Long-term effects of 3,4-methylenedioxymethamphetamine (MDMA) on 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptor functions in the control of vigilance and motor activity 6 months after treatment with neurotoxic dose of MDMA

PhD theses

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1. INTRODUCTION

The recreational drug „ecstasy” has become second in popularity to cannabis in Hungary. Its active compound is a ring-substituted amphetamine derivative, the 3,4-methylenedioxymethamphetamine or MDMA. The unique psychopharmacological profile of this drug is due to its ability to promote the release of serotonin (5-HT), but it enhances also the release of dopamine, norepinephrine and acethylcholine in multiple brain regions.

MDMA causes long-term alterations in parameters of serotonergic system in rodents and non-human primates, and there is evidence that ecstasy is able to cause similar changes in human users. MDMA is metabolised principally by cytochrome P450 2D6 (CYP2D6) or debrisoquine hydroxylase. It is reported that between 5 - 9% of Caucasians are considered "poor metabolizers" and have lower levels of activity of the liver enzyme CYP2D6, and are at greater risk of adverse reactions. In rats, MDMA is metabolised by an analogous isoenzyme, CYP2D1. The Dark–Agouti rat strain possesses decreased microsomal CYP2D1 isoenzyme activity, and thus the Dark–Agouti strain can be considered as a good model organism for the poor metabolizer human population.

Our laboratory has showed that a single dose of 15 mg/kg of MDMA (i.p.) causes decrease in serotonin transporter and tryptophan-hydroxylase density in almost all brain regions in 3-7 days after the treatment. Tryptophan-hydroxylase is the rate-limiting enzyme of the serotonin synthesis. Although regeneration of the serotonergic system was observed in most parts of the brain, thalamus, hypothalamus, parts of the dorsal striatum and hippocampus still showed alterations six months after the treatment.

Although the neurotoxic effects of MDMA are well-documented, the long-term consequencies of the 5-HT neurodegeneration on serotonergic receptor functions remains elusive. Serotonergic system plays a prominent role in the control of sleep-wake cycle and motor activity, and the serotonin-1B, -2, and -3 receptors ($5\text{-}HT_{1B}$, $5\text{-}HT_2$, $5\text{-}HT_3$) are important in the control of vigilance and movement. They posseses different signal transduction pathways: the $5\text{-}HT_{1B}$ receptor is coupled to $G_{i/o}$ protein, signaling of $5\text{-}HT_2$ receptors goes via G_q protein, and the $5\text{-}HT_3$ receptors belongs to the ligand-gated cation channel family. The importance of these receptors is underscored by the fact that they are used as therapeutic targets.

2. AIMS

There is a lot of evidence that MDMA has neurotoxic effect on the brain serotonergic system, but studies describing the long-term functional consequencies are missing. There is no controlled human studies due to ethical problems, and the avaiable animal data are contradictory. We dont know yet whether the long-term alteration of the serotonergic system is able to cause changes in 5-HT receptor function in the control of vigilance and movement. Our studies were carried out in Dark Agouti rat strain that is a useful experimental tool to model a genetically-defined human sub-population called „poor-metaboliser". Our aim was to study whether the long-lasting damage of the serotonergic system would be manifested in changes in the role of the 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors in the control of sleep/wake cycle and motor activity.
The main objectives of our studies were to answer the following questions:

1) How does the systemic activation of 5-HT_{1B} receptors influence the parameters of vigilance and motor activity? Does MDMA pre-treatment six months earlier cause any changes in the acute effects of the activation of 5-HT_{1B} receptors on sleep/wake cycle and movement?

2) How does the systemic activation of 5-HT_2 receptors influence the parameters of vigilance and motor activity? What is the contribution of 5-HT_{2C} receptors to such effects? Does MDMA pre-treatment six months earlier cause any changes in the acute effects of the activation of 5-HT_2 receptors on sleep/wake cycle and movement?

3) How does the systemic activation of 5-HT_3 receptors influence the parameters of vigilance and motor activity? Does MDMA pre-treatment six months earlier cause any changes in the acute effects of the activation of 5-HT_{1B} receptors on sleep/wake cycle and movement?

3. MATERIALS AND METHODS

3.1 Animals and drugs

All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). Permission was given by the local ethical committee.

