INTRODUCTION

Tablets called “ecstasy” primarily contain 3,4-methylenedioxymethamphetamine (MDMA) and are occasionally mixed with varied amounts of 3,4-methylenedioxyamphetamine (MDA), methamphetamine, ephedrine, caffeine, and/or ketamine (Makino et al., 2003). Ecstasy is widely abused as a psychoactive recreational drug (Parrott, 2002). MDMA was first synthesized and patented by a German pharmaceutical company that planned to market an anorectic drug or an appetite suppressor (Freudemann et al., 2006); however, the drug was never marketed. Apart from some US Army trials in the 1950s, MDMA disappeared until the 1970s when it was evaluated by an American biochemist, Shulgin (Parrott, 2001). Subsequently, MDMA was subjected to limited use as an adjunct in psychotherapy. MDMA was considered beneficial by enhancing patients’ communicative skills and thus by making patients more accessible to psychotherapy (Downing, 1986). In addition, MDMA was sporadically used as a recreational drug in nontherapeutic settings (Parrott, 2001). Therapeutic use of MDMA was gradually abolished during the 1970s, and the substance was banned in the UK in 1977, in the USA in 1985, and in Japan in 1989.

Acute MDMA administration induces a rapid release of serotonin (5-HT) (Gudelsky and Nash, 1996), eventually causing a decrease in 5-HT concentration in brain tissues (Colado et al., 1993). MDMA binds to the 5-HT transporter with high affinity, thereby inhibiting the reuptake of 5-HT. MDMA also binds to noradrenaline and dopamine transporters with lower affinities (Liechti et al., 2000; Rothman and Baumann, 2002). MDMA inhibits the enzyme tryptophan hydroxylase, a rate-limiting enzyme in 5-HT synthesis, and produces a degeneration of serotonergic nerve terminals (Battaglia et al., 1987, 1987; Schmidt and Taylor, 1987, O’Shea et al., 1998). In addition, MDMA is a weak inhibitor of both A and B subtypes of monoamine oxidase (de la Torre et al., 2004; Parrott, 2005). These observations indicate that MDMA affects brain activity by altering neurotransmission, particularly the serotonergic system.

Although the use of MDMA by the young generation is widely spread, the developmental toxicity of the drug is not obvious. Other drugs such as cocaine, heroin, amphetamines, and nicotine are reportedly associated with...
impacts fetal growth and acute withdrawal syndrome during the neonatal period when mothers are exposed to the drug (Wagner et al., 1998). Individuals using these drugs during pregnancy are associated with poor obstetric and perinatal outcomes, with exposed infants more likely to be born preterm, with lower birth weight, and smaller head circumference. These babies require resuscitation, should be admitted to special care nurseries, and have longer hospital stays (O’Donnell et al., 2009).

Therefore, the effect of MDMA on fetal growth must be determined. We have previously demonstrated the effect of MDMA on nerve cells in vitro, showing that MDMA inhibits NGF-induced neurite outgrowth and suggesting that the recreational drug may cause impaired neuronal development (Kaizaki et al., 2010). The aim of the current study was to determine whether MDMA affects the ontogenetic development and growth of mouse pups.

MATERIAL AND METHODS

Animals and treatments
Male and female BALB/c mice were purchased from Sankyo Lab Service Corporation (Tokyo, Japan). Mice were housed in plastic cages in a temperature controlled room (22 ± 1°C) and maintained on a 12-hr light-dark cycle with free access to food and water. Nulliparous female mice were mated with male mice in the phase of their estrous cycle. The day the vaginal plug was observed was regarded as gestational day 1 (G1). MDMA-HCl was dissolved in ultra pure water. Dams were given MDMA per orally (p.o.) at a dose of 20 mg/10 ml/kg, daily from G1 to P21. Ultra pure water (10 ml/kg, p.o.) was given to control dams from G1 to P21. We gave MDMA to dams, there-
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as follows: score 0, pup falls immediately; score 1, grips the wire with forelimbs; score 2, grips the wire with forepaws and tries to support itself with its hind paws; score 3, grips the wire with 3 or 4 paws; score 4, grips the wire with 4 paws and twists its tail around the wire; score 5, grips the wire with 4 paws, twist its tail around the wire, and moves to the pole. The maximum time permitted was 60 sec. If the pup moved to the pole within 60 sec, the latency time was recorded as 60 sec. This test was carried out from P10 to P19.

