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2-Phenylethylamine in combination with *l*-deprenyl lowers the striatal level of dopamine and prolongs the duration of the stereotypy in mice

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Abstract

2-Phenylethylamine (PEA)-induced stereotypy in rodents is suggested to model psychotic symptoms of schizophrenia. It is reported that PEA induces dopamine release in the striatum in vivo and in vitro. The present study analyzed the PEA-induced stereotypy and possible associated brain dopamine metabolism in mice. Using male ICR mice treated with a combination of PEA (100 mg/kg, i.p.) and increasing doses of *l*-deprenyl (0–10 mg/kg, s.c.), we examined (1) the behavioral profile of stereotypy (rating the scores), and (2) the tissue levels of dopamine and its metabolites by high-performance liquid chromatography. The stereotypic scores reached a plateau level at 10 min which lasted until 30 min after a single administration of 100 mg/kg PEA. The stereotyped behavior completely disappeared 45 min after PEA administration. Pretreatment with *l*-deprenyl (0.1, 1, and 10 mg/kg, s.c.) dose-dependently prolonged the duration of PEA-induced stereotypy. Notably, pretreatment with *l*-deprenyl dose-dependently increased the continuous sniffing. Treatment with PEA in combination of *l*-deprenyl (1 and 10 mg/kg) significantly reduced the level of dopamine in the region of the striatum and nucleus accumbens, compared with control animals. These results suggest that PEA in combination with *l*-deprenyl prolonged the duration of the stereotypy (particularly, continuous sniffing) while reducing the striatal level of dopamine.

Keywords: 2-Phenylethylamine; *l*-Deprenyl; Stereotypy; Dopamine; Monoamine oxidase; Striatum

1. Introduction

2-Phenylethylamine (PEA) is one of the endogenous so-called trace amines found throughout the brain of mammals. It is formed by the enzymatic decarboxylation of the precursor molecule L-phenylalanine, and is degraded rapidly by monoamine oxidase (MAO)-B (Sabelli et al., 1978; Boulton et al., 1990; Paterson et al., 1990; Berry, 2004). An increasing body of evidence suggests that PEA acts physiologically and pathologically as a neuromodulator of classical monoamine neurotransmitters (dopamine, 5-hydroxytryptamine (5-HT), and noradrenaline) in the brain under certain conditions, regulating neuronal excitability. However, the physiological relevance of PEA has not been established, because the tissue level of PEA is as low as 1–2 ng/g tissue (Berry, 2004).

Similar to amphetamines in structure, PEA when administered in high doses (in some cases, in combination with MAO-B inhibitors) induces amphetamine-like behavioral responses in rodents, such as hyperlocomotion, behavioral sensitization, and stereotypy (Jackson, 1972; Braestrup et al., 1975; Moja et al., 1976; Borison et al., 1977; Dourish, 1981, 1982; Ortmann et al., 1984; Timár and Knoll, 1986; Lapin, 1996). In general, amphetamine-induced stereotypy in rodents is considered a model of the psychotic symptoms of schizophrenia (Seiden et al., 1993; Gainetdinov et al., 2001). Borison et al. (1977) suggested that rodents displaying stereotypy induced by PEA are a better animal model for schizophrenia, since thioridazine and clozapine, antipsychotics with fewer extrapyramidal effects, preferentially inhibit PEA- but not amphetamine-induced stereotypy. The finding of the abnormal excretion of PEA in the urine of schizophrenics supports Borison's hypothesis (Potkin et al., 1979; Yoshimoto et al., 1987).

The mechanism(s) by which PEA exerts stereotypy in rodents have not been fully elucidated. The primary molecular

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target of PEA is assumed to be a dopamine transporter (DAT) (Sotnikova et al., 2004), leading to the release of dopamine in the region of the striatum (Bailey et al., 1987; Nakamura et al., 1998), similar to the case of amphetamines (Seiden et al., 1993). Enhancement of striatal dopamine neurotransmission initially activated by the DAT-mediated release of dopamine is a possible pathway through which PEA induces the stereotypy. However, the association of the PEA-induced stereotypy with brain dopamine metabolism is still unclear.