Male Dark Agouti rats (Harlan, Olac Ltd, United Kingdom) were used in the experiments. Rats were kept in single cages with food and water available *ad libitum*, and maintained in a 12 h light/dark cycle (lights on from 0900 to 2100 hours) at an ambient temperature of 21±1°C. Rats were randomly injected with 15 mg/kg MDMA (i.p.) or saline (1ml/kg) as pre-treatment. Five and a half months after the pre-treatment, animals were equipped with chronic epidural EEG electrodess (fronto-parietal recordings) and chronic EMG electrodes in the neck. After a 7-day recovery period the rats were attached to the polygraph by a flexible recording cable and an electric swivel, fixed above the cages, permitting free movement of the animals. The animals remained connected to the recording cables throughout the study. An electromagnetic transducer activated by cable movements was used to record motor activity. In order to habituate the animals to the recording conditions, the rats received intraperitoneal (i.p.) injections of physiological saline for 7 days before the experiments. The rats were connected to the recording cables throughout the study. To reveal the possible long-term alteration of 5-HT receptor functions the rats received intraperitoneal injection of the selective **5-HT$_{1B}$ agonist** 5-propoxy-3-(1,2,3,6-tetrahydro-4-pyrinzidyl)-1H-pyrrolo([3,2-b])pyridine hydrochloride (CP-94,253; 5 mg/kg), the **5-HT$_{2A/2B/2C}$ agonist** 2,5-dimethoxy-4-iodoamphetamine (DOI; 0.2 mg/kg), the selective **5-HT$_3$ agonist,** m- chlorophenylbiguanide (mCPBG; 1 mg/kg) or saline (1 ml/kg) at light onset 6 months after MDMA pre-tratment. In order to investigate the contribution of the activation of 5-HT$_{2C}$ receptors to the control of the sleep-wake cycle and movement, male Sprague-Dawley rats were treated with the **5-HT$_{2C}$ antagonist** 6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline (SB-242084, 0.1, 0.3, and 1.0 mg/kg i.p.) without MDMA pre-treatment. After the acute treatment, polygraphic recording and motor activity measurements were performed for 24 hours. Rats were undisturbed throughout the recordings. Data were stored on computer for further analysis.

The polygraphic recordings were classified with sleep analysis software SleepSign for Animal (Kissei Comtec America Inc.). The vigilance states were scored as follows:

Active wake (AW): EEG characterized by low amplitude activity at alpha (8–13 Hz) and beta (14–30 Hz) frequencies accompanied with high EMG and motor activity.

Passive wake (PW): EEG characterized by low amplitude activity at alpha (8–13 Hz) and beta (14–30 Hz) frequencies accompanied with high EMG and without motor activity.

Light slow wave sleep (SWS-1): EEG characterized by high-voltage slow cortical waves (0.5–4 Hz) interrupted by low-voltage fast EEG activity (spindles, 6–15 Hz) accompanied with reduced EMG and motor activity.

Deep slow wave sleep (SWS-2): continuous high-amplitude slow cortical waves (0.5–4 Hz) with reduced EMG and motor activity.

Paradoxical sleep (PS): EEG characterised by low amplitude and high frequency EEG activity with regular theta waves (5–9 Hz) accompanied by silent EMG and motor activity with occasional twitching.

3.2. Statistical analysis

For statistical analysis, STATISTICA 7.0 (Statsoft Inc., Tulsa, OK) software was used. Data were evaluated by factorial analysis of variance (ANOVA) with multiple factors. Tukey honest significant difference test was used for post-hoc comparisons. Level of significance was set at $p<0.05$. Cosinor analysis provides a mathematical method for evaluating and estimating parameters of a rhythm. In order to quantify diurnal rhythm of behavioural and sleep parameters, **amplitude**, **acrophase** (the time at which the peak of a rhythm occurs) and **mesor** (average) values (±confidence limits) were calculated by the software Time Series Analysis Seriel Cosinor 6.0 Lab View (Expert Soft Technologie, 1996–2004).

4. RESULTS

The selective 5-HT_{1B} agonist CP-94,253 increased active and passive wake, and decreased light and deep slow wave sleep in the first 2-6 hours after treatment. The effect of CP-94,253 on vigilance gained by sleep scoring was confirmed by the parameters of diurnal rhythms analysed with cosinor analysis. The peak time (acrophase) for PW, SWS-1, SWS-2 was also significantly altered, shifting to an earlier time for wakening states and a later time for sleep stages. The mesor (average) of PS was decreased, and the mesor of PW was increased significantly. CP-94,253 treatment abolished the diurnal rhythm of AW and PS. Effects of CP-94,253 on SWS-1, SWS-2 and PS were unaltered 6 months after MDMA pre-treatment. However, the effect on AW was absent in rats pre-treated with MDMA. This means that active wake was not increased by CP-94,253 treatment in animals treated with MDMA 6 months earlier. MDMA pre-treatment significantly attenuated the effects of CP-94,253 on diurnal patterns of AW, i.e., CP-94,253 was unable to abolish the diurnal rhythm of AW (significant rhythm was found) or alter the acrophase of AW 6 months after MDMA treatment.

