Beta-Phenylethylamine (PEA): An Endogenous Anxiogen? Three Series of Experimental Data

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Like the anxiogenic drugs caffeine, pentylenetetrazole, and yohimbine, the endogenous neuroactive monoamine beta-phenylethylamine (PEA) is effective in three tests for anxiogens in mice. In a social interaction test it reduced both the number and duration of contacts. In a conflict situation test (a dark-light chamber) it reduced the number of transitions between dark and light compartments. Diazepam, a standard anxiolytic, prevented both effects of PEA. Intracerebroventricular administration of PEA induced generalized clonic seizures which were antagonized by various anxiolytics but not by the tested doses of butyrophenone neuroleptics and standard anticonvulsants effective in other tests for convulsants.

Introduction

The idea of this study stems from our experiments on the mode of action of phenibut (beta-phenyl-GABA), a tranquilizer with nootropic effect, manufactured in the USSR (Lapin 1987; Khaunina and Lapin 1989). Until recently, this drug, like its muscle relaxant chlorine derivative baclofen, has been investigated only as a derivative of GABA. However, the two drugs are derivatives of both GABA and beta-phenylethylamine (PEA) (Figure 1). Does a PEA moiety take part in the anxiolytic action of phenibut? Are there any interactions between PEA and phenibut? In experiments on mice we (Lapin 1987) have observed that phenibut and baclofen diminished all studied effects of the injected PEA, namely, the locomotor excitation (higher doses of PEA—50 mg/kg, 2–5 min after injection) and inhibition (lower doses of PEA—10–25 mg/kg, 15 min after injection), hyperthermia and hypothermia, and seizures. The seizure effect of PEA was observed after administration of PEA into brain ventricles [100 μg, intracerebroventricular (ICV)]. The standard anxiolytic drug diazepam was effective against PEA only in the seizure test whereas the standard neuroleptic haloperidol was an effective antagonist of PEA in the other tests. This effect of diazepam prompted us to test other anxiolytics against PEA-induced seizures. A group of anxiolytics appeared to be antagonists of PEA in this test (Lapin 1987). The results suggest that PEA is a putative anxiogen. A similar situation occurs in the pair "benzodiazepine–pentylenetetrazole." It is generally known that the

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anxiolytic effect of benzodiazepine tranquilizers in humans correlates with their anticonvulsant activity in a pentylenetetrazole seizure test in mice (Cook and Sepinwall 1975; Rudenko et al 1982). The convulsant effect of pentylenetetrazole in this test exhibits an excitation typical of this drug as an anxiogen in humans.

In the present study, we compared the effect of PEA with those of the known anxiogens pentylenetetrazole, caffeine, and yohimbine.

Methods

Experiments were performed with male albino SHR (bred from Swiss) mice weighing 20–22 g and with C57BL/6 and CC57Br strains of the same body weight. Seizures were induced by pentylenetetrazole (80 mg/kg, IP), caffeine (250 mg/kg, IP), or yohimbine (40 μg, ICV). ICV injection to conscious mice was accomplished with semiautomatic apparatus according to the method described elsewhere (Lapin 1987). The social interaction test (Pellow and File 1984) developed in rats was adapted for mice in our laboratory. One day of isolation was found to be sufficient to increase the number of contacts in a pair of animals (Table 1). In rats, the procedure includes 5 days of isolation. Mice were isolated in boxes of 18 × 6 × 6 cm with free access to food and water. Testing was made in a box 20 × 15 × 10 cm for 5 min. Before testing, a single mouse was placed into a similar box for 5 min for adaptation and extinction of exploratory motor activity. Three types of contacts were measured, i.e., sniffings of nose, body, and genitalia (for the number of contacts see Table 1), by means of digit counters. The total (cumulative) time of contacts was registered by stop watch (Casio J-30) with a manual accuracy of 0.08–0.1 sec. Short contacts lasted 0.15–0.25 sec, long ones 5–8 sec. Locomotion and rearings were measured in each experiment simultaneously with measurings of contacts as described elsewhere (Lapin 1987). The test of conflict situation (Crawley 1981) in the dark-light chamber has proved to be a reliable model for studying benzodiazepines and other anxiolytics. We have attempted to use this test for anxiogens. The standard anxiogens caffeine, pentylentetrazole, and yohimbine showed an effect opposite to that of anxiolytics, namely, a decrease in number of transitions between dark and light compartments. In addition, they diminish dark preference (time spent in the dark compartment). Anxiolytics are known not to modify dark preference (Crawley 1981).