PEA-induced stereotypy is potentiated in rodents when the animals are treated with PEA in combination with selective MAO-B inhibitors (Braestrup et al., 1975; Moja et al., 1976; Ortmann et al., 1984; Timár and Knoll, 1986). This is because PEA is selectively deaminated by MAO-B (Yang and Neff, 1973; Paterson et al., 1990; Berry, 2004) and therefore the duration of the action of PEA might be extended. Using *l*-deprenyl as a MAO-B inhibitor (Gerlach et al., 1996), we examined the behavioral profile of stereotypy induced by PEA in combination with increasing doses of *l*-deprenyl and its association with dopamine metabolism in the striatum of the mouse.

2. Methods

2.1. Subjects

Male ICR mice (5 weeks old at purchase; Japan SLC, Shizuoka, Japan) were housed in groups of 8 (cage size, 37 × 22 × 15 cm) in a temperature— (22 ± 2 °C) and humidity— (50 ± 10%) controlled environment under a 12-h light/dark cycle (lights on at 0700 h) with food and water available ad libitum except during the observations of stereotyped behavior and measurements of locomotor activity using the Animex apparatus (see Section 2.2). Animal handling and care were conducted according to the *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH publication number 85–23, revised 1996) and all experiments were approved by the Institutional Animal Research Committee. Every effort was made to minimize the number of animals used and their suffering. After at least 7 days' habituation in this facility, mice were used in the experiments as follows.

2.2. Locomotor activities

Mice were weighed (30–38 g on Day 1) and divided randomly into eight groups. All mice were injected intraperitoneally (i.p.) with 0.1 ml/10 g of sterile saline on Day 1. This procedure was required to reduce the variance of the data on locomotor activity on Day 2 (Kitanaka et al., 2003, 2005). On Day 2, the mice in each group were subjected to treatment as follows: Group S/S ($n = 14$ and $n = 8$ for A and B of Figs. 1–3, respectively), 0.1 ml/10 g saline injection (i.p.) 2 h after 0.05 ml/10 g saline injection (s.c.); S/P ($n = 16$), 100 mg/kg PEA injection (i.p.) 2 h after 0.05 ml/10 g saline injection (s.c.);

