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Ph.D. thesis

EFFECTS OF MDMA ON VIGILANCE AND PHARMACOLOGICAL CONSEQUENCES OF MDMA NEUROTOXICITY

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INTRODUCTION

Ecstasy is widely used, mainly by young people, as a recreational drug all over the world. Tablets sold as ecstasy primarily contain the hallucinogenic and sympathomimetic ring-substituted amphetamine derivative, MDMA (3,4-metilenedioxymethamphetamine). Its stimulant and psychoactive effects, ease of ingestion and the simple synthesis of ecstasy also contribute to its popularity.

Acute effects of MDMA are short-lived, lasting approximately 24 hours, while longer-term neurotoxic effects may extend up to 12 months or more in experimental animals. MDMA produces its unique behavioural effects by stimulating the release of 5-HT (5-hydroxytryptamine, serotonin), dopamine, noradrenaline and acetylcholine from presynaptic nerve terminals. As a substrate of monoamine transporters it also inhibits the reuptake of monoamines. Furthermore, its inhibitory effects on the monoamine metabolizer MAO_A (monoamine oxidase type A) and MAO_B (monoamine oxidase type B) enzymes contribute to the enhancement in extracellular monoamine concentrations as well. Acutely it induces hyperthermia, as well as the "serotonin behavioural syndrome" characterized by hyperactivity, tremor, ataxia, head-weaving, hindlimb abduction, piloerection, salivation and defecation. Additionally it causes tachycardia and increases arterial blood pressure. Its acute effects in humans are also very similar to those seen in experimental animals.

The primary targets of MDMA are serotonergic neurons in which persistent neurotoxic effects have been demonstrated following MDMA administration. In general, its effects are consistent across species: it causes long-term reductions in 5-HT and 5-hydroxyindoleacetic acid (5-HT metabolite) concentrations, and in the density of 5-HT reuptake sites in several brain areas. MDMA also reduces the activity of tryptophan hydroxylase, the rate-limiting enzyme of 5-HT synthesis. Additionally, there is immunocythochemical

evidence that it produces morphological changes to serotonergic neurones as well.

It is well established that different strains of rats have different sensitivity to both the acute and the long-term neurotoxic effects of MDMA. The most sensitive strain is the Dark Agouti, which only requires a single dose (10-15 mg/kg) of MDMA to produce a clear 30 to 50% or greater loss in cerebral 5-HT content. The Dark Agouti rats (mainly females but with a lesser extent males as well) are deficient in the CYP2D1 isozyme and therefore exhibit a poor metabolizer phenotype for MDMA. In humans the polymorphic CYP2D6 is involved in the metabolism of MDMA and 5-10% of Caucasians are deficient in this enzyme. Consequently, the Dark Agouti rat strain provides a model for the genetically-defined, poor metabolizer human sub-population in which clinical complications of MDMA may be more likely to occur.

Serotonin plays a central role in the regulation of several physiological processes, such as feeding, thermoregulation, pain, sexual function, neuroendocrine regulation, learning, memory, sleep, circadian rhythms and motor activity. Not surprisingly, in animals MDMA-induced 5-HT depletion is often accompanied by a number of functional consequences. A growing body of data suggests that humans who use ecstasy as a recreational drug can also sustain serotonergic neurotoxicity. Furthermore several clinical studies reported that the recreational use of ecstasy is associated with a range of psychiatric symptoms and psychobiological problems.

OBJECTIVES

Despite the well documented neurochemical actions of MDMA, short- and long-term effects on sleep-wake cycle have not been extensively explored. No information is available on the acute sleep effects of a second MDMA dose on MDMA-pretreated animals and the effect of the selective serotonin reuptake inhibitor citalopram on sleep patterns has not been explored under these conditions either. In our experiments we used Dark Agouti rats, the model for the poor metabolizer human sub-population. We aimed to assess possible functional changes in the 5-HT system after administration of a single dose of MDMA which is firmly equivalent to the recreational dosage range for humans. The main objectives in our studies were to answer the following questions:

1) How does a single dose of 15 mg/kg MDMA change motor activity and sleep parameters in Dark Agouti rats acutely?
2) What kind of subacute and long-term alterations of the measured parameters are associated with the treatment?
3) Are there any differences in the acute motor activity and vigilance effects of MDMA in rats exposed to the drug 3 weeks earlier compared to drug-naïve animals?
4) Does the MDMA-pretreatment cause any changes in the acute vigilance effects of citalopram (2.5 mg/kg)?

METHODS

Male Dark Agouti rats (Harlan, Olac Ltd, United Kingdom, aged 4–5 weeks and weighing 50–80 g upon arrival) were used in the experiments. Rats were kept four/cage before surgery and individually in glass cages after surgery, with food

and water available ad libitum, maintained on a 12 h light and a 12 h dark cycle (lights from 09:00 to 21:00 h) and at an ambient temperature of 21± ^{o}C.

Animals were chronically equipped with epidural EEG electrodes (one over the left frontal cortex and one over the left parietal cortex) and EMG electrodes (sewn in the neck muscle) in order to record EEG and EMG activity. An electromagnetic transducer activated by cable movements was used to record motor activity. The vigilance states were classified by SleepSign for Animal sleep analysis software (Kissei Comtec America, Inc., USA) for 4-s periods according to the conventional criteria.

