics [1–4]. On the other hand, some drugs may affect cytochrome P450 activity by working at the level of a hepatocyte via a direct interaction with the enzyme protein, or by interfering with protein expression [5,6]. However, the effect of a drug on cytochrome P450 may also be the result of its pharmacological action on other organs/tissues (e.g. the brain), which affects the synthesis or release of endogenous substances taking part in the physiological regulation of the enzyme, such as endogenous hormones or cytokines [5,7]. It is suggested that changes observed in cytochrome P450 expression after repeated administration of antidepressants [8–11] or neuroleptics [12–14] may be attributed (at least partly) to their pharmacological action in the brain, involving the stimulation of monoaminergic neurotransmission by antidepressants or the inhibition of dopaminergic neurotransmission by neuroleptics [5,7].

Recent studies demonstrated the importance of the nervous system of the brain to the neuroendocrine regulation of the expression of cytochrome P450 in the liver. The research conducted after intracerebral administration of specific neurotoxins or agonists indicated that catecholaminergic systems of the brain, i.e. the dopaminergic system [15–17] and the noradrenergic

Abbreviations: CYP, cytochrome P450; POR, NADPH-P450 oxidoreductase; HPLC, high performance liquid chromatography; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HTP, 5-hydroxytryptophan; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic; HVA, homovanillic acid; NA, noradrenaline; GHRH, growth hormone-releasing hormone; GH, growth hormone; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; T_3, triiodothyronine; T_4, thyroxine; SSRI, selective serotonin reuptake inhibitor.

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system [18–20] play an important role in the neuroendocrine regulation of cytochrome P450 (CYP1A, CYP2B, CYP2C and CYP3A) in the liver. By acting on their receptors in the hypothalamus, dopamine and noradrenaline influence the secretion of endogenous factors which stimulate (CRH, TRH, GHRH) or inhibit (somatostatin) the release of the respective pituitary hormones (ACTH which stimulates corticosterone, TSH which stimulates thyroid hormones, and GH), thus leading to changes in blood hormone levels and, in effect, to the physiological regulation of cytochrome P450 in the liver. Blood corticosterone, thyroid hormones (T_3, T_4) and growth hormone are important regulators of liver cytochrome P450 expression [21–25].

The indoleaminergic system (serotonergic system) of the brain seems also to be involved in the central neuroendocrine regulation of cytochrome P450 expression. Our most recent data show that injection of the specific serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the anterior raphe nuclei of the brainstem (the dorsal and medial raphe nuclei) decreases serotonin concentration in all brain structures (including the hypothalamus), followed by a significant rise in the serum concentration of the growth hormone, corticosterone and testosterone, paralleled by a drop in triiodothyronine concentration. Simultaneously, the expression (mRNA and protein) and activity level of the hormone-dependent isoforms CYP1A1/2, CYP2C11 and CYP3A1 increases [26].

Therefore, in the present study we aimed at investigating the effect of stimulation of brain serotonergic system on the regulation of cytochrome P450 expression in the liver. To this end we administered the serotonin precursor 5-hydroxytryptophan (5-HTP) for 5 days to the lateral ventricles of rat brain. Afterwards, the brain concentrations of serotonin and its metabolite 5-hydroxyindoleacetic acid 5-HIAA, serum hormone levels and liver cytochrome P450 expression and activity were measured. Our study focused on CYP isoforms involved in the metabolism of clinically important drugs, in particular on isoforms belonging to the subfamilies CYP1A, CYP2C, CYP2D and CYP3A [6]. The obtained results provide evidence that brain serotonergic system is engaged in the neuroendocrine regulation of liver cytochrome P450 expression and suggest mechanisms by which this enzyme may be regulated by some antidepressant drugs.

2. Materials and methods

2.1. Animals

Male Wistar Han rats (Charles River Laboratories, Sulzfeld, Germany) weighing 280–300 g were individually kept under standard laboratory conditions (a 12:12 h light/dark cycle; a temperature of $22 \pm 2\,^{\circ}C$; room humidity of $55 \pm 5\%$). The animals had free access to food and tap water, but 18 h before decapitation they were deprived of food to avoid any effect of the digestive process on enzymatic activity. All the experimental procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Local Bioethics Commission at the Institute of Pharmacology of the Polish Academy of Sciences (Kraków).

