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European Journal of Pharmacology 566 (2007) 120–123
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Short communication

Chronic 3,4-methylenedioxymethamphetamine treatment suppresses cell proliferation in the adult mouse dentate gyrus

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Received 25 January 2007; received in revised form 28 March 2007; accepted 1 April 2007
Available online 20 April 2007

Abstract

We investigated the influence of chronic 3,4-methylenedioxymethamphetamine (MDMA) treatment on cell proliferation in the adult dentate gyrus. Mice were orally treated with MDMA (1.25 mg/kg–40 mg/kg) or saline for 30 days. To label dividing cells, mice were given 5-bromo-2′-deoxyuridine (BrdU) for 4 days from the day after the last administration of MDMA, and their brains were examined 24 h later. MDMA dose-dependently induced a decrease in the number of BrdU-positive cells in the male and female dentate gyrus. Our results suggest that chronic exposure to MDMA suppresses cell proliferation in the dentate gyrus.

Keywords: MDMA; Ecstasy; Cell proliferation; Dentate gyrus; Hippocampus

1. Introduction

The hippocampal dentate gyrus generates neurons throughout life (Eriksson et al., 1998; Gould et al., 1997). Adult hippocampal progenitor cells are located at the subgranular zone between the dentate hilus and the inner margins of the dentate granule cell layer (Sharp et al., 2002). Newly formed cells in the hippocampus migrate into the dentate granular cell layer, where most of the cells differentiate into neurons that assume a granule cell phenotype (Cameron et al., 1993; Gage et al., 1998).

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy), a widely used recreational drug, is defined as a "substrate-type 5-hydroxytryptamine (5-HT) releaser" (Rothman and Baumann, 2002). Its repeated administration can cause depletion of monoamines such as serotonin (Aguirre et al., 1997; Zhang et al., 2006), leading to neurotoxicity in the serotonin system (O'Hearn et al., 1988; Zhang et al., 2006) and memory impairment (McCann et al., 1999; Morley et al., 2001). Meanwhile, serotonin via activation of the 5-HT_{1A} receptor is a well-known mediator to stimulate granule cell proliferation (Gould, 1999). Therefore, the present study was carried out to determine whether chronic exposure to MDMA influences hippocampal cell proliferation in male and female mice.

2. Materials and methods

2.1. Animals and drug schedule

All animal procedures were approved by the Catholic Ethics Committee of the Catholic University of Korea and were carried out in accordance with the European Community guidelines for the protection of vertebrate animals used for experimental and other scientific purposes. Male and female C57BL/6 mice weighing 22–25 g received either 3,4-MDMA HCl (p.o., Sigma, St. Louis, MO) or saline once a day for 30 days. The dose of MDMA was 1.25 mg/kg, 5 mg/kg, 20 mg/kg or 40mg/kg. One day after the last administration of MDMA, BrdU

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doi:10.1016/j.ejphar.2007.04.008