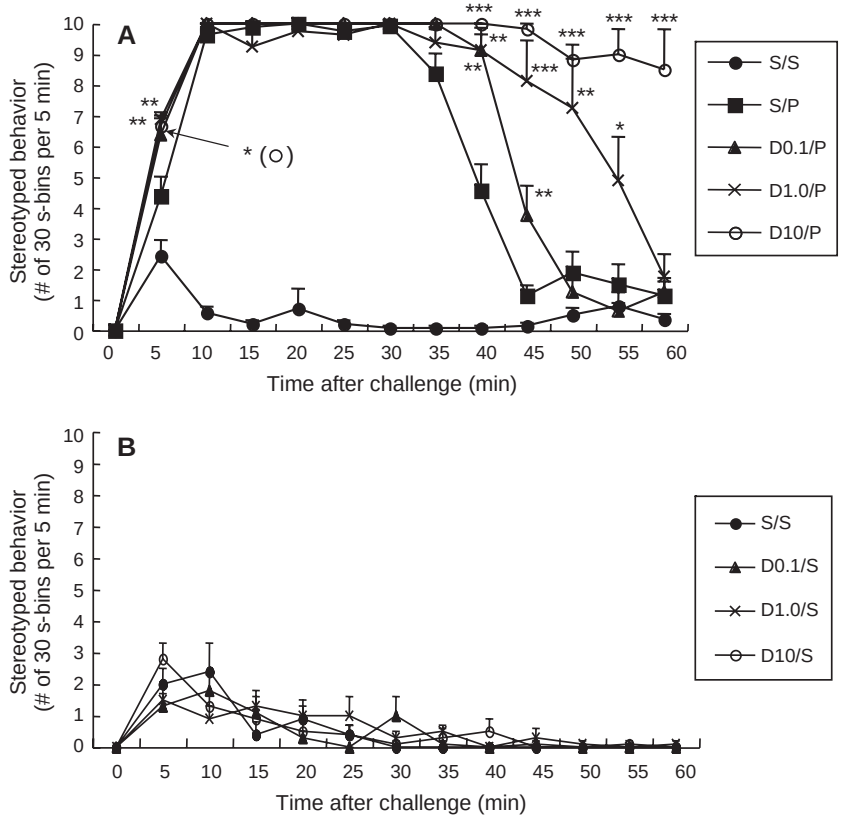

Fig. 1. Stereotypic scores after a single administration of PEA (100 mg/kg, i.p.) (A) or saline (B) in mice that were pretreated for 2 h with 0.1, 1, or 10 mg/kg (s.c.) of *l*-deprenyl or saline on Day 2. Values are shown as means with the standard errors of the means. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, compared with Group S/P (Student's *t* test).

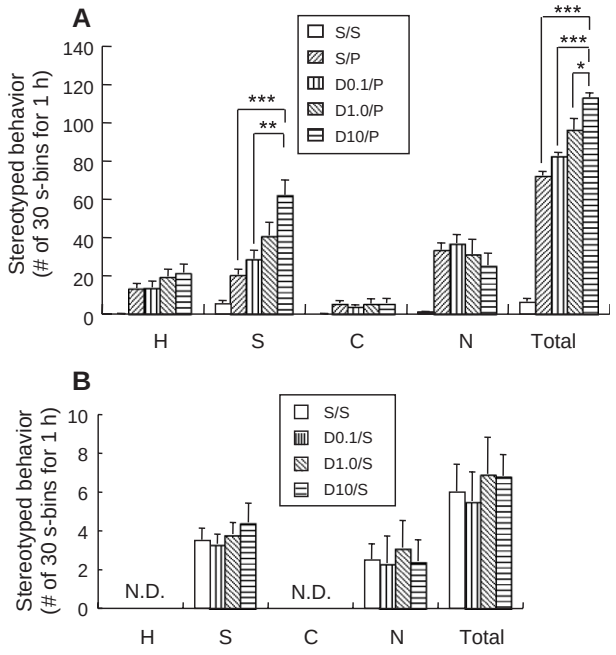

Fig. 2. Stereotyped behavior in mice in response to 100 mg/kg PEA (A) or saline (B) in combination with 0.1, 1, and 10 mg/kg *l*-deprenyl. Behavior was scored in 30-s bins, and total values for 1 h are shown. H, head bobbing; S, sniffing; C, circling; N, nail/wood chip biting. Values are shown as means with the standard errors of the means. $*p<0.05$, $**p<0.01$, $***p<0.001$, significantly different between the two groups indicated (Student's *t* test). N.D., not detected.

D0.1/P ($n=8$), 100 mg/kg 2-PEA injection (i.p.) 2 h after 0.1 mg/kg *l*-deprenyl injection (s.c.); D1.0/P ($n=8$), 100 mg/kg PEA injection (i.p.) 2 h after 1 mg/kg *l*-deprenyl injection (s.c.); D10/P ($n=8$), 100 mg/kg PEA injection (i.p.) 2 h after 10 mg/kg *l*-deprenyl injection (s.c.); D0.1/S ($n=8$), 0.1 ml/10 g saline injection (i.p.) 2 h after 0.1 mg/kg *l*-deprenyl injection (s.c.); D1.0/S ($n=8$), 0.1 ml/10 g saline injection (i.p.) 2 h after 1.0 mg/kg *l*-deprenyl injection (s.c.); D10/S ($n=8$), 0.1 ml/10 g saline injection (i.p.) 2 h after 10 mg/kg *l*-deprenyl injection (s.c.). After the final injection, all mice were subjected to measurements of locomotor activity. The doses of drugs refer to the weight of salt. All drugs were dissolved in sterile saline. *l*-Deprenyl was administered s.c. in a volume of 0.05 ml/10 g of body weight. The same volume of saline was used for the control. PEA was administered i.p. in a volume of 0.1 ml/10 g of body weight. The horizontal locomotor activity was measured in a transparent acrylic test box ($30\times30\times35$ cm) with ca. 25 g of wood chips on the Animex Auto apparatus (System MK-110; Muromachi Kikai Co., Ltd., Tokyo, Japan) in a quiet room as described previously (Kitanaka et al., 2003). All experiments were conducted between 9:00 and 16:00.

