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Repeated Exposure to β-phenylethylamine Produces Locomotor Sensitization to Amphetamine, but Not Vice Versa, in the Rat

Hye Kyoung Park†, Joon Chae Na†,
Ju Kyong Jang and Jeong-Hoon Kim*

Department of Physiology, Brain Korea 21 Project for Medical Science, Brain Research Institute, Yonsei University College of Medicine, Seoul 120-752, Korea

ABSTRACT

Repeated administration of amphetamine (AMPH) produces behavioral sensitization, a proposed model for the escalation of drug use characteristic of human addicts. β-Phenylethylamine (PEA) is an endogenous trace amine found in mammalian brain and resembles AMPH both structurally and behaviorally. Previously, it has been reported that chronic PEA administration produces behavioral sensitization to the challenges of AMPH. However, these data were obtained with very high amount of PEA for a relatively long period of time. Further, the effect of PEA challenge on the expression of behavioral sensitization developed by AMPH pre-exposures has not been tested yet. Thus, we examined in the present experiment the expression of behavioral sensitization with AMPH challenge after a mild chronic PEA treatment. Rats were repeatedly administered with systemic injections of saline, β-phenylethylamine (PEA) (10 or 50 mg/kg), or amphetamine (AMPH) (1.5 mg/kg). When challenged a week after the last pre-injection, rats pre-exposed to either PEA or AMPH showed behavioral sensitization to AMPH (1.0 mg/kg), while these effects were not observed to PEA (50 mg/kg) itself. These results demonstrate that repeated exposure to PEA produces behavioral sensitization to AMPH challenge, while PEA challenge has no effect on the expression of behavioral sensitization developed by AMPH pre-exposures, suggesting that PEA may play a role in the development of locomotor sensitization to AMPH, but not in the expression of it.

Key words: β-phenylethylamine, amphetamine, behavioral sensitization, addiction, schizophrenia

INTRODUCTION

β-Phenylethylamine (PEA) is an endogenous trace amine that is found in mammalian brain with highest levels in regions such as the caudate-putamen, olfactory tubercles, and nucleus accumbens (Berry, 2004). Both structurally and behaviorally, PEA resembles a psychostimulant drug amphetamine (AMPH) and has been implicated in human psychiatric diseases like depression and schizophrenia, leading to propose it to be called as an 'endo-

*To whom correspondence should be addressed.
†These authors contributed equally to this work.
Tel: 82-2-2228-1704, Fax: 82-2-393-0203
e-mail: jkim1@yuhs.ac
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genous AMPH' (Burchett and Hicks, 2006).

Repeated intermittent administration of AMPH produces behavioral sensitization, which is a proposed model for the escalation of drug use and craving characteristic of human addicts (Robinson and Berridge, 1993). Evidence indicates that the development of sensitization by drugs like AMPH can be separated as two phases of induction and expression, in which different neuronal processes involved mediating distinct neuronal substrates in the brain (Cador et al., 1995; Vanderschuren and Kalivas, 2000). It has been reported that PEA increases locomotor activity in rodents similar to AMPH but with the less potency and duration of action (Dourish, 1981; Popplewell et al., 1986; Lapin, 1996). Interestingly, it has been also shown that chronic systemic PEA administration produces behavioral sensitization to the challenges of AMPH as well as of PEA itself (Gianutsos and Chute, 1986; Kuroki et al., 1990). However, these data were obtained after more than 21 days of daily PEA administrations, in which the amount of PEA used was very high compared to that of AMPH generally used in the procedure of developing AMPH sensitization. Furthermore, the effect of PEA challenge on the expression of locomotor sensitization developed by AMPH pre-exposures has not been tested yet. Thus, we examined in the present experiment the expression of locomotor sensitization with AMPH challenge after a mild chronic PEA treatment, and vice versa, with a pre-exposure scheme of just several times of intermittent injections.

MATERIALS AND METHODS

Subjects

Male Sprague-Dawley rats weighing 230～260 g on arrival were obtained from Samtako (Osan, Korea). They were housed three per cage in a 12-hr light/dark cycle room with food and water available at all times. All procedures involving animals were conducted according to an approved IACUC protocol.

Drugs

PEA hydrochloride (Sigma Chemical, St. Louis, MO, USA) and d-AMPH sulfate (United States Pharmacopeial Convention, Inc., Rockville, MD, USA) were dissolved in sterile 0.9% saline. Their doses refer to the weight of the salt.

Locomotor activity

Locomotor activity was measured in a bank of 6 activity boxes (35×25×40 cm) (IWOO Scientific Corporation, Seoul, Korea) made of translucent Plexiglas and individually kept in larger PVC plastic sound attenuating cubicles. The floor of each box consisted of 21 stainless steel rods (5 mm diameter) spaced 1.2 cm apart center-to-center. Two infrared light photocells (Med Associates, St. Albans, VT, USA) positioned 4.5 cm above the floor and spaced evenly along the longitudinal axis of the box estimated locomotor activity.