The 5-$HT_{2A/2B/2C}$ agonist DOI increased the time spent in active wake in control animals. The selective 5-HT_2 agonist DOI shifted the acrophase of AW to a later time. The effect of DOI on time spent in AW was absent in rats pre-treated with MDMA, although the effect of DOI on diurnal rhythm of AW was not altered in MDMA pre-treated group. The passive wake was increased after teratment with DOI, and the selective 5-HT_2 agonist increased mesor and shifted acrophase to an earlier time in rats pre-treated with saline. The effect of DOI on passive wake was not altered in rats pre-treated with MDMA 6 months earlier. We found that DOI shifted acrophase of SWS-1 to a later time in control rats, and effects of DOI on SWS-1 was not altered after MDMA pretreatment. The selective 5-HT_2 agonist DOI decreased the time spent in SWS-2 and PS, and shifted acrophase to a later time in control rats. Altough the values of SWS-2 and PS gained by sleep scoring were not altered in MDMA pre-treated group, cosinor analysis showed that effect of DOI changed on diurnal rhythm of SWS-2 and PS.

The duration of different vigilance states was not considerably affected by lower doses (0.1 and 0.3 mg/kg) of the selective 5-HT_{2C} antagonist SB-242084. The highest dose (1.0 mg/kg) of SB-242084 increased wake and decreased SWS-2 compared with vehicle in the 1st

hour. In the 3rd and 4th hour, a significantly increased SWS-1 was found. SB-242084 did not altered motor activity.

The selective 5-HT_3 agonist mCPBG did not altered diurnal rhythm of any of the vigilance state studied. However, mCPBG increased AW in the 2nd hour after treatment in control rats. Effect of mCPBG on AW was absent in rats pre-treated with MDMA. Time spent in PW was altered by mCPBG treatment neither in the control group, nor in the animals pre-treated with MDMA. We found that mCPBG treatment decreased SWS-1 in vehicle pre-treated animals, and MDMA treatment six months earlier attenuated the effect of mCPBG on SWS-1. Treatment with mCPBG did not changed the time spent in PS and SWS-2 in control group, and this effect of mCPG was not altered 6 months after MDMA treatment.

Based on our data, we can state that MDMA treatment (15 mg/kg) 6 months earlier causes long-term decrease in the effect of the activation of 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors on motor activity (AW), and changes the effect of the activation of 5-HT_2 and 5-HT_3 receptors on sleep/wake cycle.

5. CONCLUSIONS

The results of our study lead us to the following conclusions:

1. Our findings suggest that the activation of 5-HT_{2C} receptors contributes to the effect of 5-HT_2 agonist DOI on light slow wave sleep (SWS-1). Based on our data, it seems that the effect of DOI on other vigilance states is not due to the activation of 5-HT_{2C} receptors, because 5-HT_{2C} receptor activation has opposite effects on these states.

2. We may conclude that MDMA is able to develop long-term cross-tolerance with other hallucinogens, and this raises the possibility that usage of ecstasy leads to increasing dosage of more dangerous hallucinogenic drugs.

4. It is possible that the functional alteration of 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors contributes to the tolerance to MDMA reported in human ecstasy users and suggests a danger for impairment in the control of sleep-wake cycle and movement in these people.

5. Our data raise the possibility that effects of medicines acting on receptors studied are altered in ecstasy users and may encourage to find alternative treatments.

6. PUBLICATIONS

6.1. Publications relevant to the dissertation

6.2. Journal articles

Norbert Gyongyosi, Brigitta Balogh, Eszter Kirilly, Tamas Kitka, Sandor Kantor, Gyorgy Bagdy (2008). MDMA treatment 6 months earlier attenuates the effects of CP-94,253, a 5-HT_{1B} receptor agonist, on motor control but not sleep inhibition. Brain Res 1231:34-46

Norbert Gyongyosi, Brigitta Balogh, Zita Katai, Eszter Molnar, Rudolf Laufer, Kornelia Tekes, Gyorgy Bagdy (2010). Activation of 5-HT_3 receptors leads to altered responses 6 months after MDMA treatment. J Neural Transm 117(3):285-92