2.3. Stereotyped behavior

Animals in the transparent acrylic test box undergoing locomotion measurements were simultaneously observed for stereotypy as scored for 1 h after the drug administration by an

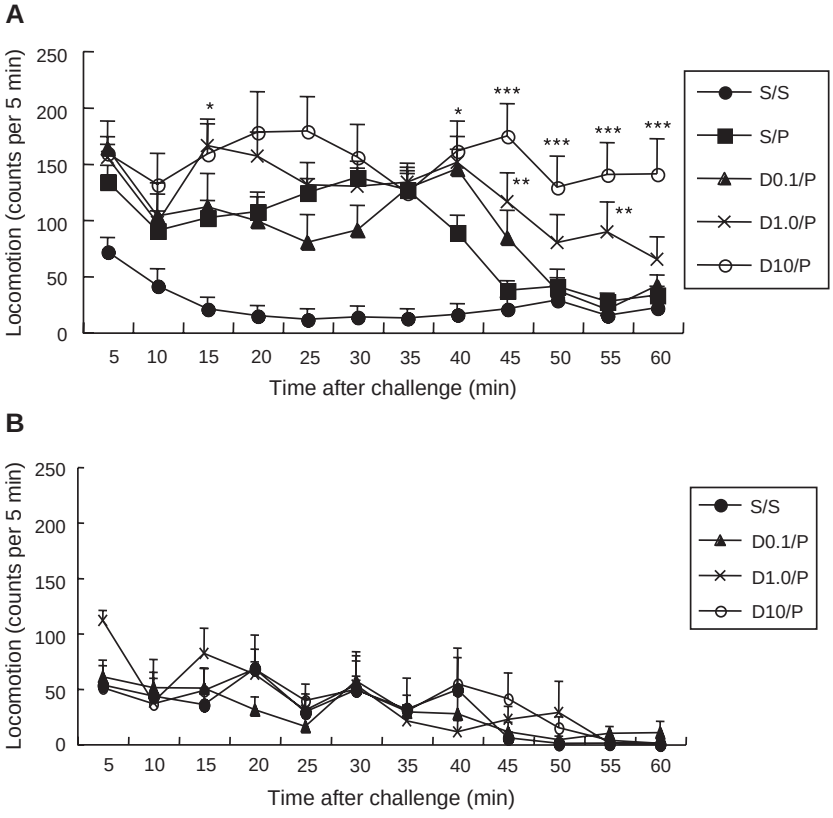

Fig. 3. Horizontal locomotor activity after a single administration of PEA (100 mg/kg, i.p.) (A) or saline (B) in mice pretreated with 0.1, 1, and 10 mg/kg (s.c.) of *l*-deprenyl or saline. Values are shown as means with the standard errors of the means. $*p<0.05$, $**p<0.01$, $***p<0.001$, compared with Group S/P (Student's *t* test).

observer blind to the treatments, using a rating scale for stereotypies described below. Behavior was broken down into 30-s bins, and a predominant behavior was recorded for each bin. Behaviors scored were quiet wake/sleeping, ambulating, rearing, vigorous grooming, head bobbing (up- and down-movements of the head), continuous sniffing with apparent exploratory behavior, circling, and nail and/or wood chip biting, according to the method of Weinshenker et al. (2002) with a slight modification. Ambulating and rearing were considered locomotor/exploratory behaviors and the last four were considered stereotypies. The cumulative numbers of bins in which stereotypies were observed for every 5 min are shown (maximal value = 10).