Design and procedure

Animals were treated for experimental procedures as follows. *Experiment 1:* Different four groups of rats were administered systemic injections of either saline, PEA (10 or 50 mg/kg), or AMPH (1.5 mg/kg) once a day with an interval of 2 or 3 days for a total of five times. Locomotor activities after injection were measured in activity boxes only on Day 1 and 5, and they were home cage-injected for the rest of days during this drug pre-exposure phase. A week of drug-free withdrawal period after the last injection, they were all AMPH (1 mg/kg, i.p.) challenged, then immediately returned to the boxes and their locomotor activity measured for 1 hr. *Experiment 2:* Additional three groups of rats, administered systemic injections of either saline, PEA (50 mg/kg), or AMPH (1.5 mg/kg), followed the same pre-exposure treatments as Experiment 1 above. A week after the last injection, they were all challenged by PEA (50 mg/kg, i.p.) and their locomotor activity measured for 1 hr. Throughout the procedures, whenever locomotor activity was measured, rats were always first habituated to the locomotor activity boxes for 1 hr before drug injections given.

Statistical analyses

The data were analyzed with one-way or two-way between-within ANOVA (analysis of variance). Post-hoc Scheffé comparisons were made according to Kirk (1968). Differences between experimental conditions were considered statistically significant when $p < 0.05$.

RESULTS

Fig. 1A shows the locomotor activity counts obtained in rats systemically injected with either saline, one of two doses of PEA (10 or 50 mg/kg) or AMPH during pre-exposure phase. The two-way between-within ANOVA conducted on these data revealed multiple significant effects of different pre-exposure groups [$F(3,34)=83.91$, $p<0.001$], days [$F(1,34)=9.98$, $p<0.01$] and a group×days interaction [$F(3,34)=8.12$, $p<0.001$]. As expected, AMPH compared to saline significantly increased locomotor activity response on day 1 and these effects were further enhanced when measured on day 5 ($p<0.001$). PEA, however, produced no effects on locomotor activity both acutely on day 1 and repeatedly on day 5. After a week of drug-free withdrawal period, AMPH compared to saline pre-exposed group of rats showed again a sensitized locomotor activity to AMPH challenge. Interestingly, PEA compared to saline pre-exposed group also showed a sensitized locomotor activity to this challenge (Fig. 1B). The ANOVA conducted on these data showed significant effect of groups [$F(3,34)=5.65$, $p<0.004$]. *Post hoc* Scheffé revealed that significant effects appeared on high dose of PEA and AMPH ($p<0.05\sim0.01$). Time-course data in Fig. 1C shows that the sensitized locomotor responses to AMPH challenge in PEA pre-exposed rats persisted apparently for up to the 30 min of testing similar to AMPH.

In a separate experiment, when challenged with PEA, however, rats pre-exposed a week earlier to either PEA (50 mg/kg) or AMPH showed locomotor activity that was not different from that displayed by saline pre-exposed rats (Fig. 2). The ANOVA conducted on these data showed no significant effects of groups [$F(2,15)=0.09$, $p<0.92$].

DISCUSSION

The present results demonstrate that repeated exposure to PEA produces locomotor sensitization to AMPH challenge, while PEA challenge has no effect on the expression of locomotor sensitization developed by AMPH pre-exposures. Previous studies have shown that chronic daily PEA injections develops behavioral sensitization which appeared after more than 21 days of treatments to the challenges

