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Antagonism by CPP, (±)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic Acid, of β-Phenylethylamine (PEA)-Induced Hypermotility in Mice of Different Strains

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LAPIN, I. P. *Antagonism by CPP, (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, of β-phenylethylamine (PEA)-induced hypermotility in mice of different strains.* PHARMACOL BIOCHEM BEHAV **55**(2) 175–178, 1996.—In male C57BL/6, BALB/c, and SHR (bred from Swiss) mice, pretreatment with (±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), a competitive antagonist of *N*-methyl-D-aspartate (NMDA) receptor, attenuated the hyperlocomotion induced by β-phenylethylamine (PEA). This effect of CPP was blocked by intracerebroventricularly (ICV) administered NMDA (0.2 nM). CPP did not alter the hyperlocomotion induced by *d*-amphetamine. PEA rarely inhibited spontaneous motor activity in those strains. Two other competitive antagonists of NMDA, 2-amino-5-phosphonopentanoic acid (AP-5) and 2-amino-7-phosphonoheptanoic acid (AP-7), ICV at doses of 0.01 − 0.1 μg, were ineffective. The noncompetitive antagonists of NMDA, dizocilpine (MK-801) and phencyclidine, at subthreshold doses of 0.1–0.5 mg/kg, potentiated the stimulant effect of PEA. In earlier studies we also observed antagonism between CPP and PEA in NIH-Swiss mice, a strain in which PEA inhibits locomotion. Relationships between the stimulant and the anxiogenic effects of PEA are discussed. **Copyright © 1996 Elsevier Science Inc.**

Phenylethylamine (PEA) CPP Dizocilpine (MK-801) Phencyclidine NMDA Locomotion Mice Strains

DETAILED information on the effective dosage of *N*-methyl-D-aspartate (NMDA) agonists and antagonists in NIH-Swiss mice (10,11) prompted us to use this strain in our studies on the NMDA receptor system. Although it is generally accepted that beta-phenethylamine (PEA) produces hyperactivity in both mice (1,5,7) and rats (2,3), we found that PEA paradoxically suppressed both horizontal and vertical components of the spontaneous activity of NIH-Swiss mice in a dose-dependent manner as automatically recorded with a Digiscan Animal Activity Monitor (8). Indeed, at the PEA doses (25, 50, and 100 mg/kg, IP) and observation times (5, 15, and 30 min) used, no evidence for stimulation of activity could be detected. Moreover, CPP, ((+)-3-(2-carboxypiperazin-4-yl)-1-phosphonic acid), a competitive antagonist of the NMDA receptor, very selectively antagonized this inhibitory action of PEA. It seemed important to determine whether CPP could also antagonize PEA-induced hyperactivity. The present article reports on the effects of CPP and other NMDA ligands on the PEA-induced changes in the locomotor activity of strains responding to PEA with hyperactivity (5,7).

METHOD

Animals

Male C57BL/6, BALB/c, and SHR (bred from Swiss mice) from Rappolovo farm (near St. Petersburg) weighing 20–22 g and about 8 weeks old, were used. Animals were housed in groups of 35–40 and received standard diet. In the laboratory, mice were kept in groups of eight in 20 × 15 × 10 cm. cages. Earlier (7) these mouse strains had been used in studies of anxiety testing PEA as a putative endogenous anxiogen.

Motor Activity

The locomotion and rearings of individual mice were measured in two chambers (20 × 15 × 10 cm and 24 × 35 × 18