2.4. Measurement of levels of dopamine and metabolites

After the behavioral analyses, the mice were sacrificed by cervical dislocation and decapitation 1 h after the drug injection. The brains were immediately removed, and the striata were isolated, weighed, and frozen in liquid nitrogen. Tissue levels of dopamine and the metabolites were quantified by high-performance liquid chromatography (HPLC) with electrochemical detection as described previously (Kitanaka et al., 2005) as follows: each frozen brain sample was homogenized with a Teflon/glass homogenizer in 10–20 vol. (w/v) of ice-cold 0.1 N perchloric acid with 30 μM Na_2EDTA containing 3,4-dihydroxybenzylamine hydrobromide and isoproterenol as internal standards for the catechols and for the indoles, respectively. The homogenates were centrifuged at 10,000 $\times g$ for 10 min at 4 °C and the supernatants were filtered through a 0.20-μm membrane filter (Millipore Co., Bedford, MA, USA). The filtrates (10 μl) were injected directly into an HPLC system (system controller, model SCL-10A; auto-injector, model SIL-10A; pump, model LC-10AD; Shimadzu Co., Kyoto, Japan) equipped with a reversed-phase ODS-column (MCM column 150; 4.6 × 150 mm; MC Medical, Inc., Osaka, Japan) and an electrochemical detector (Coulochem Model 5100A, ESA, Inc., Chelmsford, MA, USA). The column temperature was maintained at 24 °C, and the detector potentials were set at +0.40, +0.15 and −0.35 V on the conditioning cell, and Detectors 1 and 2, respectively. The mobile phase was a 1000 : 35.2 : 85.8 (v/v) mixture of a buffer (50 mM Na_2HPO_4, 50 mM citric acid, 4.4 mM 1-heptanesulfonic acid and 0.1 mM Na_2EDTA, pH 3.0), acetonitrile and methanol, and the flow rate was set at 0.9 ml/min.

2.5. Reagents

PEA hydrochloride was purchased from Wako (Osaka, Japan). *R*-(−)-deprenyl hydrochloride (*l*-deprenyl) and all standard reagents for HPLC were from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of the highest purity commercially available.

2.6. Statistical analysis

Values are shown as means with the standard errors of the means (SEM). Statistical analysis was performed using a one-way or two-way analysis of variance (ANOVA) with or without repeated measures followed by the Tukey–Kramer test or Student's *t* test as indicated. A *p* value of less than 0.05 was considered a statistically significant difference.

3. Results

3.1. Stereotypic scores after the administration of PEA and l-deprenyl in combination to mice

In our preliminary experiments, single administrations of 5 and 10 mg/kg (i.p.) of PEA had no effect on spontaneous locomotion in male ICR mice (data not shown). The hyperactive locomotion, but not stereotypy, was observed when mice were administered 50 mg/kg of PEA. A single administration of PEA (100 mg/kg) induced stereotypy as described below, although convulsions with high frequency were observed when mice were administered 200 mg/kg of PEA. Therefore, a dose of 100 mg/kg of PEA was chosen in the present study.

The stereotypic scores reached a plateau level 10 min after a single administration of 100 mg/kg PEA which lasted until 30 min after the administration in S/P mice (Fig. 1A). The stereotyped behavior completely disappeared 45 min after the administration in S/P mice. Pretreatment with *l*-deprenyl (0.1, 1, and 10 mg/kg, s.c.) dose-dependently prolonged the PEA-induced stereotypy (repeated-measures two-way ANOVA followed by Tukey–Kramer test, Time × Pretreatment, $F(36,468)=13.706$, $p<0.001$) (Fig. 1A). The stereotypic scores in D10/P mice maintained a plateau level until at least 1 h after the PEA administration when mice were pretreated with 10 mg/kg of *l*-deprenyl. In S/S mice (control animals), a stereotypy-like continuous sniffing with apparent exploratory behavior was scored by the observer blind to the treatments between 0 and 5 min after the second saline injection, but the behavioral response disappeared 10 min after the injection (Fig. 1A).

Pretreatment with *l*-deprenyl per se did not induce stereotyped behaviors at the dose between 0.1–10 mg/kg (s.c.) ($F(36,364)=1.571$, $p=0.229$) (Fig. 1B).

3.2. Observed stereotyped behaviors

The observed stereotyped behaviors were classified into four groups: head bobbing (H), continuous sniffing with apparent exploratory behavior (S), circling (C), and nail and/or wood chip biting (N). The frequency of each observed stereotyped behavior for 1 h and the total count of all observed stereotyped behaviors (H + S + C + N) are shown for each mice group (Fig. 2A). Pretreatment with *l*-deprenyl (0.1, 1, and 10 mg/kg, s.c.) dose-dependently increased both the PEA-induced continuous sniffing and the total count (one-way ANOVA followed by Tukey–Kramer test, $F(3,36)=13.003$, $p<0.001$ and $F(3,36)=34.81$, $p<0.001$, respectively), but not head-bobbing, circling, or nail and/or wood chip biting ($F(3,36)=1.409$, $p=0.2561$, $F(3,36)=0.199$, $p=0.8962$, and $F(3,36)=0.708$, $p=0.5534$, respectively). In mice pretreated with *l*-deprenyl per se (no PEA challenge), no significant