2.2. Drugs and chemicals

The following compounds were used for the study: serotonin (5-hydroxytryptamine, 5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), noradrenaline (NA), dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), 5-hydroxytryptophan (5-HTP), ascorbic acid, NADPH, NADP, glucose-6-phosphate-dehydrogenase and glucose-6-phosphate, caffeine and its metabolites (theobromine, paraxanthine, theophylline, and 1,3,7-trimethyluric acid); all the compounds were purchased from Sigma (St. Louis, MO, USA). Testosterone and its hydroxy-metabolites (2α-, 2β-, 6β-, 7α-, 16α- and 16β-hydroxy-testosterone) were provided by Steraloids (Newport, KY, USA). Warfarin was donated by Merck (Darmstadt, Germany), while 7-hydroxywarfarin was synthesized at our Institute [8]. Bufuralol and 1-hydroxybufuralol was a gift from Dr. Y. Funae of the Osaka University, Japan. The polyclonal primary anti-rat CYP1A1 antibody, a secondary antibody (anti-IgG) and rat cDNA-expressed CYPs were obtained from Gentest Corp. (Woburn, MA, USA). The polyclonal primary anti-rat CYP2C11 antibody was purchased from Abcam (Cambrige, UK); the anti-rat CYP3A1 and CYP3A2 antibodies were obtained from Millipore (Temecula, USA). The polyclonal primary anti-human CYP2A13, monoclonal anti-rat CYP2B1/2 and polyclonal anti-rat β-actin antibodies were purchased from Santa Cruz (Dallas, USA). The chemiluminescence reagents LumiGlo kit came from KPL (Gaithersburg, USA). ELISA kits for serum hormones (growth hormone and testosterone) were purchased from DRG, MedTek (Warsaw, Poland), and those for corticosterone, T_3 and T_4 came from Endocrine Technologies (Newark, CA, USA). ELISA kits for the interleukines IL-2 and IL-6 were obtained from R&D Systems (Minneapolis, USA). All the organic solvents were of HPLC purity and were supplied by Merck (Darmstadt, Germany). For RNA isolation a mirVana kit purchased from Life Technologies (Carlsbad, CA, USA) was used. To perform reverse-transcription a Transcriptor High-Fidelity cDNA synthesis kit was applied (Roche Diagnostics, Indiana, IN, USA). TaqMan assays and TaqMan Gene Expression Master Mix were also purchased from Life Technologies (Carlsbad, CA, USA). RNA-free water was obtained from Sigma (St. Louis, MO, USA). Ketamine (ketamine hydrochloride) and Sedazin (xylazine hydrochloride) came from Biowet (Puławy, Poland).

2.3. Surgery and intracerebral injection of 5-HTP

The rats were anesthetized with ketamine HCl (65 mg/kg i.p.) and xylazine HCl (5 mg/kg i.p.) and were placed in the Kopf stereotaxic apparatus (Tujunga, CA, USA). Stainless-steel guide cannulas were implanted bilaterally in the lateral ventricles of the brain. The coordinates were based on a rat brain atlas [27] and were as follows: AP −0.8, L ± 1.5 from the bregma and V −3.0 from the dura. Intracerebroventricular (icv) injections were made through an inner cannula (V −3.5) 7 days after implanting the guide cannula. The freshly prepared 5-HTP (a serotonin precursor) at a concentration of 10 μg/μl was administered (5 μl, infused at a rate of 1 μl/min) into both lateral ventricles of the brain (50 μg per ventricle). The applied dose of 5-HTP was chosen on the basis of our preliminary experiment (using icv 5-HTP doses of 10–50 μg), which indicated the dose-dependent efficacy of 5-HTP in increasing serotonin level in the brain (data not shown). First of all, the experimentally chosen dose of 5-HTP (50 μg/ventricle) was administered as a single intracerebral injection to confirm its short-term (30 min) effectiveness in the brain. In that case only the brain neurotransmitter level was measured. Then, the confirmed dose of 5-HTP (50 μg/ventricle) was injected for five days (once a day) to evoke neuroendocrine-produced changes in cytochrome P450 (CYP) expression in the liver. In the latter case the brain neurotransmitter, serum hormones and liver cytochrome P450 were investigated. Samples were collected 3 h after the last 5-HTP injection to give the serotonergic signal evoked in the brain enough time to be transformed (via endocrine system) into a peripheral effect in the liver, i.e. the altered expression of CYP protein. The results obtained after repeated intracerebral injection of 5-HTP were compared with those for the control (sham-operated animals) which received repeated intracerebral injection of a vehicle (a 0.9% NaCl with a 0.05% ascorbic acid) instead of 5-HTP.