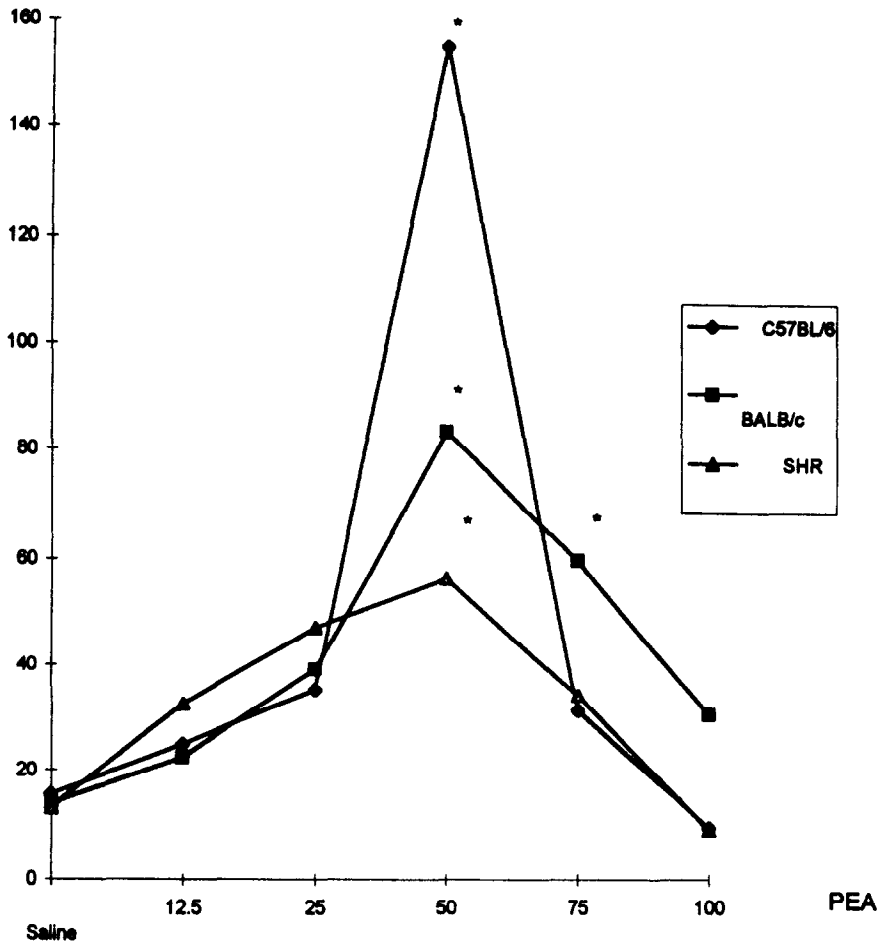

FIG. 1. Dose–response curves for PEA-induced hyperlocomotion in three strains of mice: C57BL/6, BALB/c, and SHR. Doses (IP) of PEA in mg/kg. Ordinate: counts of the actometer. $*p < 0.05$ (comparison with saline in the same strain).

cm) as described elsewhere (5). Early studies (5,7,9) used the smaller chamber to measure PEA-induced hyperactivity. The larger chamber was introduced in this study both to see if it improved the occurrence of PEA-induced hyperactivity and to assess the influence of chamber size on results. The duration of the test period was 5 min. Experiments were performed between 1000 and 1500 h. The illumination within the chambers was constant-60 (in corners) and 100 (at center) lx.

Drugs

CPP, dizocilpine hydrogen maleate (MK-801), 2-amino-5-phosphonopentanoic (AP-5), and 2-amino-7-phosphonoheptanoic (AP-7) acids and *N*-methyl-D-aspartic acid (NMDA) were from Research Biochemical Inc. (RBI, Natick, MA), PEA and *d*-amphetamine sulfate-from Sigma Chemical Co. (St. Louis, MO), phencyclidine (PCP) from the Research Technology Branch, National Institute on Drug Abuse (Research Triangle Park, NC). All drugs were freshly dissolved in saline and administered either intraperitoneally (IP) in a volume of 1% of body weight or intracerebroventricularly (ICV) in a volume of 0.005 ml. Drugs were injected ICV to conscious mice by a semiautomatic apparatus as described elsewhere (6). All solutions were pH of 6.5–7.0. Controls were treated with the same volumes of saline.

Procedure

PEA and *d*-amphetamine were injected 5 min before testing. This interval is optimal for recording the stimulant effect of PEA (1,5). The maximal effect of *d*-amphetamine was observed 15–20 min after injection. However, 5 min was used for both drugs to compare them. Equipotent doses of both drugs were used. Pretreatments with CPP, PCP, and MK-801 were made 15 min before PEA or *d*-amphetamine. The maximal anticonvulsant effect of CPP, PCP, and MK-801 in mice after IP administration occurs in 15 min (10,11). NMDA was injected ICV 5 min after CPP.

Statistical Analysis

Data are presented in the tables as the mean ± SEM. Comparisons of groups were made by the Mann–Whitney *U*-test and the Dunnet formula (ANOVA). Both procedures gave essentially identical results.

RESULTS

PEA stimulated exploratory locomotion in a dose-dependent manner in all three strains of mice (Fig. 1). The dose–response curve was cupola-like in shape; decreased hyperactiv-

TABLE 1

REPRODUCIBILITY OF THE STIMULANT AND THE INHIBITORY EFFECT OF PEA

	Mice		
	C57BL/6	BALB/c	SHR
Locomotor hyperactivity*	17/17	2/2	11/16
Inhibition of locomotion**	0/4	2/5	2/6

Columns 2, 3, and 4 indicate number of experiments with statistically significant difference with control/total number of experiments.