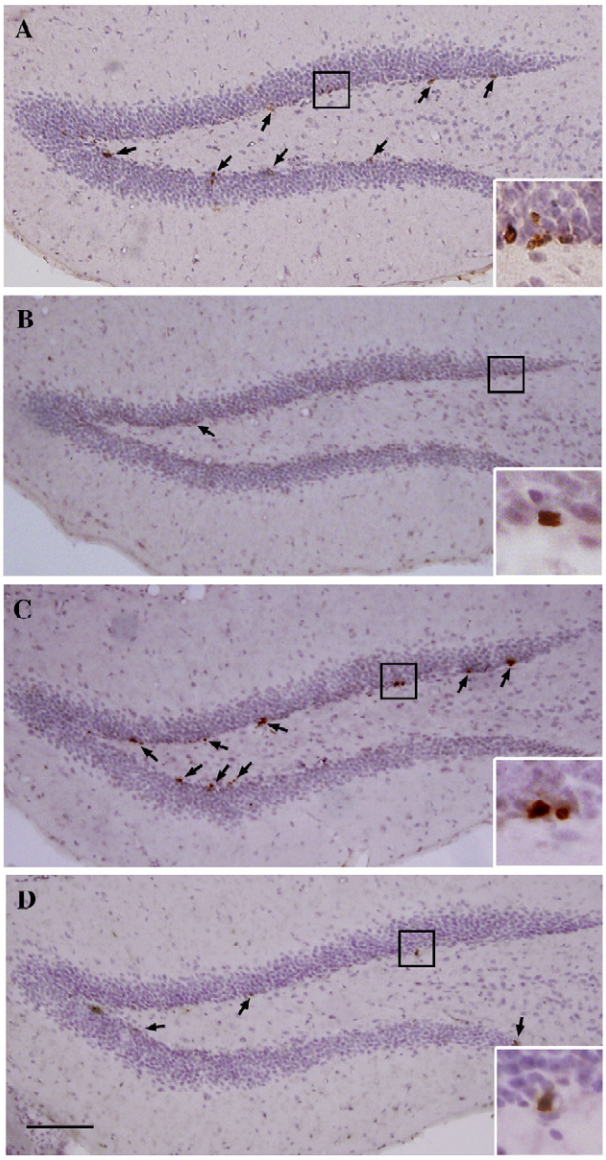

Fig. 1. Effects of chronic exposure to MDMA for 30 days on cell proliferation rates in the male and female dentate gyrus. A, B: Photomicrographs of representative dentate gyruses from a male control and a mouse treated with 40 mg/kg of MDMA. C, D: Photomicrographs of representative dentate gyruses from a female control and a mouse treated with 40 mg/kg of MDMA. Each rectangle in A–D is magnified to show BrdU-labeled cells. Note that chronic exposure to MDMA for 30 days reduced BrdU-positive cells (arrows) in the subgranular zone. Bar = 200 μm.

(120 mg/kg, i.p.) was injected once a day for 4 days. At 24 h after the last BrdU injection, the animals were euthanized with 15% chloral hydrate.

2.2. Immunohistochemistry

Animals were perfused with 150 ml of a fixative containing 4% paraformaldehyde. To detect BrdU labeling, DNA denaturation was conducted by incubation for 2 h in 50% formamide/2× SSC at 65 °C. Sections (20 μm thick) were incubated for 1 h in 2 N HCl at 37 °C and then for 10 min in boric acid. Sections were incubated with anti-mouse BrdU (1:100; DAKO, Glostrup, Denmark) overnight at 4 °C. Sections were then incubated for 2 h with secondary antibody (biotinylated horse anti-mouse; Vector Laboratories, Burlingame, CA) followed by amplification with an avidin–biotin complex (Vector) for 1 h. Cells were visualized with 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide. After BrdU labeling, tissues were stained with hematoxylin for 2 min and bleached with 1% HCl. The slides were rinsed with distilled water, dehydrated and mounted.

2.3. Cell counting and statistics

Every fifth section (i.e. a total of three) was processed for BrdU labeling. BrdU-labeled cells in the dentate gyrus were counted bilaterally in each section using a light microscope (BX51, Olympus, Tokyo, Japan). Numbers of BrdU-positive cells were expressed as means ± S.E.M., and one-way ANOVA and post-hoc Bonferroni's test were performed. Differences were assumed to be statistically significant at $P<0.05$.

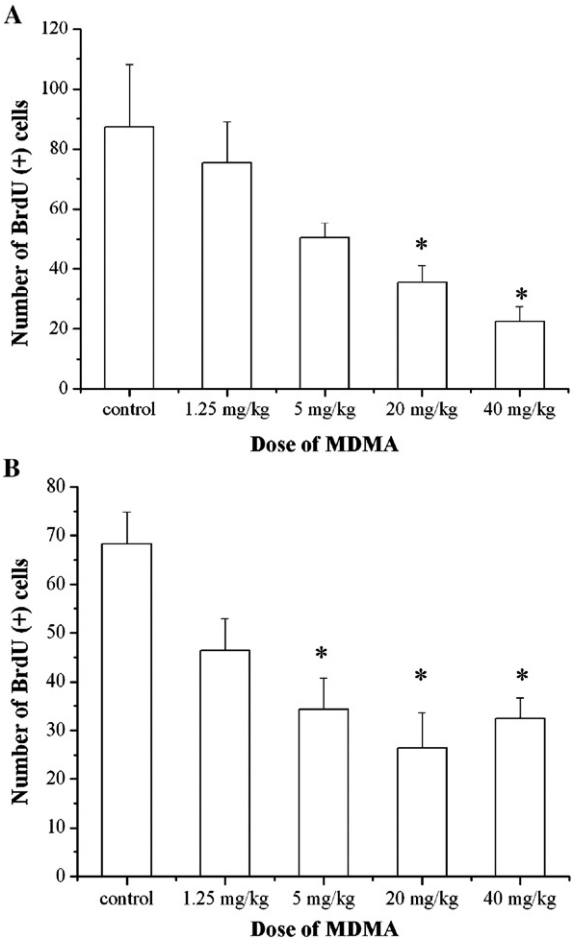

Fig. 2. Numbers of BrdU-positive cells in male and female dentate gyruses. A: Data from male dentate gyrus. B: Data from female dentate gyrus. Daily MDMA administration of more than 20 mg/kg (male) or 5 mg/kg (female) for 30 days decreased the number of BrdU-positive cells in the subgranular zone of the hippocampus in a dose-dependent manner. *$P<0.05$, ANOVA.

