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BRIEF COMMUNICATION

Local Application of β-Phenylethylamine to the Caudate Nucleus of the Rat Elicits Locomotor Stimulation

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DOURISH, C T *Local application of β-phenylethylamine to the caudate nucleus of the rat elicits locomotor stimulation.* PHARMACOL BIOCHEM BEHAV 22(1) 159–162, 1985 —The behavioural effects of bilateral injections of β-phenylethylamine (PEA) into the caudate nucleus of male rats were examined PEA in doses of 200 and 300 μg increased locomotion with maximal stimulation being evident 15–25 min after injection. In addition 300 μg PEA increased rearing 20–25 min post injection and produced increases in sniffing This is the first report of a behavioural stimulant effect of intracranially administered PEA without concurrent monoamine oxidase inhibition The data suggest that the stimulant action of systemically administered PEA may be mediated, at least in part, by striatal mechanisms

β-Phenylethylamine Caudate nucleus Locomotor activity Rearing Rat

β-PHENYLETHYLAMINE (PEA) is an endogenous constitutent of mammalian brain tissue which may act as a neurotransmitter or neuromodulator [2] In rodents and man, the highest concentrations of the amine are found in the caudate nucleus, a brain region which is important in the control of movement [2,5] PEA is very similar in structure to amphetamine (α-methyl PEA) and it has been claimed that endogenous PEA could play a major role in mediating the central actions of amphetamine [1]

Recent studies in this laboratory have demonstrated that systemically administered PEA can mimic the actions of amphetamine on spontaneous motor activity in rodents [8]. Both compounds elicit stereotyped behaviour in rats which is generally preceeded by a phase of locomotor stimulation [4,9] However, in order to elicit behavioural stimulation, PEA must be given in much larger doses than amphetamine since PEA is rapidly metabolised by the enzyme monoamine oxidase (MAO) to which amphetamine is resistant [2]. Striatal dopaminergic mechanisms have been strongly implicated in the mediation of the stimulant and stereotypic effects of PEA and amphetamine [3,5]. Thus, it has been shown that intrastriatal application of 100 μg amphetamine elicits hyperactivity and stereotyped behaviour in rats pretreated with an MAO inhibitor [6]. Surprisingly, under the same experimental conditions, it was claimed that intrastriatal application of 100 μg PEA had no effect on behaviour [6] It is important to note however, that behavioural assessment in the Costall study was carried out using a rating scheme for hyperactive/stereotyped behaviour. The limitations and lack of sensitivity of such methods for assessing motor activity have been repeatedly demonstrated [12,16]. In addition, it is advantageous to examine the effects of PEA without MAO inhibitor pre-treatment since MAO inhibitors given alone can induce behavioural stimulation (see [4,10] and references therein). In the present study, the effects of intrastriatal PEA administration were assessed using a novel photobeam system which records total horizontal activity, ambulatory movements and rearing [10]. In addition, individual elements of behaviour were recorded by direct observation.

METHOD

Animals

The subjects were adult male Sprague-Dawley rats obtained from Charles River, Montreal, Quebec, Canada. They were housed individually in a room in which temperature was maintained at 21°C, under a 12 hr dark-light cycle (lights on 6 a.m.). Tap water and standard food pellets were continually available and the rats weighed 300–350 g at testing.

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Surgery

The rats were anaesthetized with sodium pentobarbital (Nembutal 40–50 mg/kg, IP), placed in a stereotaxic frame, and implanted bilaterally with stainless steel guide cannulae (22 gauge, Plastic Products Co , Roanoke, VA) aimed at the caudate nucleus. The stereotaxic coordinates were AP +0 11 cm, L + or −0.2 cm, V −0.55 cm chosen on the basis of previous studies in this laboratory using the atlas of Konig and Klippel [14] with bregma and the skull surface as reference points The cannulae were secured to the skull using 3 stainless steel screws and dental acrylic The skin was sutured around the implant and the animals were returned to their home cages for a minimum one week recovery period before testing