Sixteen mice in each group.

Groups are compared according to chi-square method.

* PEA (50 mg/kg) 5 min prior to registration of motor activity.

** The same dose of PEA 15 and 30 min (the only intervals where the inhibition of locomotion was observed) prior to registration of motor activity.

TABLE 3

EFFECT OF NMDA ON THE ANTI-PEA EFFECT OF CPP

	Treatment		Locomotion
	I—IP	II—ICV	III—IP
I Vehicle	Vehicle	Vehicle	14.4 ± 2.5
II Vehicle	Vehicle	PEA	92.5 ± 8.1*
III CPP	Vehicle	Vehicle	13.9 ± 2.4
IV Vehicle	NMDA	Vehicle	15.0 ± 1.6
V CPP	NMDA	Vehicle	12.6 ± 1.8
VI Vehicle	NMDA	PEA	97.9 ± 7.3
VII CPP	Vehicle	PEA	30.1 ± 4.8**
VIII CPP	NMDA	PEA	93.5 ± 8.7***

C57BL/6 mice (groups of 12 mice).

Doses: CPP—10 mg/kg, PEA—50 mg/kg, NMDA—0.2 nM.

*Significant difference with Group I, **with Group II, ***with Group VII.

ity was associated with the appearance of stereotypies. The pattern was similar for the effects of amphetamine (not shown in the figure). The results also show that the stimulant effect of PEA and *d*-amphetamine was most pronounced in the smaller chamber.

The reproducibility of PEA-induced hyperlocomotion was absolute in the C57BL/6 strain, near absolute in BALB/c mice, and reliable in SHR (Table 1). An inhibitory effect of PEA on locomotion was rarely seen with BALB/c and SHR mice and never with C57BL/6 (Table 1).

CPP, at a dose of 10 mg/kg (1 and 5 mg/kg were essentially ineffective), attenuated the stimulatory effect of PEA on locomotion (Table 2). CPP had no effect on *d*-amphetamine-induced locomotor stimulation (Table 2).

NMDA (0.2 nM, ICV) blocked CPP inhibition of PEA-induced hyperactivity (Table 3). ICV administered CPP, AP-5, and AP-7 (all at 0.01 to 0.1 μg) did not affect PEA stimulation of the locomotion of C57BL/6 or SHR mice (BALB/c was not tested). The same doses of CPP and AP-7, but not AP-5, had an anticonvulsant effect against pentylenetetrazole (60 mg/kg IP); seizure latency was prolonged from 8.1 ± 1.1 to 23.7 ± 2.4 and 16.5 ± 3.3 min, respectively (at a dose of 0.1 μg). The number of seizures (25 ± 0.2) and lethality (6 or 7 of 32 mice) was unchanged.

Pretreatment with subthreshold doses of MK-801 (0.1 mg/kg) and PCP (0.5 mg/kg) potentiated the stimulant effect of PEA and *d*-amphetamine (Table 4). In controls the threshold stimulant doses of MK-801 and PCP were 0.25 and 2 mg/kg, respectively.

DISCUSSION

The stimulatory effect of PEA on locomotion, which has been repeatedly reported in the literature (1–3,5,7,9), was confirmed in this study on three strains of mice: C57BL/6, BALB/c, and SHR. The most effective dose of PEA was 50 mg/kg (Fig. 1). PEA had least effect on SHR mice (Fig. 1). Perhaps the failure to observe PEA stimulation of the motor activity of NIH-Swiss mice (8), which are genetically related to SHR, suggests a genetic peculiarity in that strain.

Behavioral antagonism between CPP and PEA-induced locomotion was independent of the direction of the PEA effect; CPP inhibited both PEA-stimulatory and inhibitory effects. This antagonism was rather selective for PEA because in a previous study (8) CPP did not affect inhibition of motor activity by diazepam, haloperidol, baclofen, or phenibut, while in this study it did not alter locomotor stimulation by amphetamine. The inhibitory effect of PEA (on NIH-Swiss mice in our previous study—8) was not altered by pretreatment with

