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The reverse role of the hypothalamic paraventricular (PVN) and arcuate (ARC) nuclei in the central serotonergic regulation of the liver cytochrome P450 isoform CYP2C11

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ABSTRACT

Our recent work showed that the brain serotonergic system negatively regulated liver cytochrome P450. The aim of our present research was to study the effect of damage to the serotonergic innervation of the paraventricular (PVN) or arcuate nuclei (ARC) of the hypothalamus on the neuroendocrine regulation of cytochrome P450 (CYP). Male rats received bilateral injections of the serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the PVN or ARC. One week after the injection brain neurotransmitters, serum hormones (growth hormone, testosterone, corticosterone, thyroid hormones), pituitary somatostatin and liver cytochrome P450 expression and activity were measured. Lesion of the serotonergic innervation of the PVN decreased serotonin level in the hypothalamic area containing the PVN, causing an increase in growth hormone and testosterone concentrations in the blood and, subsequently, an increase in the expression (mRNA and protein level) and activity of isoform CYP2C11 in the liver. In contrast, damage to the serotonergic innervation of the ARC, which caused a decrease in serotonin level in the hypothalamic area containing the ARC, reduced the concentration of growth hormone and the expression and activity of CYP2C11. In conclusion, the obtained results show a reverse effect of the serotonergic innervation of the hypothalamic paraventricular (a negative effect) and arcuate nuclei (a positive effect) on growth hormone secretion and growth hormone-dependent CYP2C11 expression. They also suggest that CYP2C11 expression may be changed by drugs acting *via* the serotonergic system, their effect depending on their mechanism of action, route of administration (intracerebral, peripheral) and distribution pattern within the hypothalamus.

1. Introduction

Cytochrome P450 (CYP) enzymes are members of the superfamily of heme-containing monooxygenases that play an important role in the oxidative metabolism of endogenous (eg. steroids) and exogenous substrates (drugs, toxins). The most important role in physiological regulation of the expression of cytochrome P450 in the liver is played by such hormones as growth hormone (GH), thyroid hormones (triiodothyronine T_3 and thyroxine T_4), glucocorticoids, and sex hormones, which – *via* activation of the nuclear/cytoplasmic receptors – regulate the transcription of CYP coding genes [1–7].

The research conducted in our laboratory in the last decade was aimed at demonstrating the importance of brain nervous system in the regulation of the expression of cytochrome P450 in the liver. Our earlier studies showed an important role of brain dopaminergic and noradrenergic systems in the above-mentioned regulation *via* neuroendocrine mechanisms [8–13]. Our more recent work showed that brain serotonergic system also contributed to the regulation of hepatic cytochrome P450, yet in a different way (yielding mostly opposite results) than that described for catecholaminergic brain systems [14,15].

Thus injection of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) into the frontal raphe nuclei (the median – MRN and dorsal raphe nuclei – DRN) decreased serotonin concentration in

Abbreviations: ARC, arcuate nucleus; PVN, paraventrucular nucleus; CYP, cytochrome P450; HPLC, high performance liquid chromatography; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; 5,7-DHT, 5,7-dihydroxytryptamine; 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; CRT, corticosterone; SST, somatostatin; TRH, thyrotropin-releasing hormone; T_3, triiodothyronine; T_4, thyroxine; TST, testosterone.

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some brain structures (including the hypothalamus), followed by a significant rise in the concentrations of growth hormone, corticosterone and testosterone, and a drop in triiodothyronine concentration in the serum. At the same time, the expression (mRNA and protein) and activity levels of the hormone-dependent isoforms CYP1A1/2, CYP2C11 and CYP3A1 rose [14]. Accordingly, repeated administration of the serotonin precursor 5-hydroxytryptophan (5-HTP) into the lateral ventricles of the brain significantly increased serotonin levels in the brain (especially in the hypothalamus), which resulted in a decreased concentration of growth hormone and an increased thyroxine concentration in the blood. Those hormonal changes led to reduction in the expression and activity of the cytochrome P450 isoforms CYP1A2, CYP2C11 and CYP3A1/2 [15]. The two complementary experimental approaches described above, i.e. the damage or the activation of brain serotonergic system, constitute well-documented proof of the negative regulation of liver cytochrome P450 by brain serotonergic system.