Apparatus

Testing was conducted in 4 individual Perspex cages (40 cm square, 23 cm high) positioned in automatic activity recording devices (Opto Varimex Minor, equipped with option VS, Columbus Instruments, Columbus, OH) These photocell devices measured total horizontal activity, ambulation and rearing. Total horizontal activity (including grooming, scratching, head swaying, tail movements, etc.) was determined by the interruption of any one of 12×12 infrared photobeams (3 cm apart) in any order Ambulation (locomotion), on the other hand, was determined by the interruption of consecutive photobeams. Rearing was determined by the interruption of a separate series of 12 photobeams (3 cm apart) which were suspended (15 cm above the cage base) from the walls of the cage Interruption of any photobeam produced a 1 msec pulse which was counted by a microprocessor/Apple II plus microcomputer system (see [10] for further details)

Procedure

The injection assembly consisted of two 30 gauge internal cannulae (Plastic Products Co., Roanoke, VA) each of which was connected by PE-10 tubing to a 5 μl syringe (SGE, Melbourne, Australia) For injection each rat was removed from the cage and the injection cannulae lowered into the brain tissue on both sides to a depth of 0.5 mm below the tips of the guides The bilateral injection solutions of β-phenylethylamine hydrochloride (Sigma Chemical Co , St Louis, MO) or the saline vehicle were administered manually in a volume of 1 μl per cannula over a period of 1–2 min The cannulae were left in situ for an additional 30 sec to allow for diffusion of the solution away from the tip of the injection cannulae The animal was then placed in the test cage (without prior habituation) for a 30 min test Activity data were recorded automatically at 5 min intervals by the microcomputer and, in addition, an observer continuously observed the animals and at 5 min intervals recorded the frequency of occurrence of grooming, sniffing and head movements on a five point scale. (0=absent, 1=mild intensity or present 1–2 times during observation; 2=moderate intensity or present 3–4 times during observation; 3=high intensity or present 5 or more times during observation; 4=severe or present for prolonged periods).

Experimental Design and Statistics

There were three treatment conditions which consisted of saline, 200 μg PEA and 300 μg PEA and each of the seven subjects was tested under all three conditions in a counterbalanced order

Activity counts were analysed by two factor ANOVA (drug × time) with repeated measures on both factors [18] Individual group differences were located using the two tailed *t* test for correlated means Observational data were analysed by Wilcoxon test [17]

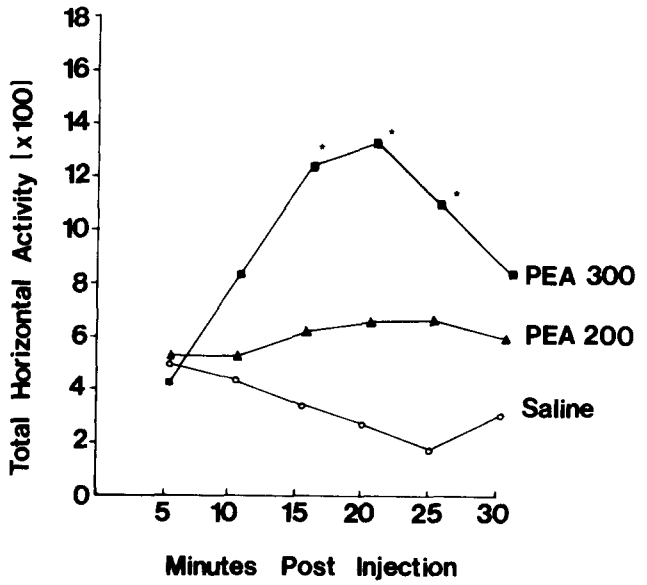

FIG 1 Time course of the effects of 200 and 300 μg PEA injected bilaterally into the caudate nucleus on total horizontal activity in rats (n=7) Significant differences were determined by 2 tailed correlated *t* test following a significant ANOVA result *$p<0.05$ vs saline treatment

Histology

Injection loci were determined at the end of the experimental schedule by injecting (under deep anaesthesia) 1% fast green dye, using the same technique as for drug administration The animals were sacrificed and the brains removed and fixed in formaldehyde (20%, v/v) for at least 12 hours Subsequently, the brains were sectioned at 60 μm on a freezing microtome and the slices mounted on glass slides and inspected microscopically The positions of the tips of the cannulae were verified with reference to the atlas of Konig and Klippel [14] and only animals in which injection tracks entered the caudate nucleus (and not the nucleus accumbens) were used in the analysis of results