TABLE 2

EFFECT OF PRETREATMENT WITH CPP ON THE PEA- AND *d*-AMPHETAMINE-INDUCED HYPERLOCOMOTION IN THREE STRAINS OF MICE

Treatments (IP)			Strains		
First		Second	C57BL/6	BALB/c	SHR
Vehicle	+	Vehicle	13.6 ± 2.1	20.8 ± 3.8	16.4 ± 3.6
Vehicle	+	PEA	98.2 ± 10.3*	169.2 ± 22.3*	88.9 ± 14.9*
CPP	+	Vehicle	12.4 ± 2.7	28.6 ± 2.3	19.5 ± 4.4
CPP	+	PEA	39.0 ± 6.7**	8.0 ± 3.5**	27.5 ± 10.8**
Vehicle	+	*d*-amphetamine	81.3 ± 5.2*	66.2 ± 4.7*	54.7 ± 6.8*
CPP	+	*d*-amphetamine	78.8 ± 8.3	62.6 ± 5.9	57.3 ± 9.4

Doses: PEA 50 mg/kg in all strains; CPP—10 mg/kg (in C57BL/6 and SHR) and 1 mg/kg (in BALB/c; in this strain doses of 5 and 10 mg/kg induced hyperlocomotion in control); *d*-amphetamine—10 mg/kg in all strains. Significance of the difference: *with Group I, **with group II.

TABLE 4

EFFECT OF NONCOMPETITIVE ANTAGONISTS OF NMDA RECEPTOR, DIZOCILPINE (MK-801) AND PHENCYCLIDINE (PCP) ON THE STIMULANT EFFECT OF PEA AND *d*-AMPHETAMINE IN C57BL/6 MICE

Pretreatment		Treatment		
IP	mg/kg	IP	mg/kg	Locomotion
Vehicle	—	Vehicle	—	23.6 ± 3.9
Vehicle	—	PEA	50	101.0 ± 15.4*
Vehicle	—	*d*-amphetamine	10	143.8 ± 13.8*
MK-801	0.1	Vehicle	—	26.3 ± 2.4
MK-801	0.1	PEA	50	171.6 ± 24.3**
MK-801	0.1	*d*-amphetamine	10	228.1 ± 32.7***
PCP	0.5	Vehicle	—	22.4 ± 3.6
PCP	0.5	PEA	50	201.2 ± 22.3**
PCP	0.5	*d*-amphetamine	10	305.5 ± 41.7***

Significant difference: * with Group I, ** with Group II, *** with Group III.

the noncompetitive NMDA receptor antagonists MK-801, PCP, or PCA (1-phenylcyclohexylamine) nor was PEA-induced hyperactivity (in this study) blocked by haloperidol, baclofen, or phenibut. It seems reasonable to assume then that CPP antagonizes PEA at its initial site of action, the PEA receptor.

The role of the NMDA receptor in CPP inhibition of PEA-induced locomotion is ambiguous. A role for the NMDA receptor is suggested by the finding that NMDA itself antagonizes the effects of CPP on PEA-induced hyper- or hypolocomotion. On the other hand, two other competitive inhibitors of NMDA, AP-5 and AP-7, were ineffective. Further, CPP itself was ineffective when administered ICV. However, AP-7 was only slightly effective in our experiments with pentylenetetrazole in this study and AP-5 was ineffective. In other studies (4) AP-7 and AP-5 (ED_{50} = 64.8 mg/kg IP) antagonized NMDA (ICV)-induced seizures in mice in a dose-dependent manner. However, NMDA-induced hyperactivity was not altered. The authors suggest the existence of two subtypes of NMDA receptors in mice, one related to seizures and the other related to hyperactivity. In rats AP-7 (ICV) attenuated both seizures and hyperactivity induced by NMDA. However, it is difficult to compare the data on rats and mice because AP-7 was given IP to the rats and ICV to the mice.

The efficacy of CPP against PEA when given IP compared to its ineffectiveness when given ICV suggests it acts at a peripheral site. How such a peripheral antagonism alters PEA-induced hyperlocomotion is puzzling. However, inactivity of a drug administered ICV only suggests the drug does not act a sites adjacent to brain ventricles. Systemically administered CPP might well act centrally at other sites despite its poor penetrance through the blood–brain-barrier. ICV AP-5 and AP-7 may also have been ineffective against PEA for analogous reasons. They were not given systemically because they do not penetrate to brain.

Recent unpublished experiments using an elevated plus-maze showed CPP attenuated the anxiogenic effect of PEA in mice. In animal models of anxiety, PEA has a pharmacological profile like that of standard anxiogens such as pentylenetetrazole, caffeine, and yohimbine (7,9). It is noteworthy that these standard anxiogens all have convulsant actions on mice at doses 10 times higher than their anxiogenic dose. The same is true for PEA. The significance of this tenfold relationship has been discussed in detail elsewhere (6) as has the suggestion that elevated central excitability may be a precondition for the genesis of anxiety.

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