The level of serotonin in the hypothalamus significantly changed after injection of the neurotoxin 5,7-DHT into the raphe nuclei, as well as after intraventricular administration of the serotonin precursor 5-HTP [14,15]. The hypothalamus contains two kinds of nuclei essential for neuroendocrine regulation, i.e. the paraventricular nuclei – PVN (producing corticotropin-releasing hormone – CRH, thyrotropin-releasing hormone – TRH and somatostatin – SST) and the arcuate nuclei – ARC (producing growth hormone-releasing hormone – GHRH) which act differently on the secretion of anterior pituitary gland hormones involved in the regulation of CYP expression. Both the hypothalamic nuclei, the PVN and ARC, receive serotonergic innervation from the frontal raphe nuclei [16–19]. Therefore the aim of our present research was to cause damage to the serotonergic innervation of hypothalamic areas containing the PVN or ARC and, subsequently, to study its effect on serum and pituitary hormone levels and cytochrome P450 expression in the liver.

2. Materials and methods

2.1. Animals

Male Wistar Han rats (Charles River Laboratories, Sulzfeld, Germany) weighing 280–300 g were kept under standard laboratory conditions (a 12:12 h light/dark cycle; the temperature of 22 ± 2 °C; the room humidity of 55 ± 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 our 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), 5,7-dihydroxytryptamine (5,7-DHT, a creatinine sulfate salt), ascorbic acid, NADP, NADPH, glu cose-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-hydroxytestosterone) were provided by Steraloids (Newport, KY, USA). The polyclonal primary anti-rat CYP2C11 antibody was obtained from Abcam (Cambridge, UK). The polyclonal anti-rat β-actin antibody was purchased from Santa Cruz (Dallas, TX, USA). The chemiluminescence reagents LumiGlo kit came from KPL (Gaithersburg, MD, 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 pituitary somatostatin were obtained from MyBiosource (San Diego, CA, USA). ELISA kits for the interleukins IL-2 and IL-6 were purchased from R&D Systems (Minneapolis, USA). All the organic solvents were of the 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 used (Roche Diagnostics, Indianapolis, IN, USA). TaqMan assays and the 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 infrastructural injection of 5,7-DHT

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). 5,7-DHT (a toxin specific to serotonergic neurons) was dissolved in a 0.9% NaCl with a 0.05% ascorbic acid and injected into the paraventricular or arcuate nuclei (PVN or ARC) of the hypothalamus at a concentration of 6 μg/μl (1 μl infused at a rate of 1 μl/min). The rats received bilateral injections of 5,7-DHT into the PVN (6 μg per side) or the ARC (6 μg per side). Control rats (sham-operated animals, $n = 8$) were subjected to the same procedure as were the 5,7-DHT-treated group ($n = 8$), except that they received a vehicle (a 0.9% NaCl with a 0.05% ascorbic acid) instead of 5,7-DHT. The following coordinates were used in accordance with the Paxinos and Watson atlas [20]: AP −1.6 (anterior-posterior from the bregma), L ± 0.3 (lateral) and V −7.2 (ventral from the surface of the dura) for the PVN, and AP −2.6, L ± 0.4 and V −9.4 for the ARC.

The vehicle, 1 μl, or a 5,7-DHT solution was administered into the PVN or ARC using a Hamilton syringe (at a flow rate of 1 μl/min), which was left in place for 5 min after injection before it was slowly removed. One week after injection the rats were killed by decapitation. Their brains were rapidly removed and the middle hypothalamus (its upper or lower part, containing the PVN or ARC, respectively) was dissected and frozen on dry ice. The placement of the needle was histologically verified in a preliminary experiment carried out on ten rats (using a color marker and a tray of the needle). The site specificity and neurotransmitter selectivity of lesion was checked by measuring concurrently serotonin and catecholamine neurotransmitter concentrations in the PVN and ARC areas after 5,7-DHT injection into the PVN or ARC. After injection of 5,7-DHT into the PVN or ARC, a significant decrease in serotonin levels was observed in the hypothalamic area containing the PVN or ARC, respectively.

2.4. Collection of brain, liver and serum samples

One week after the injection of 5,7-DHT the rats were decapitated and their brains and livers were removed. Samples of liver and brain tissues were stored at −80 °C until they were further analyzed. The blood was collected and the serum was separated by centrifugation and stored at −20 °C. Liver microsomes were prepared from individual animals by differential centrifugation in a