This test was performed on mice of C57BL/6 and CC57Br strains because the albino mice strains that we tested (SHR and BALB/c) did not demonstrate stable dark preference. Of 98 control groups (each of 8–10 animals), only in 40 was there statistically significant dark preference. This was observed in 1 day ("blank controls" when all groups received a vehicle; i.e., there were groups with and without dark preference) or in consecutive days. The chamber consists of dark (27 × 15.5 × 27 cm, illumination 1–6 lux) and light (27 × 29 × 27 cm, illumination 80–100 lux) compartments. During 5-min sessions we measured time spent in each compartment and the number of transitions. Locomotion and rearings were measured separately in both compartments and expressed as a ratio of activity to time (in sec) spent in a certain compartment (multiplied by 100). PEA was tested in three series of experiments and compared with the standard anxiogens caffeine, pentylenetetrazole, and yohimbine. Statistical significance of differences was tested according to the Student's *t*-test, U-test of Wilcoxon, and chi-square method.

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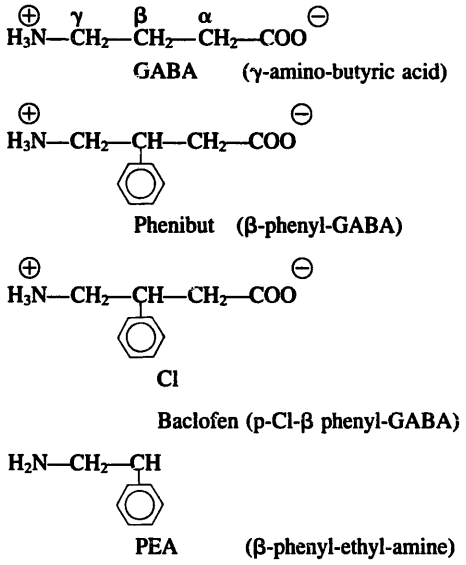

Figure 1. Phenibut and baclofen as derivatives of both GABA and PEA.

Results

Anxiolytic-sensitive Seizures Induced by PEA

Data are presented in Table 1. The following drugs with anxiolytic and/or antipanic action appeared to be effective in preventing PEA-induced seizures (ED 100, mg/kg, IP, except ethanol PO, 15 or 30 min prior to PEA): benzodiazepines: diazepam (5), chlordiazepoxide (25), oxazepam (25), and phenazepam (2); GABA derivatives phenibut (100), baclofen (10), and sodium hydroxybutyrate (200); buspirone (10), ethanol (4000). Drugs diminishing the number of mice with seizure and/or increasing the latency of seizure were beta-adrenoblockers propranolol (50) and alderlin (50), the α2-adrenoceptor agonist clonidine (1), and the tricyclic antidepressant amitriptyline (10). Ineffective drugs (maximal doses tested) were the anticholinergics benactyzine (10) and methylbenactyzine (5), the tricyclic antidepressant imipramine (50), neuroleptics haloperidol (0.5) and spiroperidol (0.5), and the anticonvulsants phenobarbital (60), diphenylhydantoin (80), primidone (50), and depakiene (300).