3. Results

3.1. Chronic exposure to MDMA decreases cell proliferation rates in the male dentate gyrus in a dose-dependent manner

The number of newly generated cells in the subgranular zone of the male dentate gyrus affected by 40 mg/kg of MDMA administration for 30 days was much lower than the number of newly produced cells in the control tissue (Fig. 1A and B). Higher magnification of the subgranular zone reveals that the BrdU-positive cells in the control samples significantly outnumbered those in the MDMA-treated group and were more closely associated with one another than those in the MDMA group. One-way ANOVA shows that more than 20 mg/kg of exposure to MDMA (Fig. 2A) caused marked downregulation of cell proliferation rates compared with control ($P=0.002$).

3.2. Chronic exposure to MDMA influences cell proliferation rates in the female dentate gyrus in a dose-dependent manner

Chronic exposure to 40 mg/kg of MDMA (Fig. 1D) for 30 days resulted in a sharp decline in the number of BrdU-positive cells in the female hippocampal dentate gyrus when compared with control (Fig. 1C). Higher magnification of each rectangle in Fig. 1C and D shows that MDMA-treated mice had fewer and less clustered BrdU-labeled cells than did control mice. Statistically, more than 5 mg/kg of exposure to MDMA (Fig. 2B) significantly diminished cell proliferation rates compared with control ($P=0.001$, one-way ANOVA).

4. Discussion

In the present study, we have found that chronic exposure to MDMA reduces cell proliferation in the adult dentate gyrus in a dose-dependent manner. We have also shown that this effect is observed both in male and female mice.

Lines of evidence suggest clarifying effects of addictive drugs on cell proliferation of neural precursor cells in the brain. For example, cocaine (Dominguez-Escriba et al., 2006) or methamphetamine (Teuchert-Noodt et al., 2000) treatment has been shown to decrease cell proliferation in the subgranular zone of the dentate gyrus. The activity of these psychomotor stimulants was comparable to that of MDMA in the present study. On the contrary, Hernandez-Rabaza et al. (2006) reported that intraperitoneal injections of MDMA 4 times a day for 2 days resulted in impaired survival of neural progenitor cells without affecting the rate of cell proliferation in the adult hippocampus of rats. This discrepancy between our data and theirs seems to be derived from the different duration, route and schedule of MDMA administration, schedule of BrdU injections as well as different species used.

In the present study, oral doses of MDMA (1.25 mg/kg–40 mg/kg) were administered for 30 consecutive days. Because there are few published reports for this type of long-term daily dosing in human MDMA users, the applicability of this animal study to humans is unclear, particularly since the large majority of MDMA use is of a non-dependent variety (Jansen, 1999). Nevertheless, a subcutaneous dose of 10 mg/kg in the monkey is equivalent to 1.4 mg/kg in humans (Montoya et al., 2002), an amount similar to that used for recreational purposes (Burgess et al., 2000). In addition, Slikker et al. (1988) reported that monkeys may be more sensitive than rodents to the persistent serotonergic neurotoxicity of MDMA, suggesting that humans may be more sensitive to MDMA than mice. Studies show that individuals such as club promoters have used large quantities of MDMA for extended periods (Jansen, 1999; Krystal et al., 1992; McGuire and Fahy, 1991; Pichini et al., 2006). Recently, the U.S. Food and Drug Administration approved pilot studies in chronic posttraumatic stress disorder patients who have failed to obtain relief from at least one course of conventional treatment (Doblin, 2002). Taken together, although rare among MDMA users, high-frequency MDMA use for 1 month or longer may be possible in an occupational or clinical situation.

In conclusion, chronic MDMA administration led to marked deficiencies in the proliferative activities of cells in the hippocampus in a dose-dependent manner. Further studies are needed to elucidate the mechanisms and long-term consequences of MDMA addiction on hippocampal progenitor cells.

Acknowledgement

This research was supported by a grant (06132485) from the Korea Food and Drug Administration in 2006.

References

Aguirre, N., Frechilla, D., Garcia-Osta, A., Lasheras, B., Del Rio, J., 1997. Differential regulation by methylenedioxymethamphetamine of 5-hydroxytryptamine$_{1A}$ receptor density and mRNA expression in rat hippocampus, frontal cortex, and brainstem: the role of corticosteroids. J. Neurochem. 68, 1099–1105.

Burgess, C., O'Donohoe, A., Gill, M., 2000. Agony and ecstasy: a review of MDMA effects and toxicity. Eur. Psychiatr. 15, 287–294.

