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Abstracts of the doctoral dissertation in English

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Title of the doctoral dissertation:	Significance of glutamatergic NMDA receptors in the regulation of hepatic cytochrome P450

Abstract of the doctoral dissertation in English:

The majority of currently prescribed drugs are metabolized by enzymes belonging to the CYP1–3 families, which, in addition to their role in xenobiotic biotransformation, are also involved in the metabolism of endogenous compounds such as arachidonic acid, steroid hormones, and monoaminergic neurotransmitters. Variations in the expression levels and functional activity of individual CYP enzymes can significantly influence the metabolism of drugs and other chemical substances, thereby leading to pharmacokinetic interactions. The expression of cytochrome P450 enzymes is influenced by factors such as sex, tissue type, and developmental stage. Their regulatory control is mediated by hormones that activate specific ligand-dependent membrane, nuclear, or cytoplasmic receptors. Hormone secretion itself is regulated by the hypothalamus, which is innervated by various neurotransmitter systems – such as the noradrenergic, dopaminergic, and serotonergic pathways – that have already been studied in the context of cytochrome P450 regulation.

The aim of the present study was to investigate the effect of CP-101,606 – a pharmacological tool compound that acts as a selective antagonist of the GluN2B subunit of the NMDA receptor – on the neuroendocrine regulation of hepatic cytochrome P450 enzymes *via* the glutamatergic system, with particular emphasis on identifying the brain structures and hormones involved in this process. The NMDA receptor has emerged as a promising target in the search for novel therapeutic agents for neurodegenerative diseases and depression. However, candidate drugs targeting the NMDA receptor have encountered several challenges, including poor blood–brain barrier permeability and adverse

side effects. Nevertheless, due to their therapeutic potential, new compounds of this class continue to be developed. Preliminary studies involving intraperitoneal administration of CP-101,606 to male Wistar Han rats indicated a possible involvement of the glutamatergic system in the neuroendocrine regulation of hepatic cytochrome P450 enzymes. However, in the case of peripheral administration, the observed effects may result from various direct or indirect interactions between the compound and the enzyme, making it difficult to determine whether a neuroendocrine mechanism is predominant. To clarify the nature of these effects, repeated administrations of the selective GluN2B subunit antagonist were performed into the lateral ventricles of the rat brain, followed by targeted injections into the paraventricular and arcuate nuclei of the hypothalamus – structures involved in the control of hormone secretion that modulates hepatic CYP enzyme expression. Additionally, the direct effects of CP-101,606 on hormonally regulated cytochrome P450 enzymes were also examined.

Five-day administration of CP-101,606 into the lateral ventricles of the rat brain (at doses of 6, 15, or 30 µg/brain) exerted a dose-dependent effect on hepatic cytochrome P450 enzymes as well as on hypothalamic and pituitary hormones. The lowest dose of the antagonist resulted in increased activity, protein level, and mRNA expression of the CYP2C11 compared to the control group. The enzymatic activity of CYP2A, CYP2B, CYP2C11, CYP2C6, and CYP2D, as well as the protein levels of CYP2B and CYP2C11, were elevated at the lowest dose relative to the highest dose. Additionally, CP-101,606 increased CYP1A protein levels and upregulated mRNA expression of *CYP1A1* and *CYP1A2*, without affecting their enzymatic activity. The antagonist reduced somatostatin levels in the pituitary and elevated serum growth hormone concentrations at the lowest dose, while consistently decreasing serum corticosterone levels regardless of the dose applied.

Five-day administration of CP-101,606 into the paraventricular nucleus of the hypothalamus increased somatostatin levels both in this nucleus and in the pituitary gland, decreased serum concentrations of GH and corticosterone, and elevated triiodothyronine levels. A reduction in the expression (mRNA and protein) and activity of hepatic CYP1A1/2, CYP2A1/2, CYP2B1/2, CYP2C11, and CYP3A enzymes was also observed. In turn, repeated administration of CP-101,606 into the arcuate nucleus of the hypothalamus led to a decrease in growth hormone–releasing hormone levels in the arcuate nucleus and the pituitary gland, as well as reduced serum GH and corticosterone concentrations, without affecting thyroid hormone levels. Furthermore, a decrease in expression and activity of hepatic CYP1A1/2 and CYP2C11, as well as a reduction in *CYP3A2* mRNA levels, was noted.

The results obtained after five-day administration of the selective GluN2B subunit antagonist into the paraventricular or arcuate nuclei of the hypothalamus were consistent with the effects previously observed following intraperitoneal administration of the compound, whereas divergent results were obtained after administration into the lateral ventricles. Additionally, *in vitro* studies involving incubation of CP-101,606 with rat liver microsomes demonstrated that the NMDA receptor antagonist does not exert a direct effect on hormonally regulated CYP enzymes, including CYP2A, CYP2B, CYP2C11, and CYP3A. Therefore, it appears that the effects of peripheral administration of the antagonist on hepatic cytochrome P450 are primarily mediated through its action at the level of the central nervous system and neuroendocrine pathway.

The obtained results indicate a significant role of NMDA receptors containing the GluN2B subunit, located in the paraventricular and arcuate nuclei of the hypothalamus, in the central neuroendocrine regulation of cytochrome P450 expression and activity in the rat liver. The observed changes in the expression and activity of cytochrome P450 enzymes following NMDA receptor blockade (e.g., by novel pharmacological agents) may be of medical importance, both for the metabolism of endogenous compounds (such as steroid hormones) and the biotransformation of concurrently administered drugs.