Data were evaluated by multivariate analysis of variance (MANOVA) and by factorial analysis of variance (ANOVA). Tukey honest significant difference test was used for post-hoc comparisons. Amplitude, acrophase and mesor values (±confidence limits) were calculated by cosinor analysis in order to quantify circadian patterns of motor activity and sleep parameters.

The neurodegenerative effect of our MDMA-dose was confirmed by measuring [^{3}H]-paroxetine binding. Using this method we examined the quantity of tritium-labelled paroxetine bound to 5-HT transporter in the occipital cortex, striatum, substantia nigra pars reticulata and pars compacta and compared results of rats treated with MDMA or vehicle 3 weeks previously. Data were analyzed statistically by using Student's t-test.

RESULTS

1. Acute effects of MDMA: first 24 hours

MDMA caused marked increase in motor activity and wake, and inhibited sleep in the first 5-11 hours, potentially to its acute monoamine- and acetilcholine-releasing potency. Sleep latency (time from light onset until the beginning of sleep) of MDMA-treated animals was much higher compared to controls.

Interestingly, MDMA caused a longer inhibition on REM (rapid eye movement) sleep than NREM-1 (light slow wave sleep) or NREM-2 (deep slow wave sleep). 10-12 hours later adverse subacute effects emerged: motor activity and wake were reduced, while length of NREM-1, NREM-2 and REM increased. Cosinor analysis confirmed these massive changes in circadian patterns of parameters on the day of MDMA treatment.

2. Short-term effects of MDMA: day 3 and 5

On day 3 motor activity decreased mainly during dark (active) phase; a reduction in mesor values (this parameter shows the mean of timevalues) confirmed this finding. While on day 5 motor activity was still decreased, wake increased (because of the increase of passive wake) and REM sleep decreased. These changes may be explained by the second, smaller 5-HT releasing effect of MDMA. On the same day, circadian patterns of MDMA-treated animals were markedly different from controls: amplitudes of motor activity, wake and REM sleep values were decreased, min-max differences between timevalues are decreased.

3. Long-term effects of MDMA

Circadian patterns of motor activity continued to be disturbed (mesor increased, motor activity transiently increased around dark onset and light onset) on day 14 and 28. Neurodegenerative effect of MDMA causes long-term changes in the circadian clock (nucleus suprachiasmaticus) function in rats, possibly this fact could explain our findings. Significant reductions in [^{3}H]paroxetine binding in the occipital cortex of MDMA-treated animals provided evidence for axonal damage, although no significant change was observed in motor areas. 3 weeks following MDMA treatment, an increase in the length of REM was observed at

the beginning of the sleep-cycle. 5-HT inhibits the REM-initiating cholinergic REM-on neurons, therefore such finding is likely to be caused by decreased 5-HT-neurotransmission due to neurodegeration after MDMA.

4. Acute effects of MDMA (first 24 hours) in rats previously exposed to the drug

In rats exposed to MDMA 3 weeks earlier, acute effects induced by MDMA on motor activity and vigilance had a shorter duration. After a single dose of MDMA challenge sleep latency was lower and value of mesor of REM sleep was higher in MDMA-pretreated animals compared to drug-naive rats. Difference between REM mesors are possibly caused by a longer REM-inhibiting effect of MDMA in drug-naive rats. Furthermore, a chronic, REM-initiating effect of MDMA (see previous section) could also implicated in that.

5. Acute vigilance effects of citalopram

Citalopram decreased REM in drug-naïve rats. This effect was present for several hours, since 24 hour mesor value of REM sleep was significantly decreased after treatment. Furthermore citalopram increased passive wakefulness in drug-naïve rats. Possibly both changes were due to markedly increased synaptic 5-HT caused by the serotonin-reuptake inhibiting effect of citalopram.

6. Acute effects of citalopram in rats previously exposed to MDMA

In rats exposed to MDMA 3 weeks earlier citalopram did not increase passive wakefulness and its REM-inhibiting effect was attenuated compared to drug-

naïve rats. These findings may be explained by a smaller synaptic 5-HT-increasing effect of citalopram in rats previously exposed to MDMA.

CONCLUSIONS

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

- Even a single dose of MDMA can result in long-term changes in motor activity and sleep/vigilance parameters in the poor metabolizer Dark Agouti rats.
- A single dose of MDMA caused persistent changes in the 5-HT system of Dark Agouti rats and these resulted in a reduction of acute effects of the next MDMA-dose as well as those of citalopram.
- All these consequently outline the potential dangers of human ecstasy-use.

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ORIGINAL PUBLICATIONS AND ABSTRACTS RELATED TO THE SUBJECT OF THE PHD THESIS

Original Publications

1. **Balogh B**, Molnár E, Jakus R, Quate L, Olverman HJ, Kelly PA, Kántor S, Bagdy G (2004). Effects of a single dose of 3,4-methylenedioxymethampheta-mine on circadian patterns, motor activity and sleep in drug-naive rats and rats previously exposed to MDMA. Psychopharmacology (Berl) 173: 296-309.
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2. Sandor Kantor, Rita Jakus, **Brigitta Balogh**, Anita Benko, Gyorgy Bagdy: Increased wakefulness, motor activity and decreased theta activity after selective blockade of the 5-HT_{2B} receptor by the subtype-selective antagonist SB-215505. Br J Pharmacol, 142(8):1332-42, 2004. IF= 3,325

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