Sandor Kantor, Rita Jakus, Eszter Molnar, **Norbert Gyongyosi**, Attila Toth, Laszlo Detari, Gyorgy Bagdy (2005). Despite similar anxiolytic potential, the 5-hydroxytryptamine 2C receptor antagonist SB-242084 [6-chloro-5-methyl-1-[2-(2-methylpyrid-3-yloxy)-pyrid-5-yl carbamoyl] indoline] and chlordiazepoxide produced differential effects on electroencephalogram power spectra. J Pharmacol Exp Ther 315(2): 921-30

6.3 Posters and presentations

N. Gyongyosi, B. Balogh, S. Kantor, G. Bagdy: Effects of the 5-HT_{1B} receptor agonist CP94253 in rats treated with MDMA (Ecstasy) 6 months earlier. 11th of Congress of the Hungarian Society of Neuroscience, Pécs, Hungary, January 26-29, 2005., Clinical Neuroscience 58, 1. különszám, 2005

N. Gyongyosi, B. Balogh, S. Kantor, G. Bagdy: Long term effects of MDMA on 5-HT_{1B} receptor functions. Spring Symposium of the Hungarian Society for Experimental and Clinical Pharmacology, June 6-7 2005, Budapest, Hungary

S. Kantor, R. Jakus, E. Molnar, **N. Gyongyosi**, G. Bagdy: Effect of potent anxiolytic doses of the 5-HT_{2C} receptor antagonist SB-242084 and chlordiazepoxide on vigilance states in freely moving conscious rats. 19th Annual Meeting of the Associated Professional Sleep Societies, June 18-23, 2005 Denver, Colorado, USA. Sleep 28, P. 120, 2005.

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6.4 Book chapters

Gyöngyösi Norbert, Lazáry Judit, Ádori Csaba: A másnap, harmadnap tapasztalt pszichés hatások biológiai alapjai. In: Bagdy György (szerk.), Amit az Ecstasyról tudni kell iskolásoknak, szülőknek, tanároknak, partizóknak. Akadémiai kiadó, Budapest, 2006:124-130

Ádori Csaba, **Gyöngyösi Norbert**, Lazáry Judit: Az idegrendszer általános felépítése. In: Bagdy György (szerk.), Amit az Ecstasyról tudni kell iskolásoknak, szülőknek, tanároknak, partizóknak. Akadémiai kiadó, Budapest, 2006:38-46

Ádori Csaba, **Gyöngyösi Norbert**, Lazáry Judit: Az idegrendszer nagy működési egységei. In: Bagdy György (szerk.), Amit az Ecstasyról tudni kell iskolásoknak, szülőknek, tanároknak, partizóknak. Akadémiai kiadó, Budapest, 2006: 46-49

Andó Rómeó Dénes, **Gyöngyösi Norbert**, Lazáry Judit: Az első néhány órában kialakuló pszichés hatások biológiai alapjai. In: Bagdy György (szerk.), Amit az Ecstasyról tudni kell iskolásoknak, szülőknek, tanároknak, partizóknak. Akadémiai kiadó, Budapest, 2006:98-105

Ádori Csaba, **Gyöngyösi Norbert**, Lazáry Judit: Szakkifejezések szótára. In: Bagdy György (szerk.), Amit az Ecstasyról tudni kell iskolásoknak, szülőknek, tanároknak, partizóknak. Akadémiai kiadó, Budapest, 2006:200-215

6.5 Other publications

Posters and presentations

T Kitka, **N Gyongyosi**, B Balogh, S Kantor, G Bagdy. Suppression of REM as a measure of antidepressant pharmacotherapy: studies with citalopram. Semmelweis Egyetem Gyógyszerésztudományi Kar önnállóvá válásának 50. évforulója alkalmából Pharmacy: Smart Molecules for Therapy címmel rendezett konferenciája. MTA. Budapest 2005. 10. 12-14.

Katai, Z., Kitka, T., **Gyongyosi, N**., Bagdy, G.: Effects of risperidone on vigilance and EEG power spectra, A Magyar Experimentális Farmakológia III. Szimpóziuma, Budapest, 2007. június 1-2.

Katai, Z, Kitka, T, **Gyongyosi, N**, Bagdy, G. Effects of risperidone on vigilance and EEG power spectra. Joint Meeting of the Slovak Physiological Society and The Physiological Society and The Federation of European Physiological Societies, Bratislava, September 11-14 2007; Acta Physiologica, Volume 191, Suppl. 658, 2007, pp. 47.

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N. Gyöngyösi, A .P. Sándor, K. Koi, K. Káldi: Contribution of ROS and RASGEF-mediated signaling to the control of circadian rhythm in *Neurospora crassa*. XI. Congress of the European Biological Rhythms Society. August 22-28, 2009, Strasbourg, France