change in the number of stereotyped behavior was observed between each group ($F(3,28)=0.454$, $p=0.7162$, $F(3,28)=0.106$, $p=0.9557$, and $F(3,28)=0.188$, $p=0.904$ for continuous sniffing, nail and/or wood chip biting, and the total count, respectively) (Fig. 2B).

3.3. Horizontal locomotor activity

The horizontal locomotion was measured simultaneously with stereotypic scores, and the cumulative counts for every 5 min were plotted until 1 h after PEA administration (Fig. 3A). The time courses of the locomotion in D1.0/P and D10/P mice were significantly different from S/P (repeated-measures two-way ANOVA followed by Tukey–Kramer test, Time × Pre-Pretreatment, $F(33,432)=1.796$, $p<0.01$). As shown in Fig. 3B, pretreatment with *l*-deprenyl per se had no stimulatory effect on spontaneous locomotion at the dose between 0.1–10 mg/kg (s.c.) ($F(33,336)=1.018$, $p=0.4453$).

3.4. Tissue levels of dopamine and metabolites and apparent dopamine turnover

Treatment with PEA in combination with *l*-deprenyl significantly reduced the level of dopamine in the striatum+nucleus accumbens of D1.0/P and D10/P mice, compared with S/P mice (one-way ANOVA followed by Tukey–Kramer test, $F(4,43)=20.549$, $p<0.001$) (Table 1). The tissue level of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) did not differ among the groups ($F(4,43)=2.265$, $p=0.0778$ and $F(4,43)=0.495$, $p=0.7395$, respectively).

Table 1
Tissue levels of dopamine and its metabolites in the striatum+nucleus accumbens 1 h after the final drug/saline challenge

	DA	DOPAC	HVA
PEA challenge			
S/S (10)	4.67±0.34	0.227±0.063	0.250±0.036
S/P (14)	5.14±0.30	0.153±0.073	0.204±0.032
D0.1/P (8)	5.45±0.56	0.063±0.037	0.349±0.144
D1.0/P (8)	2.32±0.27[a,b]	0.032±0.023	0.318±0.194
D10/P (8)	1.75±0.31[a,b]	0.013±0.003	0.349±0.144
Saline challenge			
S/S (8)	6.18±0.31	0.472±0.023	0.421±0.041
D0.1/S (8)	6.57±0.57	0.479±0.030	0.360±0.036
D1.0/S (8)	6.35±0.29	0.477±0.024	0.416±0.017
D10/S (8)	6.98±0.60	0.359±0.022[a]	0.366±0.040

The brains were dissected 1 h after the 2-phenylethylamine (PEA) challenge (100 mg/kg, i.p.) or saline. Values are expressed as nanograms per milligram of wet tissues (mean±SEM, $n=8–14$).
S/S, 0.1 ml/10 g saline injection (i.p.) 2 h after 0.05 ml/10 g saline injection (s.c.); S/P, 100 mg/kg PEA injection (i.p.) 2 h after 0.05 ml/10 g saline injection (s.c.); D0.1/P, 100 mg/kg PEA injection (i.p.) 2 h after 0.1 mg/kg *l*-deprenyl injection (s.c.); D1.0/P, 100 mg/kg PEA injection (i.p.) 2 h after 1 mg/kg *l*-deprenyl injection (s.c.); D10/P, 100 mg/kg PEA injection (i.p.) 2 h after 10 mg/kg *l*-deprenyl injection (s.c.); D0.1/S, 0.1 ml/10 g saline injection (i.p.) 2 h after 0.1 mg/kg *l*-deprenyl injection (s.c.); D1.0/S, 0.1 ml/10 g saline injection (i.p.) 2 h after 1 mg/kg *l*-deprenyl injection (s.c.); D10/S, 0.1 ml/10 g saline injection (i.p.) 2 h after 10 mg/kg *l*-deprenyl injection (s.c.).
[a] $p<0.05$, compared with S/S group (one-way ANOVA followed by Tukey–Kramer test).
[b] $p<0.05$, compared with S/P group (one-way ANOVA followed by Tukey–Kramer test).

