Gene expression studies for the evaluation of molecular interactions between ecstasy and antidepressants

PhD thesis booklet

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INTRODUCTION

The active ingredient of the recreational drug ecstasy, 3,4-methylenedioxy-methamphetamine (MDMA), is a ring-substituted amphetamine derivative. Following its entry into the central nervous system MDMA can bind the serotonin (5-HT), dopamine (DA) and noradrenaline (NA) transporters (SERT, DAT, NAT, respectively). After the binding MDMA is transported into the cells via these proteins and its uptake is paralleled by the release of the mentioned monoamines. The released 5-HT, DA and NA are responsible for the acute positive effects of ecstasy, like euphoria, tirelessness, and the so called entactogenic effect, which means an elevated sociability. At the same time, MDMA also initiates processes able to impair the neurons acutely, e.g. hyperthermia, free radical production and impairment of the cerebral autoregulation, thereby the elevated neuronal demand for glucose and oxygen will be accompanied with vasoconstriction. On the long-run MDMA causes a decrease in serotonergic markers, which is interpreted as a selective serotonergic toxicity. The levels of tryptophan-hydroxylase or SERT is decreased in

brain regions, where 5-HT containing neurons, originating in the raphe nuclei, project. Parallel to the alterations within the serotonergic system, functional impairments also occur, and in users elevated depression, disturbed cognitive and motor functions can be observed. Through animal experiments in recent years, changes in the serotonergic system became partially delineated, however, the non-specific neurotoxic mechanisms raise the possibility of other alterations underlying the MDMA-induced chronic, functional impairments.

Venlafaxine (VLX) is a therapeutically effective antidepressant on the market, which through binding to SERT and NAT can elevate 5-HT and NA levels, but to a lesser extent than MDMA. Most of the antidepressants can also bind acutely to these transport proteins. However, acute elevations in monoamine concentrations cannot be exclusively responsible for the therapeutic effects, because 1) antidepressant efficacy is first visible after weeks, 2) approximately 30-40% of patients are resistant to current antidepressant therapies, 3) combination with psychotherapy was more effective than pharmacological treatments alone. Several theories tried to explain these discrepancies. One of them suggested

chronically elevated monoamine levels through the desensitization of 5-HT1B, 5-HT1A and α2 receptors, while another, the so called synaptic theory of depression, stated that in depression the number of synapses may be substantially decreased and antidepressants may reinstate impaired network functions through the induction of synapse formation. While alterations in depression and subsequent antidepressant treatments in the so called depression circuitry, the prefrontal cortex, hippocampus and amygdala, were evaluated by multiple experiments, studies in other brain regions, like the frontal cortex, which has dense serotonergic innervations and a confirmed role in depression, are scarce. Furthermore, the synaptic theory of depression raises the possibility of the use of antidepressants in other pathological states, e.g. stroke, where cortical regions also play a role. Indeed, fluoxetine, a selective 5-HT reuptake inhibitor showed efficacy in a clinical study evaluating motor recovery following stroke and VLX activated motor cortices of healthy individuals in another experiment. It remains unclear, however, what molecular changes can be responsible for the therapeutic actions of VLX, which

possess an extended mechanism of action via the inhibition of both 5-HT and NA reuptake, since these alterations are unevaluated at therapeutically relevant time points in the frontal cortex.

In the light of the above, the two substances, MDMA and VLX, can both acutely, through alterations of monoaminergic levels, and chronically influence each other's effects. The functional consequences, like motor and cognitive disturbances and depression in MDMA users and elevated motor and cognitive functioning and antidepressant effects after chronic VLX administration also raise the possibility of interactions. Furthermore, in an experiment previous MDMA administration attenuated the effects of chronic fluoxetine. However, according to our knowledge, effects of chronic VLX administration following acute MDMA administration remained unevaluated.

OBJECTIVES

1. Evaluation of the molecular mechanisms three weeks after a single dose MDMA administration, which may play a part in functional impairments

or a possible regeneration in the frontal cortical regions.

2. Discovery of signaling pathways, which may underlie the therapeutic effects of VLX after a 3-weeks long chronic treatment in the frontal cortex.
3. To examine, if and how 3-weeks long VLX treatment may influence the MDMA-caused alterations, and whether a previous MDMA administration may have impact on the changes induced by VLX in frontal cortical regions.

METHODS

We used 42, eight weeks old Dark Agouti rats. These animals possess an enzyme polymorphism, which results in a slower metabolism rate of MDMA, and thus, represent the human population which may be especially vulnerable to neurotoxic effects of the drug. The animals were divided in two groups and received single saline injections (SAL) or MDMA (15 mg/kg) intraperitoneally. Thereafter two further subgroups were made, one of them received three weeks long VLX treatment (40 mg/kg via

minipumps), while the control group contained animals with sham operations or saline containing minipumps (SHAM). This protocol resulted in four treatment groups: control animals (SAL/SHAM), MDMA treated- (MDMA/SHAM) and VLX treated animals (SAL/VLX), and those receiving a combined treatment (MDMA/VLX).

