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Effects of long-term exposure of 3,4-methylenedioxymethamphetamine (MDMA; "ecstasy") on neuronal transmitter transport, brain immuno-regulatory systems and progression of experimental periodontitis in rats

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ABSTRACT

The present study was designed to investigate the effects of long-term exposure (4 weeks) to the widely used narcotic drug and putative neurotoxicant 3,4-methylenedioxymetamphetamine (MDMA; "ecstasy") on neuronal transmitter transport and progression of experimental periodontitis in male Wistar rats. The rats were exposed to MDMA (10 mg/kg/day i.p.) or saline five days a week for four consecutive weeks. Exposure to MDMA induced a significant reduction in the synaptosomal reuptake of serotonin, while the uptake of dopamine was significantly increased 24 h after the last injection of MDMA. In contrast, the synaptosomal uptake of noradrenaline and the vesicular uptake through the vesicular monoamine transporter 2 were not affected. In the experiments of periodontitis development, ligature-induced periodontitis was induced three days prior to MDMA administration. Compared to controls, MDMA-treated rats developed significantly more periodontitis. In conclusion, our results show that long-term exposure to MDMA affects the serotonergic and dopaminergic transport systems in the rat brain and increased the susceptibility to the psychosomatic ailment periodontitis following disturbances of brain immune-regulatory systems. These results are interesting with respect to recent research showing that changes in neurotransmitter signalling may alter the reactivity of brain-controlled immunoregulatory systems controlling pathogenic microorganisms colonizing mucosal surfaces.

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA; "ecstasy") is a ring-substituted amphetamine and a popular drug of abuse, mainly used among young people. In addition to the ability to induce euphoria, MDMA abuse is associated with a range of acute and some long-term hazardous effects. The reported adverse effects of MDMA intoxication include cardiac arrhythmias, hyperthermia, increased heart rate, anxiety, serotonin (5-HT) syndrome, liver complications, seizures, coma, and in rare cases death (Schifano, 2004; Grunau et al., 2010; Green et al., 2003). After long-term MDMA abuse, the greatest adverse effect is its impact on cognition and mood disturbances, which can last for months after cessation of drug intake (Morgan, 2000; Parrott et al., 2002; Parrott, 2006). The *acute* effects of MDMA on brain neurotransmitter systems have been well documented (White et al., 1996; Capela et al., 2009; Cole and Sumnall, 2003; Green et al., 1995, 2003; Mayerhofer et al., 2001). These studies show that MDMA blocks the 5-HT, dopamine (DA), and noradrenaline (NA) transporters, and stimulates the release of monoamines by reverse transport. We have previously shown that MDMA reduces both synaptosomal and vesicular uptake of 5-HT and DA in a dose dependent manner in vitro, while ex vivo studies demonstrated reduced vesicular uptake of 5-HT by inhibi-

Abbreviations: MDMA, 3,4-methylenedioxymetamphetamine; 5-HT, serotonin; DA, dopamine; NA, noradrenaline; SERT, serotonin transporter; HPA, hypothalamus–pituitary–adrenal axis; CEJ, cemento-enamel junction.

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tion of the vesicular monoamine transporter 2 (VMAT2) (Bogen et al., 2003). However, the effects of *long-term* exposure (several weeks) to MDMA on neurotransmitter signalling, and in particular at doses leading to increased DA signalling, have not yet been adequately tested (see e.g. Schenk, 2011).

Case studies have indicated that MDMA use may lead to periodontitis (Brazier et al., 2003; Miranda-Rius et al., 2009), which is a tissue destructive inflammatory condition of the tooth supporting tissues that may lead to tooth loss in the most severe cases. The disease is initiated by increased colonisation of pathogenic microorganisms, including gram-negative bacteria, in the subgingival dental biofilm (Socransky and Haffajee, 2005), which may indicate that immune system responses vital for clearing these pathogens in gingival pockets are inappropriately regulated. MDMA has been reported to have impact on the functioning of the immune system, where both the innate and adaptive arms may be affected, and thus, to influence the susceptibility to infectious diseases. These effects may partly be due to alterations in the activation of brain-controlled immuno-regulatory system, including the hypothalamus–pituitary–adrenal (HPA) axis (Boyle and Connor, 2010; Stankevicius et al., 2012). We have previously studied the impact of different immune aberrations in a rat model and found that agents which drive adaptive immunity and inhibit innate immunity, are protective in periodontitis (Breivik and Rook, 2000, 2003; Breivik et al., 2005a, 2011a). In addition, our studies show that rats that differ in their reactivity or responsiveness of brain-controlled immunoregulatory pathways, including the HPA axis, the sympathetic and parasympathetic nervous system, as well as the sensory peptidergic nervous system, also differ in their susceptibility to periodontitis (Breivik et al., 2000, 2001, 2005b, 2009, 2011b). Thus, environmental stimuli and/or pharmacological agents that alter the reactivity of brain-controlled immunoregulatory systems may alter the outcome of immune system responses and the development and progression of immune-related diseases.