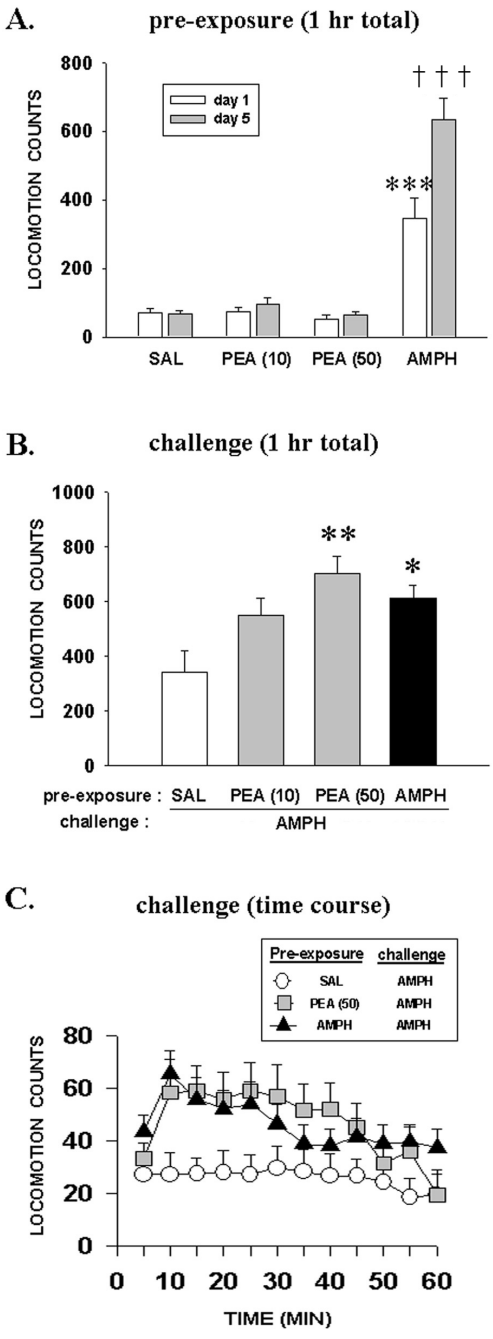

Fig. 1. Repeated exposure to PEA produces locomotor sensitization to AMPH challenge. Data are illustrated as group mean (+SEM) locomotor activity counts obtained for 1 hr following the drug injection during the pre-exposure period (A) and at the time of challenge (B). Time-course data at the challenge (C) are shown for only three groups of the four tested to clarify the effects of high dose of PEA. Numbers of rats in each group are 9 to 10. Symbols indicate significant differences as revealed by *post-hoc* Scheffé comparisons following two-way between-within (for pre-exposure) and one-way (for challenge) ANOVA's. ***$p<0.001$; AMPH compared with saline pre-exposed on day 1. **$p<0.01$, *$p<0.05$; PEA (50 mg/kg) or AMPH compared with saline pre-exposed after AMPH challenge. $^{\dagger\dagger\dagger}p<0.001$; Day 5 compared with day 1 in rats AMPH pre-exposed.

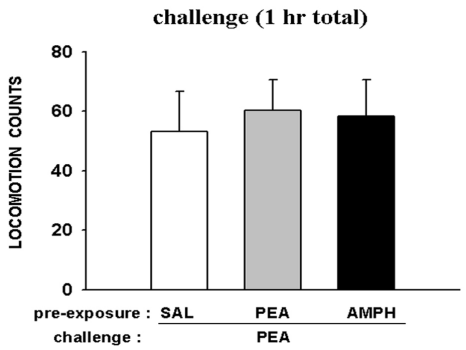

Fig. 2. PEA challenge a week after repeated exposure to either PEA or AMPH shows no difference in locomotor activity. Data are illustrated as group mean (+SEM) locomotor activity counts obtained for 1 hr following the drug injection at the time of challenge (n=6/group).

of AMPH as well as of PEA itself (Gianutsos and Chute, 1986; Kuroki et al., 1990). Our results are consistent with those in that chronic PEA produces behavioral sensitization to AMPH challenge. Further, it is evident in our results that only several times of intermittent PEA injections are sufficient enough to induce locomotor sensitization which is demonstrated by AMPH challenge after a week of withdrawal. However, contrary to the previous studies, PEA challenge did not evoke sensitized locomotion with our PEA pre-exposure procedures (Fig. 2), suggesting that PEA-induced behavioral sensitization may require the heavier pre-exposure treatments (e.g., daily over 21 days) to express its development to PEA itself. More interestingly, our results also showed that the same dose of PEA (50 mg/kg) is not able to evoke the expression of locomotor sensitization induced by AMPH pre-exposures, although several injections of that dose are enough to induce behavioral sensitization as revealed by AMPH challenge a week after. These results suggest that PEA may have a more significant role in the induction than in the expression of behavioral sensitization by AMPH. As evidence indicates that the induction and expression of behavioral sensitization are mediated by distinct neuronal substrates (e.g., the nucleus accumbens and ventral tegmental area) in the brain (Cador et al., 1995; Vanderschuren and Kalivas, 2000), it may be interesting in the future to further look at the effects of PEA in these sites on different phase of sensitization.

The precise mechanisms by which PEA influences the development of behavioral sensitization remain unknown. It may possibly exert its effects by influencing dopaminergic neurotransmission in the brain similar to AMPH (Kalivas and Stewart, 1991). For example, it has been shown in rodents that PEA increases extracellular levels of dopamine in the nucleus accumbens and striatum (Nakamura et al., 1998; Sotnikova et al., 2004), as well as in the ventral tegmental area (Ishida et al., 2005). However, others demonstrated that PEA-induced hyper-locomotor activity was inhibited by CPP, a competitive NMDA receptor antagonist (Lapin, 1996), suggesting that other neurotransmission than dopamine may also be involved in mediating PEA effects on the locomotion as also shown for AMPH (Kalivas and Stewart, 1991; Kim et al., 2001). Due to recent identifications of receptors that are specifically activated by PEA (Borowsky et al., 2001), it may soon be revealed how PEA mediates information and thereby influences the development of behavioral sensitization. Because AMPH has been known to significantly increase PEA concentrations in brain regions importantly implicated in drug addiction such as frontal cortex, striatum, and the nucleus accumbens (Chuang et al., 1982; Karoum et al., 1997), to know what PEA actually does in the brain will definitely contribute to better understanding for the mechanism of the development of behavioral sensitization by AMPH.

In summary, our present findings clearly show that only several times of repeated PEA injections are able to produce behavioral sensitization that is demonstrable by AMPH challenge, and further indicate, on the contrary, that PEA is not enough to evoke the expression of behavioral sensitization developed by AMPH as well as PEA with this scheme of pre-exposures. Thus, the present results may provide a new insight for the differential behavioral profile PEA may produce in relation with the development of behavioral sensitization by AMPH.

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