Cameron, H.A., Woolley, C.S., McEwen, B.S., Gould, E., 1993. Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. Neuroscience 56, 337–344.

Doblin, R., 2002. A clinical plan for MDMA (ecstasy) in the treatment of posttraumatic stress disorder (PTSD): partnering with the FDA. J. Psychoact. Drugs 34, 185–194.

Dominguez-Escriba, L., Hernandez-Rabaza, V., Soriano-Navarro, M., Barcia, J.A., Romero, F.J., Garcia-Verdugo, J.M., Canales, J.J., 2006. Chronic cocaine exposure impairs progenitor proliferation but spares survival and maturation of neural precursors in adult rat dentate gyrus. Eur. J. Neurosci. 24, 586–594.

Eriksson, P.S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., Gage, F.H., 1998. Neurogenesis in the adult human hippocampus. Nat. Med. 4, 1313–1317.

Gage, F.H., Kempermann, G., Palmer, T.D., Peterson, D.A., Ray, J., 1998. Multipotent progenitor cells in the adult dentate gyrus. J. Neurobiol. 36, 249–266.

Gould, E., 1999. Serotonin and hippocampal neurogenesis. Neuropsychopharmacology 21, 46S–51S.

Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A., Fuchs, E., 1997. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J. Neurosci. 17, 2492–2498.

Hernandez-Rabaza, V., Dominguez-Escriba, L., Barcia, J.A., Rosel, J.F., Romero, F.J., Garcia-Verdugo, J.M., Canales, J.J., 2006. Binge administration of 3,4-methylenedioxymethamphetamine ("ecstasy") impairs the survival of neural precursors in adult rat dentate gyrus. Neuropharmacology 51, 967–973.

Jansen, K.L.R., 1999. Ecstasy (MDMA) dependence. Drug Alcohol Depend. 53, 121–124.

Krystal, J.H., Price, L.H., Opsahl, C., Ricaurte, G.A., Heninger, G.R., 1992. Chronic 3,4-methylenedioxymethamphetamine (MDMA) use: effects on mood and neuropsychological function? Am. J. Drug Alcohol Abuse 18, 331–341.

McCann, U.D., Mertl, M., Eligulashvili, V., Ricaurte, G.A., 1999. Cognitive performance in (+/−) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: a controlled study. Psychopharmacology (Berl) 143, 417–425.

McGuire, P., Fahy, T., 1991. Chronic paranoid psychosis after misuse of MDMA ("ecstasy"). Br. Med. J. 302, 697.

Montoya, A.G., Sorrentino, R., Lukas, S.E., Price, B.H., 2002. Long-term neuropsychiatric consequences of "ecstasy" (MDMA): a review. Harv. Rev. Psychiatr. 10, 212–220.

Morley, K.C., Gallate, J.E., Hunt, G.E., Mallet, P.E., McGregor, I.S., 2001. Increased anxiety and impaired memory in rats 3 months after administration of 3,4-methylenedioxymethamphetamine ("ecstasy"). Eur. J. Pharmacol. 433, 91–99.

O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J., Molliver, M.E., 1988. Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J. Neurosci. 8, 2788–2803.

Pichini, S., Poudevida, S., Pujadas, M., Menoyo, E., Pacifici, R., Farre, M., de la Torre, R., 2006. Assessment of chronic exposure to MDMA in a group of consumers by segmental hair analysis. Ther. Drug Monit. 28, 106–109.

Rothman, R.B., Baumann, M.H., 2002. Therapeutic and adverse actions of serotonin transporter substrates. Pharmacol. Ther. 95, 73–88.

Sharp, F.R., Liu, J., Bernabeu, R., 2002. Neurogenesis following brain ischemia. Brain Res. Dev. Brain Res. 134, 23–30.

Slikker Jr., W., Ali, S.F., Scallet, A.C., Frith, C.H., Newport, G.D., Bailey Jr., 1988. Neurochemical and neurohistological alterations in the rat and monkey produced by orally administered methylenedioxymethamphetamine (MDMA). Toxicol. Appl. Pharmacol. 94, 448–457.

Teuchert-Noodt, G., Dawirs, R.R., Hildebrandt, K., 2000. Adult treatment with methamphetamine transiently decreases dentate granule cell proliferation in the gerbil hippocampus. J. Neural Transm. 107, 133–143.

Zhang, L., Shirayama, Y., Shimizu, E., Iyo, M., Hashimoto, K., 2006. Protective effects of minocycline on 3,4-methylenedioxymethamphetamine-induced neurotoxicity in serotonergic and dopaminergic neurons of mouse brain. Eur. J. Pharmacol. 544, 1–9.