After three weeks animals were decapitated, frontal cortical regions dissected and RNA extracted with TRIZOL method. The samples were exposed to quality measurements and 2-2 samples from the eight samples with the best values were pooled.

The 4 pooled samples per treatment group were examined with whole-genome expression microarrays (Illumina RatRef-12 v1) containing 22 523 different probes.

Raw data were background corrected and normalized using program packages for R statistical programing language. Thereafter, significantly altered genes were determined by the PUMA package, while to avoid bias from multiple hypothesis testing, significance criteria were reduced. We identified significantly altered biological pathways with gene set enrichment analysis

(GSEA). To reveal interactions ANOVA was also performed.

RESULTS

In the MDMA treated group, following the grouping of the enriched gene sets into biological pathways, protein synthesis and -localization, transmembrane- and nucleocytoplasmic transport, chromatin maintenance and mitochondrial oxidoreductase activity related gene sets were found to be downregulated, while gene sets of dendrite- and synapse development showed elevated expression 3 weeks after a single-dose treatment in the frontal cortex. Individual genes supported the findings: downregulations were evident in genes of some ribosomal proteins, while calcium/calmodulin-dependent kinases and the NMDA2B type glutamate transporter showed elevated expressions, among others.

In the case of the VLX, significantly enriched gene sets could be grouped to four main biological processes. Gene sets implicated in neurotransmitter release, synaptic plasticity and -organization, and

insulinergic signaling were upregulated, while those involved in mitochondrial antioxidant activity were downregulated. On the gene level, insulinergic (Dpp4) and glutamatergic (Grin2b, Gria) genes and calcium/calmodulin dependent kinases showed elevated expression, while those related to mitochondrial activity were downregulated. The galaninergic genes remained unaltered.

Comparison of the double treated group to the VLX treated animals showed that the expression of gene sets involved in biosynthetic processes and mitochondrial functions was decreased, while Stat3 signaling was upregulated. Comparison of the combination treatment to the MDMA treated animals revealed that gene sets related to the ribosomal subunits and translation showed underexpression, but some genes with known antidepressant activity (Ca2, Rgs9) were upregulated.

The combined treatment compared to the control group showed elevations in the expression of gene sets related to neurotransmitter release, synaptic plasticity, insulinergic- and glutamatergic signaling and a downregulation of mitochondrial functions, translation

and other biosynthetic processes, pointing out to possible additive effects of the two substances.

The ANOVA analysis demonstrated interaction effects only in case of TATA-box binding protein, confirming that the other changes are additive effects.

CONCLUSIONS

1. MDMA may cause functional deficits via the downregulation of biosynthetic processes and decreased mitochondrial antioxidant activity, however, 3 weeks after its administration growth of dendrite trees may mark ongoing recovery processes in the frontal cortical region.
2. Gene sets related to neurotransmitter release after 3-weeks long, chronic VLX administration suggest adaptive mechanisms, while upregulations of synaptic connectivity related gene sets support the synaptic theory behind its antidepressant effects. We identified insulinergic pathway as a so far undiscovered mechanism of action. VLX also attenuated mitochondrial

antioxidant activities and could not influence the expression of galanin genes.

3. In the combined treatment, we can conclude, that most of the effects are additive except TATA-box binding protein, which may be implicated in the regulation of mitochondrial and translational processes. The MDMA administration three weeks earlier could not influence the effects of chronic VLX treatment and similarly, VLX could not reverse the changes induced by MDMA. At the same time, the antidepressant could exert its wide-scale positive upregulations probably also involved in its therapeutic effects, which raises the possibility of its use in cortical diseases even in previous addicts.

PUBLICATIONS

Journal articles related to the thesis:

1. Petschner P, Vas S, Adori C, Ando DR, Balogh B, Gyongyosi N, Kirilly E, Katai Z, Kovács G, Bagdy G (2010) Az ecstasy által kiváltott neuronkárosodás és regeneráció funkcionális

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3. Vas S, Juhasz G, Kostyalik D, Laufer R, Magyar K, Petschner P, Szoko E, Tabi T, Tekes K, Tothfalusi L, Torok T, Bagdy G (2014) **A Semmelweis Egyetem Gyógyszerhatástani Intézetében folyó központi idegrendszeri kutatások. Gyógyszerészet**, 68:(Suppl. 1) p. S50.
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5. Kostyalik D, Katai Z, Vas S, Pap D, Petschner P, Molnar E, Gyertyan I, Kalmar L, Tothfalusi L, Bagdy G (2014) **Chronic escitalopram treatment caused dissociative adaptation in serotonin (5-HT) 2C receptor antagonist-induced effects in REM sleep, wake and theta wave activity**. *Exp Brain Res*, **232**(3):935-946.
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