Statistical analysis
The birth, survival, and gene expression data were analyzed by Student’s t-test. Body weight and behavioral data were analyzed by Mann-Whitney test.

RESULTS

Number of pups, survival rate, and body weight
There were no significant differences between the mean number of pups born from control and MDMA-administered dams. However, MDMA significantly decreased the number of surviving pups (Table 1). On P0, the survival rate of pups born from control (control pups) and MDMA-administered dams (MDMA pups) were 95.6% and 80.8%, respectively. On P4, the survival rate of control pups was 91.7% and that of MDMA pups was only 64.0% (Table 1).

Body weight gains were compared between control and MDMA pups (Fig. 1). Significant reductions in weight gain were observed in MDMA pups during P3-P21.

Behavioral tests
Pups were subjected to behavioral performance tests, including the righting reflex, cliff avoidance, and wire hanging tests from P4 to P19. Latency time of the righting reflex test decreased with age in both groups, indicating that muscle strength and subcortical maturation increased with age. The latency time was significantly longer in MDMA pups than in control pups during P10-P14 (Fig. 2). We defined the pups that could turn over on all four paws and touch the platform in less than 2 sec as a success in this test. Although no pups succeeded on P4, the success rate increased over time. On P10, 74.2% of control pups succeeded, but only 40.5% MDMA pups succeeded. These results indicate that MDMA pups develop the righting reflex ability later than control pups.

The latency time of cliff avoidance decreased with age in both groups, indicating that this act requires coordination, and thus, the integration of exteroceptive input and locomotor output increases with age. During P9-P15, there was a significant delay in the cliff avoidance response in MDMA pups compared with control pups (Fig. 3).

The latency time and the score of wire hanging increased with age in both groups, indicating that the hanging performance increased with age. The latency time was significantly shorter and the score was lower in MDMA pups than in control pups during P10-P19 (Fig. 4). Low ability demonstrated by the wire hanging test continued until weaning.

Table 1. Number of pups at birth, surviving pups, and the survival rate of neonates. The number at birth and survivors were counted on postnatal day 0 (P0). The number at birth includes pups that were stillborn and alive. The survival rate on P4 was calculated as follows: the number of surviving pups divided by the number at birth.

<table>
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<tr>
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<th>Control (n = 19)</th>
<th>MDMA (n = 18)</th>
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<tbody>
<tr>
<td>Number of births</td>
<td>8.63 ± 0.47</td>
<td>8.44 ± 0.73</td>
</tr>
<tr>
<td>Numbers of survivors</td>
<td>8.26 ± 0.50</td>
<td>6.78 ± 0.70 *</td>
</tr>
<tr>
<td>Survival rate on P4 (%)</td>
<td>91.72 ± 3.52</td>
<td>63.99 ± 8.91 **</td>
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Data represent the mean ± S.E.M. of examined dam.

*: P < 0.05, **: P < 0.01 compared with control dams.