In the antipentylenetetrazole test, the following drugs were ineffective: GABA derivatives, beta-adrenergic blockers, anticholinergics, and buspiron. The same was true for the anticaffeine test. In contrast to the former test, the latter appeared to be very sensitive to the anticonvulsant effect of ethanol (ED 100 was 2500 mg/kg). In the antiyohimbine test, only benzodiazepines and phenibut were effective.

Social Interaction Test in Mice

Table 2 shows that PEA reduces both the total number of contacts and the overall time of contacts. Similar effects were observed in mice treated with the standard anxiogens caffeine (50 mg/kg), pentylenetetrazole (25 mg/kg), and yohimbine (2 mg/kg). In doses of 5 and 10 mg/kg, PEA did not reduce locomotion and rearings in the same experiment.

Table 1. Antagonism to PEA-Induced Seizures in Mice

Pretreatment drugs IP	Dose mg/kg	Efficacy
Benzodiazepines	ED 100	Prevention of seizures
Diazepam	5.0	
Chlordiazepoxide	25.0	
Oxazepam	25.0	
Phenazepam	2.0	
GABA derivates		
Phenibut	100.0	
Baclofen	10.0	
Sodium hydroxybutyrate	200.0	
Buspiron	10.0	
Ethanol	4.000.0	
Beta-Adrenoblockers	ED 100	Diminution of the number of mice with seizures and/or increase of the latency of seizures
Propraholol	50.0	
Alderlin	50.0	
Adreno α_2-receptor agonist		
Clonidine	1.0	
Tricyclic antidepressant		
Amitriptyline	10.0	
Anticholinergics	Maximal dose tested	Ineffective
Benactyzine	10.0	
Methylbenactyzine	5.0	
Tricyclic antidepressant		
Imipramine	50.0	
Neuroleptics		
Haloperidol	0.5	
Spiroperidol	0.5	
Anticonvulsants		
Phenobarbital	60.0	
Diphenyldantoin	80.0	
Primidone	50.0	
Depakine	300.0	

Pretreatment with diazepam (1 mg/kg, IP, 30 min prior to PEA) prevented the inhibiting effect of PEA (Table 4). Phenibut (100–200 mg/kg, IP, 30 min prior to PEA) was ineffective.

Conflict Situation Test (Dark-Light Chamber)

PEA in doses of 5 and 10 mg/kg (the latter dose is not present in Table 3) reduces the number of transitions between dark and light compartments and the dark preference (Table 2). The anxiogens pentylenetetrazole (10 and 20 mg/kg) and caffeine (100–250 mg/kg) have a similar dose-dependent effect. Diazepam and phenibut in the doses mentioned above diminished the effect of PEA (Table 4). At effective doses, all three anxiogens did not inhibit locomotion and rearings in the same experiments. In control experiments, diazepam and phenibut in doses used did not change either the dark preference or the number of transitions.

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Table 2. Inhibitory Effect of PEA on Social Interaction in Mice

Groups of mice	Drug IP mg/kg	Active contacts: Total number mean ± SE	Active contacts: Total time (sec) mean ± SE	Locomotion mean ± SE	Rearings mean ± SE
SHR strain					
Without isolation (10)	Saline	3.0 ± 0.8	0.62 ± 0.18	42.0 ± 3.3	28.2 ± 2.2
Without isolation (10)	PEA 5	3.2 ± 0.7	0.80 ± 0.24	38.5 ± 2.6	26.4 ± 3.5
After isolation (10)	Saline	16.8 ± 2.7	16.7 ± 5.6	91.4 ± 8.7	43.8 ± 4.8
After isolation (10)	PEA 5	8.9 ± 2.7[a]	4.0 ± 1.9[b]	84.3 ± 9.2	39.5 ± 2.9
C57BL/6 strain					
After isolation (6)	Saline	14.2 ± 1.9	4.6 ± 1.0	78.7 ± 9.6	32.5 ± 2.7
After isolation (6)	PEA 10	6.2 ± 1.5[c]	1.3 ± 0.3[b]	71.6 ± 8.8	30.6 ± 4.3