Treatment with saline in combination with *l*-deprenyl significantly reduced the level of DOPAC in the striatum+nucleus accumbens of D10/S mice, compared with S/S mice ($F(3,28)=5.518$, $p<0.01$). Tissue levels of dopamine and HVA did not differ among the groups ($F(3,28)=0.558$, $p=0.6474$ and $F(3,28)=0.873$, $p=0.4667$, respectively).

4. Discussion

PEA resembles amphetamines in structure, and when administered to rodents induces amphetamine-like behavioral responses in a complex manner (Berry, 2004; Sotnikova et al., 2004). PEA-induced stereotypy has been studied in rats (Braestrup et al., 1975; Borison et al., 1977; Dourish, 1981; Ortmann et al., 1984; Timár and Knoll, 1986) and in mice (Dourish, 1982; Lapin, 1996; Sotnikova et al., 2004). Lapin (1996) reported that when mice were injected with PEA at a dose of 100 mg/kg (i.p.), stereotypy was observed instead of the hyperlocomotion observed at lower doses of PEA. In the present study, the PEA (100 mg/kg, i.p.)-induced abnormal behavioral responses in male ICR mice consisted of three phases in terms of scores of observed stereotypy; an initial phase (0–10 min after injection; forward walking, continuous sniffing, circling, and head bobbing), a plateau phase (10–30 min; continuous sniffing, nail/wood chip biting, and head bobbing), and a recovery phase (30–45 min; forward walking, continuous sniffing, and vigorous grooming) (Fig. 1A). This observation was in good agreement with that reported by Dourish (1982).

As shown in Fig. 2A, the proportion of sniffing in the PEA-induced stereotypy increased dependent on the dose of *l*-deprenyl (28.1%, 34.6%, 42.3%, and 54.9% in S/P, D0.1/P, D1.0/P, and D10/P mice, respectively), and seemed to be associated with the decrease in the proportion of nail/wood chip biting (46.1, 44.5, 32.3, and 22.3% in S/P, D0.1/P, D1.0/P, and D10/P mice, respectively). This suggests that the change in the proportion of sniffing in the PEA-induced stereotypy is under the influence of the degree of MAO-B inhibition, since *l*-deprenyl is a selective, irreversible MAO-B inhibitor (Gerlach et al., 1996). This is supported by earlier evidence that increasing doses of *l*-deprenyl potentiated PEA-induced continuous sniffing in rats (Braestrup et al., 1975; Ortmann et al., 1984).

It should be noted that there is a difference in stereotypy between PEA- and methamphetamine (METH)-treated mice, since our observations showed that a single administration of METH (10 mg/kg, i.p.) induced a stereotypy consisting of nail/wood chip biting (52% of the total stereotypic score), vigorous grooming with excess saliva production (45%), and continuous sniffing (3%) (Tatsuta et al., manuscript in press).

The effect of PEA in combination with *l*-deprenyl on spontaneous locomotor activity was examined (Fig. 3A). In S/P mice (treated with PEA alone), the data indicated no "biphasic" stimulatory effect on the spontaneous locomotor activity reported by Jackson (1972). This might be due to the

difference in the doses of PEA used (100 vs. 75 mg/kg, i.p.) or mouse strains (ICR vs. Quackenbush Swiss mice) between us and Jackson (1972). The locomotor activity in D1.0/P and D10/P mice significantly increased compared with that in S/P mice (Fig. 3A), although the locomotor activity in D0.1/S, D1.0/S and D10/S mice was similar to that of S/S mice (Fig. 3B). In D10/P mice, the increase in locomotor activity was accompanied by a significant increase in continuous sniffing (Fig. 2A). This is reasonable, because two types of behavior (i.e. horizontal stimulated locomotion and continuous sniffing) occurred simultaneously in the stereotypy period.