Fig. 1. Effect of 3,4-methylenedioxymethamphetamine (MDMA) on changes in body weight of pups. Pups were weighed from postnatal day (P)3 to P21. The dashed and solid lines represent the weight of control and MDMA pups, respectively. Values were presented as mean ± S.E.M. (control; n = 49-62, MDMA; n = 35-44) Statistical analysis was performed by Mann-Whitney test. **P < 0.01 compared with the control group.
Gene expression
Expression of the c-fos gene, which is associated with neuronal activity, and the HO-1 gene, a typical marker of oxidative stress, remained almost unchanged in the midbrains of MDMA pups when determined on P7 and P10 (Fig. 5). However, MDMA tended to decrease the expression of the TH gene, a rate-limiting enzyme involved in catecholamine synthesis, in the midbrain of MDMA pups on P10 (Fig. 5).
DISCUSSION

The present study was designed to demonstrate if MDMA promotes reproductive and developmental toxicities in mice. MDMA treatment showed no effect on the birth rate when administered after mating. However, MDMA treatment significantly decreased the number of pups that survived on P0 and weight gain of pups during P3-P21. Although the body weight of newborn pups was not measured in this study, it is possible that MDMA may affect the growth of the fetus; therefore, the number of surviving pups decreased. In addition, MDMA pups born with a low weight have less than the required muscle strength and therefore they could not efficiently feed.
had retarded growth, and eventually died at an early stage. Moreover, some of the MDMA-administered dams appeared to have little interest in rearing their pups, which may also be related to the survival rate of newborns.

The righting reflex test was used to evaluate muscle strength and subcortical maturation, the cliff avoidance test was used to assess the integration of exteroceptive input and locomotor output, and the wire hanging maneuver test was used to evaluate neuromuscular and locomotor development (Altman et al., 1971; Altman and Sudarshan, 1975; Shen et al., 1991; Hermans et al., 1992).

MDMA pups showed significantly poorer performances in these tests than the control pups (Figs. 2, 3 and 4), particularly in the wire hanging test. These results suggest that neuromuscular and locomotor development was delayed in MDMA pups and support the idea that weak muscle strength is linked to lower survival rate of the drug-treated pups.

MDMA has been shown to induce oxidative stress and thus increase expressions of c-fos and HO-1 genes in the brain (Salzmann et al., 2003; Navarro et al., 2004; Puerta et al., 2010). However, expressions of c-fos and HO-1 appeared to be unchanged in MDMA pups (Fig. 5A and B). In general, HO-1 and c-fos responses appear immediately by the exposure to heat-shock, UV radiation, inflammatory cytokines, glutathione depletors and 4-hydroxynonenal, which cause oxidative stress (Keyse and Tyrrell, 1989; Rizzardini et al., 1993; Oguro et al., 1996; Zhang et al., 2001; Numazawa et al., 2003). It is possible that the immature brain has already gained resistance to MDMA-induced oxidative stress and, therefore, the response was not observed.

TH mRNA expression on P10 showed a tendency to decrease, but without statistical significance (Fig. 5C). MDMA inhibits TH activity, resulting in impaired and damaged dopamine neurons and degeneration of serotonergic nerve terminals in adult animals (Battaglia et al., 1987; Schmidt and Taylor, 1987; O’Shea et al., 1998).

Exposure of the fetus or newborn pup to MDMA does not cause such nerve toxicities (Skelton et al., 2008). The nervous system of infants is underdeveloped, and MDMA could be less toxic to these neurons. In addition, MDMA pups possibly developed resistance to MDMA-induced neurotoxicity by continuous exposure to MDMA.

In conclusion, the present study demonstrates for the first time that perinatal administration of MDMA to mouse dams caused retarded growth and behavioral dysfunction in their pups. These results suggest that MDMA, similar to cocaine, heroin, and amphetamines, may cause impaired fetal growth in humans.

**ACKNOWLEDGMENT**

This work was supported by Ministry of Education, Culture, Sports, Science and Technology of Japan - Supported Program for the Strategic Research Foundation at Private Universities (2010-2013).

**REFERENCES**


Keyse, S.M. and Tyrrell, R.M., (1989): Heme oxygenase is the
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Parrott, A.C. (2005): Chronic tolerance to recreational MDMA (3,4-methylenedioxymethamphetamine) or ecstasy. J. Psychopharmacol., 19, 71-83.