Number of pairs in parentheses. Testing (during 5 min) was made 5 min after drug administration [a] + significant reduction of number of pairs with more than 5 contacts (10 vs. 2); [b] + significant reduction of number of pairs with contacts longer than 1 sec (10 vs. 2). [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$. Reproducibility of the effect of PEA—statistically significant difference from control at the same day (in SHR mice): 5 mg/kg (14 experiments)—reduction of the total time of contacts in 11 experiments, reduction of the total number of contacts in 8 experiments; 10 mg/kg (16 experiments), respectively, 14 and 12 experiments, reduction of both criteria in 11 experiments. Pentylenetetrazole (10 mg/kg) in all 6 experiments reduced both total number of contacts and total time of contacts. In C57BL/6 mice, the reproducibility of the effect of PEA was higher than in SHR mice.

Discussion

Data from the above three series of experiments suggest that PEA may be an endogenous anxiogen. We found no data or speculations about probable relationships between PEA and anxiety in the literature. A review devoted to PEA in neuropsychiatric disorders

Table 3. Inhibitory Effect of PEA on Dark Preference and Transitions in Dark-Light Chamber (Conflict Situation Test)

Drugs	Dose IP mg/kg	Dose ICV μg	Time spent in light chamber, sec mean ± SE	Number of transitions mean ± SE	Locomotion in light chamber mean ± SE	Locomotion in dark chamber mean ± SE	Rearings in light chamber mean ± SE	Rearings in dark chamber mean ± SE
C57BL/6 strain								
Saline	—		34.3 ± 7.6	6.5 ± 1.3	62.4 ± 9.2	40.3 ± 6.2	10.5 ± 2.1	7.3 ± 1.1
PEA	5		90.2 ± 6.6[c]	2.0 ± 0.[b]	56.8 ± 10.3	38.7 ± 4.9	10.1 ± 2.2	6.1 ± 0.8
Pentylenetetrazole	10		72.0 ± 9.2[b]	3.1 ± 0.4[a]	52.4 ± 8.5	32.6 ± 5.1	8.3 ± 1.8	5.5 ± 0.3
Saline		—	28.9 ± 10.8	7.6 ± 1.0	59.5 ± 7.3	41.4 ± 3.8	9.6 ± 2.3	6.9 ± 0.9
PEA		0.1	89.6 ± 38.7	4.6 ± 1.0	56.7 ± 6.8	39.7 ± 4.6	9.2 ± 2.7	7.2 ± 1.4
		1.0	137.0 ± 26.7[b]	4.3 ± 0.6[b]	58.1 ± 6.4	42.5 ± 5.7	10.6 ± 1.8	7.9 ± 0.8
		10.0	176.2 ± 19.3[c]	2.8 ± 0.5[c]	53.8 ± 8.2	36.1 ± 5.7	8.2 ± 1.3	5.2 ± 0.7
CC57Br strain								
Saline	—		71.0 ± 12.4	7.0 ± 1.3	89.5 ± 10.7	70.0 ± 6.9	15.5 ± 2.3	10.1 ± 1.8
PEA	5		145.0 ± 12.2	2.5 ± 0.3[b]	80.3 ± 12.1	67.6 ± 8.6	12.7 ± 2.0	9.9 ± 1.3
Pentylenetetrazole	10		120.0 ± 18.7	3.7 ± 0.6[a]	73.6 ± 10.8	61.8 ± 7.9	9.8 ± 2.4	7.6 ± 0.9

Groups of 8 mice. Animals were tested 5 min after IP administration of a drug and 15 min after ICV administration. It is impossible to test mice earlier than 15 min after ICV injection of a drug because animals are slightly inhibited due to the procedure of fixation and ICV intervention.

The effect of PEA was observed in 4 experiments with C57BL/6 mice and in 3 experiments with CC57Br mice. Reproducibility of the effect was not specially studied in various seasons of the year.