The acute effects of MDMA exposure are well studied, while the effects of long-term MDMA treatment on neuronal signalling and immune responses have not been adequately examined. To our knowledge, controlled animal studies of the effects of MDMA on periodontitis have previously not been reported. The main purpose of the present study was to investigate how long-term (4 weeks) MDMA treatment affects neurotransmitter systems in the brain, and to investigate whether such changes correlated with the development and progression of the well-defined psychosomatic ailment periodontitis.

2. Materials and methods

2.1. Animals

Male Wistar rats were obtained from Möllegaard Breeding Center (Ejby, Denmark) of which 20 rats were included in the neurotransmitter uptake study, and 30 rats were included in the periodontal disease study (200–300 g at arrival). The rats were used after 2 weeks of acclimatisation to their housing condition. The rats were housed in groups of five, and had free access to standard rat pellets and tap water. The animals were maintained under a 12–24 h light/dark cycle (light on 7.00 a.m. to 7.00 p.m.) with average temperature and humidity at 22 °C and 40–60%, respectively. The experiments were registered and approved by the Norwegian Animal Research Authority.

2.2. Experimental design

2.2.1. Experiment 1: Neurotransmitter transport

This experiment was designed to test whether long-term (4 weeks) treatment with MDMA would affect neurotransmitter uptake in the brain. The rats were randomly assigned to 2 groups, each consisting of 10 and 11 (control) rats; during the experiment one MDMA-treated rat died and was therefore not included in the analysis. MDMA (7.5 mg/kg/day) was injected i.p. for the first 5 days and the dose increased to 10 mg/kg/day for the next 15 weekdays (in total of 20 injections). The control rats were injected with 0.9% NaCl (2 ml/kg). The dehydration effects on the animals were compensated for by i.p. injections of 0.9% NaCl 1.5–2 h after each injection of MDMA. The rats were decapitated 24 h after the last injection of MDMA/NaCl.

2.2.2. Experiment 2: Periodontitis

This experiment was designed to test whether long-term (4 weeks) treatment with MDMA would affect ligature-induced periodontal breakdown. The rats were randomly assigned to 2 groups, each initially consisting of 15 rats; during the experiment 2 MDMA-treated rats from the same cage died and were therefore not included in the analysis. Periodontitis was induced in all rats. Thereafter, MDMA (10 mg/kg/day) was given 5 days a week for 4 consecutive weeks (in total 20 injections), while the control group was given saline (2 ml/kg). The dehydration effects on the animals were compensated for by i.p. injections of 0.9% NaCl 1.5–2 h after each injection of MDMA. All animals were killed by decapitation 29 days after induction of experimental periodontitis.

2.3. Chemicals

HBSS (containing $CaCl_2 \cdot 2H_2O$ (1.26 mM), KCl (5.37 mM), KH_2PO_4 (0.44 mM), $MgCl_2 \cdot 6H_2O$ (0.49 mM), $MgSO_4 \cdot 7H_2O$ (0.41 mM), NaCl (0.14 M), $NaHCO_3$ (4.17 mM), Na_2HPO_4 (0.34 mM), D-glucose (5.55 mM)) and HEPES buffer was purchased from GibcoBRL (Life Technologies Inc Gibco/Brl Division, Grand Island, NY, USA). [^{3}H] 5-hydroxytryptamine trifluoroacetate, I-[7,8-^{3}H] noradrenaline and [2,5,6-^{3}H] dopamine were purchased from Amersham Pharmacia Biotech, Little Chalfont, UK. Filter-Count was purchased from Packard Instrument Company, Meriden, CT, USA. 3,4-methylenedioxymethamphetamine (MDMA) was provided and purified by the Norwegian Criminal Investigation Department.

2.4. Preparation of neuronal subcellular fractions

For neurotransmitter uptake studies, forebrain synaptosomes and synaptic vesicles were prepared as described by Mariussen and Fonnum (2001). In brief, rats were killed by decapitation and the brains rapidly removed and stored in ice-cold 0.32 M sucrose. The cerebrum was homogenized in 0.32 M sucrose [5% (w/v)] in a glass-Teflon homogenizer at 450 r.p.m. The homogenate was centrifuged at 1000*g* for 10 min. The supernatant (S1) was divided in two for separate isolation of synaptosomes and synaptic vesicles. For the isolation of synaptosomes, the S1 fraction was mixed with 1.3 M sucrose to obtain a final concentration of 0.8 M and centrifuged at 21,000*g* for 30 min at 4 °C. The resultant crude synaptosomal fraction (P2) was resuspended in 0.32 M sucrose and neurotransmitter uptake measured the same day.

For isolation of synaptic vesicles, a method based on the work of Erickson et al. (1990) and Teng et al. (1998) was used. The supernatant (S1) was centrifuged at 21,000*g* for 30 min. The resulting supernatant was discarded, while the synaptosomal pellet was osmotically shocked by resuspension in ice-cold double-distilled water. After centrifugation for 30 min at 21,000*g*, the pellet containing synaptosomal membranes was removed while the supernatant was collected. Final concentrations of 0.1 M K-tartrate and 25 mM HEPES (pH 7.4) were added and the mixture centrifuged at 100,000*g* for 1 h. The supernatant was discarded while the crude synaptic vesicles were gently resuspended in 0.32 M sucrose and stored at −140 °C until neurotransmitter uptake studies. The pro-