The Response to Acoustic Stimulation and the Changes in Brain Amine Levels after Repeated Administration of β -Phenylethylamine in Rats

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ABSTRACT—Reverse tolerance to stereotyped behavior was induced after repeated administration of β -phenylethylamine (PEA) (50 mg/kg, i.p., daily for 10 days) in rats. The reverse tolerance was maintained for at least 4 weeks after the last administration. We studied the effects of acoustic stimulation on locomotor activity 2 days and 4 weeks after withdrawal from PEA and measured the changes in brain monoamine levels 4 weeks after the withdrawal. Locomotor activity during acoustic stimulation was increased in the saline treated group, and this response was unaffected after repeated PEA treatment. Four weeks after withdrawal, significant increases in noradrenaline levels in the cerebral cortex and decreases in 5-hydroxytryptamine levels in the hypothalamus were found. The effects of acoustic stimulation on locomotor activity and the changes in brain monoamine levels were different from those of methamphetamine treatment obtained in our previous study. In conclusion, it may be suggested that the response to acoustic stimulation after repeated PEA administration in rats cannot be a model for abnormal responsiveness to environmental stimulation that is observed in chronic paranoid schizophrenics.

β -Phenylethylamine (PEA) is a trace amine which is naturally present in the mammalian brain (1). PEA structurally resembles amphetamine (AMPH) and possesses similar pharmacological properties, which include its abilities to cause an increase in spontaneous motor activity (2–4), appearance of stereotyped behaviors (2, 3, 5) and decrease in food and water intake (3, 6). However, the physiological roles of PEA are not as fully understood.

After the repeated administration of PEA to rats, augmented stereotyped behavior is observed (5, 7, 8). This phenomenon is also observed after repeated administration of AMPH (9) or methamphetamine (MAP) (10–

12) and is called “reverse tolerance”. It is proposed that the reverse tolerance to stereotyped behavior in animals may closely resemble AMPH psychosis in man, because of the similarity between the behavioral sensitization and relapse in psychosis (13, 14); and the stereotyped behavior observed in animals treated chronically with PEA has been proposed as a pharmacological model for schizophrenia (5, 7, 15).

It is well-known that responses to environmental stimulation are abnormal in chronic paranoid schizophrenics (16, 17). In this study, we induced reverse tolerance to stereotyped behavior by repeated PEA administration in

rats. Then we investigated the effects of acoustic stimulation on locomotor activity 2 days and 4 weeks after drug withdrawal and measured the levels of brain monoamines and their major metabolites 4 weeks after the withdrawal.

MATERIALS AND METHODS

Animals

Male Wistar rats aged 7 weeks and weighing 200–250 g at the beginning of this study were used. The rats were housed in groups of six and had continuous access to food and water except during the experimental sessions. Lighting was maintained on a 12-hour light/dark cycle under standard laboratory conditions.

Groups and schedule

The rats were divided into 4 groups. In each group, reverse tolerance to stereotyped behavior was induced. In the first group, we confirmed that reverse tolerance to stereotyped behavior was maintained at least for 4 weeks. In the second and the third groups, we studied the locomotor activity response to acoustic stimulation 2 days and 4 weeks after withdrawal, respectively. In the fourth group, the contents of brain monoamines and their metabolites were measured 4 weeks after withdrawal.

Induction of reverse tolerance

For inducing reverse tolerance to stereotyped behavior, PEA (50 mg/kg, i.p.) or saline as a control was administered daily for ten days. To confirm the induction of reverse tolerance, stereotyped behavior was rated every 3 min for 45 min after each treatment on the first, second, third, fifth and tenth day. Animals were rated according to Borison et al. (5) with some modifications as follows: 0) inactive, lying down; 1) moving about cage, sniffing, rearing; 2) repetitive exploration of the cage with hyperactivity; 3) occasional side-to-side head bobbing, remains in one location; 4) continuous head bobbing, remains in one location for over five min. At least 2 hours

prior to the observation, the rats were placed individually in the experimental cages, which were the standard wire mesh type, and allowed to adapt to the observation room. The same dose of PEA (50 mg/kg, i.p.) was given 4 weeks after withdrawal, and stereotyped behavior was scored again.

Response to acoustic stimulation

After the 2-hour adaptation to the experimental room which could be sealed off from outside noises and other disturbances, the behavioral tests were carried out. We used an open field apparatus (18) that consisted of a circular floor (60 cm diameter) and a surrounding wall expanding towards the upper brim (60 cm high and 90 cm in diameter at the top). The floor was divided by black lines into 13 units of approximately equal size, and locomotor activity was counted individually for 12 min and expressed as the number of lines crossed by the rat per min. Between 4 to 5 min and between 9 to 10 min, acoustic stimulation (peak frequency: 4 kHz, mean intensity of 3 points on the floor: 95.9 db) was given by a buzzer located one meter above the floor. Statistical evaluations were determined by the Mann-Whitney *U*-test.

Contents of brain amines and their metabolites

The contents of noradrenaline (NA), 3-methoxy-4-hydroxyphenylglychol (MHPG), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) were measured using HPLC-ECD (19, 20). The rats were sacrificed by decapitation, and the whole brains were removed immediately, placed on ice and dissected into seven regions (21). The cerebral cortex, midbrain and thalamus, hypothalamus, and striatum were used for the measurements. Each region was homogenized in 0.12 M sodium acetate buffer (pH 5.0) and then centrifuged at $15,000 \times g$ for 20 min at 4°C. The supernatant was processed for enzymatic hydrolysis with sulfatase. After another centrifugation, the supernatant was washed with

chloroform and then analyzed. Statistical evaluations were performed by Student's *t*-test.