[a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

Table 4. Effect of Anxiolytics Diazepam and Phenibut on the Action of PEA

Pretreatment (IP) 30 min prior to PEA 1st		2nd			
Drug	Dose (mg/kg)	Drug	Dose (mg/kg)	Effect	
Social isolation test				Active Contacts	
				Total number mean ± SE	Total time sec mean ± SE
Vehicle	—	Vehicle	—	16.5 ± 1.8	6.2 ± 1.0
Vehicle	—	PEA	10.0	5.1 ± 1.1[a]	1.0 ± 0.2[a]
Diazepam	1.0	Vehicle	—	18.0 ± 2.3	7.1 ± 1.3
Phenibut	100.0	Vehicle	—	17.2 ± 1.7	6.1 ± 1.2
Phenibut	200.0	Vehicle	—	18.4 ± 2.2	5.3 ± 0.6
Diazepam	1.0	PEA	10.0	12.7 ± 2.1[b]	4.5 ± 1.1[b]
Phenibut	100.0	PEA	10.0	6.8 ± 0.9	2.0 ± 0.4
Phenibut	200.0	PEA	10.0	7.3 ± 1.2	1.9 ± 0.5
Conflict situation test (dark-light chamber)				Time spent in light chamber (sec) mean ± SE	Number of transitions mean ± SE
Vehicle	—	Vehicle	—	40.2 + 6.8	8.1 + 1.2
Vehicle	—	PEA	10.0	191.3 + 20.4[a]	2.5 + 0.3[a]
Diazepam	1.0	Vehicle	—	43.1 + 7.2	10.2 + 1.3
Phenibut	100.0	Vehicle	—	42.6 + 7.1	8.7 + 1.4
Phenibut	200.0	Vehicle	—	35.0 + 5.3	9.1 + 0.9
Diazepam	1.0	PEA	10.0	67.8 + 9.9[b]	6.7 + 0.8[b]
Phenibut	100.0	PEA	10.0	103.2 + 2.5[c]	3.8 + 0.4[d]
Phenibut	200.0	PEA	10.0	88.5 + 10.6[b]	5.5 + 0.7[b]

Group of 8 C57BL/6 mice were used.
[a]$p < 0.001$ (vs. control); [b]$p < 0.001$ (vs. group treated with PEA); [c]$p < 0.01$; [d]$p < 0.05$.

(Wolf and Mosnaim 1983) contains evidence for the involvement of PEA in mania, depression, and paranoid schizophrenia.

Compared with diazepam, phenibut is a weak anxiolytic drug when used in doses of 250 mg thrice daily (Khaunina and Lapin 1989). Inefficiency of phenibut in the social isolation test and low efficiency (according to doses antagonizing anxiogens in mice) versus diazepam in other tests used in the present study are in agreement with its relatively weak antianxiety action in patients. However, phenibut is particularly effective in asthenic and asthenic–depressive syndromes where diazepam is not. This is in agreement with both its experimental and clinical profile of a nootropic drug. Numerous nootropillike effects of phenibut have been reported in animals and patients (see Khaunina and Lapin 1989). No side effects of phenibut except dizziness in some geriatric patients have been reported after about 25 years of practical use of the drug. This is not surprising if one takes into account that phenibut is a close derivative of GABA, the endogenous metabolite of the brain. However, there is a loss implicated in that advantage, namely the rather rapid (3–4 weeks) tolerance to the therapeutic action of phenibut which requires increased dosage if the treatment is to be continued over 4 weeks. Our assumption that PEA is an endogenous anxiogen is in agreement with data on anxiety as a typical symptom of

amphetamine (methyl-PEA) intoxication and addiction (Schiorring 1981) and on increased excretion of PEA during stress in animals (Snoddy et al. 1985) and humans (Paulos and Tessel 1982). Concentration measurements of PEA in body fluids of anxious patients are highly desirable in order to examine a probable relationship between anxiety and PEA.

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