It is of interest to examine the association of brain dopamine metabolism with the PEA-induced stereotypy, particularly continuous sniffing. Braestrup et al. (1975) reported that *l*-deprenyl at a dose of 8 mg/kg potentiated PEA (40 mg/kg)-induced sniffing in rats and decreased the tissue level (whole brain) of DOPAC by ca. 50%. As shown in Table 1, the level of dopamine in the striatum+nucleus accumbens of D1.0/P and D10/P mice significantly decreased, compared with that in S/P mice (Table 1, PEA challenge). Pretreatment with *l*-deprenyl per se had no effect on the tissue levels of dopamine and HVA in the brain regions tested (Table 1, saline challenge), although, in the extracellular level, *l*-deprenyl treatment alone was reported to increase striatal dopamine (Melega et al., 1999; Finberg et al., 2000). The present finding that *l*-deprenyl treatment alone did not change tissue dopamine level (Table 1, saline challenge) was similar to the previous report that methamphetamine per se did not increase striatal dopamine level (Kitanaka et al., 2003). The effective concentrations of PEA might increase under the blockade of MAO-B by MAO inhibitors (Suzuki and Yagi, 1976; Philips and Boulton, 1979), resulting in an enhancement of dopamine release into synaptic cleft (Parker and Cubeddu, 1988) associated with the decrease in the tissue level of dopamine (Table 1).

In the case of amphetamines, the stimulant-induced behavioral changes in mammals are dependent primarily upon an increase in brain dopamine turnover (Randrup and Munkvad, 1970; Robinson and Becker, 1986). In addition, METH (20 mg/kg, s.c.)-induced self-injurious behavior in mice was abolished by haloperidol (0.01–1 mg/kg, i.p.) in a dose-dependent manner (Mori et al., 2004). These observations suggest that dopamine neurotransmission plays an important role in METH-induced behavioral changes. Similarly, it has been postulated that PEA-induced stereotypy is controlled by increased dopamine turnover (Paterson et al., 1990; Berry, 2004). However, the present study did not provide evidence for this, since no significant alterations in DOPAC or HVA were observed (Table 1).

There is evidence that PEA induces dopamine release in rat caudate nucleus in vivo (Bailey et al., 1987), in rat nucleus accumbens in vivo (Nakamura et al., 1998), and in rabbit striatal slices in vitro (Parker and Cubeddu, 1988). Furthermore, PEA-induced dopamine release disappeared almost completely in the striatum of mice lacking DAT (Sotnikova et al., 2004), similar to the case of *d*-amphetamine (Jones et al., 1998). These observations suggest that the mechanisms by which PEA and amphetamines produce stereotypy may overlap through a primary action on the DAT. The present study is most consistent with the hypothesis that PEA acts to increase extracellular dopamine level in the striatum, and that *l*-deprenyl potentiates the effect by blocking dopamine degradation via MAO, resulting in the decrease in the striatal dopamine level. However, other mechanism(s) are also proposed for the action of PEA because of the inhibitory effect of PEA on hyperactivity and stereotypy observed in DAT-knockout mice (Sotnikova et al., 2004).

Of observed components of the PEA-induced stereotypy, head bobbing, abnormal posture with hindlimbs splaying out, and reciprocal forepaw padding are classified as "5-HT behavioral syndrome" (Dourish, 1981). The possible involvement of 5-HT neurotransmission in the PEA-induced behavioral changes is, therefore, suggested (Dourish, 1981; Paterson et al., 1990). However, no changes in the striatal levels of 5-HT and its metabolite 5-hydroxyindolacetic acid were observed (data not shown), suggesting that the PEA-induced increase in continuous sniffing is primarily under the control of striatal dopamine neurotransmission. In rats, haloperidol (0.25 mg/kg, i.p.) completely abolished chronic PEA (50 mg/kg/day, i.p., for four weeks)-induced stereotypy, supporting the involvement of dopamine neurotransmission in PEA-induced stereotypy (Borison et al., 1977).

In conclusion, the present study indicated that: (1) PEA in combination with increasing doses of *l*-deprenyl decreased the level of dopamine in the striatum of the mouse, and (2) prolonged the duration of the stereotyped behavior. The stimulated dopamine release in the striatum might induce the prolonged expression of continuous sniffing.

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