RESULTS

Induction of reverse tolerance

Reverse tolerance to stereotyped behavior was induced after repeated PEA administration in rats. The reverse tolerance was maintained for at least 4 weeks after the last administration (data not shown).

Response to acoustic stimulation

Figure 1 shows the effects of acoustic stimulation on locomotor activity 2 days and 4 weeks after the last drug administration. As shown in Fig. 1a, 2 days after withdrawal, relatively high activity was observed during acoustic stimulation (15 counts at 4 to 5 min), but the activity was completely suppressed after the acoustic stimulation. The same effects were observed 4 weeks after the withdrawal of PEA (Fig. 1b). However, there was no difference in the response between the PEA-treated group and the saline-treated group.

Fig. 1. Response to the acoustic stimulation was examined 2 days (a) and 4 weeks (b) after the repeated PEA administration. Locomotor activity was counted individually for 12 min using an open field apparatus. Between 4 to 5 min and between 9 to 10 min acoustic stimulation was given. : with or : without acoustic stimulation. Values are mean $\pm$ S.E.M. †† : $P < 0.01$ (Mann-Whitney *U*-test).

Table 1. Contents of brain amines and their metabolites 4 weeks after PEA withdrawal

	NA	MHPG	DA	DOPAC	HVA	5-HT	5-HIAA
Cerebral cortex							
saline	265 ± 18	101 ± 60	332 ± 41	173 ± 13	125 ± 60	224 ± 26	248 ± 15
PEA	322 ± 02*	114 ± 70	347 ± 58	186 ± 18	185 ± 10	212 ± 15	245 ± 14
Midbrain and thalamus							
saline	299 ± 17	121 ± 10	123 ± 22	75 ± 06	67 ± 12	390 ± 45	229 ± 24
PEA	339 ± 10	124 ± 80	147 ± 33	86 ± 10	78 ± 50	377 ± 45	252 ± 21
Hypothalamus							
saline	1059 ± 160	154 ± 10	422 ± 42	314 ± 36	298 ± 45	482 ± 27	254 ± 36
PEA	1069 ± 128	139 ± 11	472 ± 48	374 ± 17	323 ± 33	308 ± 26**	201 ± 76
Striatum							
saline	137 ± 13	74 ± 05	5531 ± 628	1331 ± 126	798 ± 141	353 ± 26	212 ± 33
PEA	124 ± 37	76 ± 05	5415 ± 473	1499 ± 154	776 ± 194	342 ± 21	220 ± 82

Rats were decapitated 4 weeks after PEA withdrawal, and the contents of brain amines and their metabolites were measured by HPLC-ECD. Results are shown as the mean ± S.E.M. (ng/g tissue) from 6 rats. Statistical differences: *P < 0.05, **P < 0.01 (Student's *t*-test).

Contents of brain amines and their metabolites

Significant increases in NA levels in the cerebral cortex and significant decreases in 5-HT levels in the hypothalamus were found (Table 1). However, DA, DOPAC and HVA levels showed no significant changes.

DISCUSSION

We have demonstrated that reverse tolerance to stereotyped behavior was induced after repeated PEA treatment. This is consistent with previous reports by other authors (5, 7, 8).

PEA is known to induce an amphetamine-like release of DA from presynaptic neurons and an inhibition of DA re-uptake (2, 22, 23). The previous tissue studies (24, 25) suggest that the presynaptic response to PEA of the striatal DA nerve terminals is augmented in rats with repeated PEA treatment. Moreover, results from the direct measurement of extracellular levels of DA in rats using *in vivo* brain microdialysis (8) or push-pull cannula (23) indicate that the augmented behavioral response after repeated PEA treatment may be explained by enhanced DA release as observed after repeated AMPH (9, 25, 26) or MAP treatment (11, 12). On the other hand,

PEA is also reported to exert a direct stimulating effect on postsynaptic dopamine receptors (27) or its own specific binding site (28). The details of the related mechanism still remain to be studied.

It is interesting that the levels of the striatal DA, DOPAC and HVA did not change long after withdrawal from PEA treatment (Table 1), although some neurochemical changes are proposed in the striatal DA nerve terminals.

It is known that there are differences in the behavioral effects between PEA and MAP. The hyporesponsiveness to acoustic stimulation that had been observed after withdrawal from repeated MAP treatment (10) was not found after withdrawal from PEA. However, in this study, reverse tolerance to stereotyped behavior was induced after repeated PEA treatment as observed after withdrawal from MAP (10–12). This suggests that the responsiveness to acoustic stimulation is irrelevant to the induction of the reverse tolerance. Moreover, neurochemically, significant decreases in 5-HT and 5-HIAA levels in the cerebral cortex, which had been observed after withdrawal from MAP (10), were not found after PEA withdrawal.

Significant increases in NA levels in the cerebral cortex were found 4 weeks after PEA

withdrawal. However, there are no reports that have investigated the levels of NA or MHPG in the brain long after withdrawal from PEA treatment. The details of the related mechanism still remain to be studied.

PEA is an endogenous trace amine and known to play an important physiological role in the brain (1). In this study, we used a relatively high dose (50 mg/kg) of PEA. We need to use a lower dose of PEA with type B monoamine oxidase inhibitor to study the details of the effects of PEA on the related mechanisms in the brain.

In conclusion, it may be suggested that the response to acoustic stimulation after repeated PEA administration in rats can not be a model for the abnormal responsiveness to environmental stimulation that is observed in chronic paranoid schizophrenics.

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