RESULTS

It is apparent from Fig 1 that intracaudate PEA application increased total horizontal activity. ANOVA confirmed a significant effect of PEA, $F(2,18)=4.29$, $p<0.05$, and a significant interaction of drug and time factors, $F(10,90)=3.34$, $p<0.01$ Further analysis by correlated *t* test revealed that 300 μg PEA significantly increased total horizontal activity 15–25 min after injection (Fig 1) Similarly, ANOVA revealed a significant stimulant effect of PEA on ambulation, $F(2,18)=4.75$, $p<0.05$, and a significant interaction between drug and time factors on this measure, $F(10,90)=2.71$,

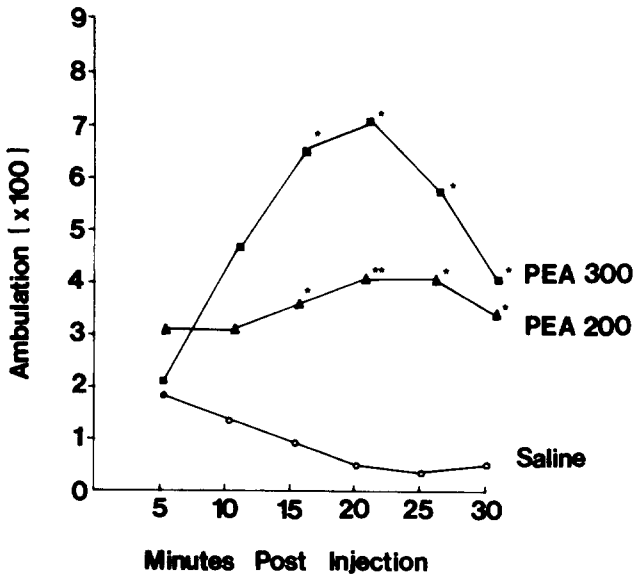

FIG 2 Time course of the effects of 200 and 300 μg PEA injected bilaterally into the caudate nucleus on ambulation in rats **$p<0.01$ vs saline treatment Other details are as in Fig 1

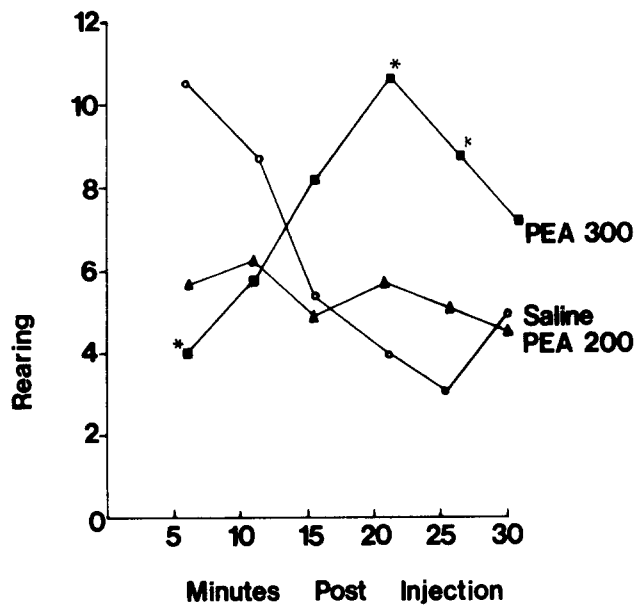

FIG 3 Time course of the effects of 200 and 300 μg PEA injected bilaterally into the caudate nucleus on rearing in rats Details are as in Fig 1

$p<0.01$. Drug effects on ambulation were more pronounced than on total horizontal activity and individual t test comparisons revealed that 200 and 300 μg PEA increased ambulation 15–30 min after injection (Fig. 2). The effects of PEA on rearing were complex (Fig 3) ANOVA revealed no significant main effects of PEA or time but a significant interaction between these two factors, $F(10,90)=2.01$, $p<0.05$. After saline treatment animals exhibited high levels of rearing at the start of the test which declined from 15–30 min after injection. In contrast, rats given 300 μg PEA reared less than controls 5 min after injection but exhibited increased rearing 20–25 min after drug treatment (see Fig. 3).

TABLE 1

EFFECTS OF BILATERAL INTRASTRIATAL INJECTION OF PEA ON VARIOUS COMPONENTS OF SPONTANEOUS MOTOR ACTIVITY IN RATS

Treatment	Grooming	Stereotyped Head Movements	Sniffing
Saline	8	0	17
PEA 200 μg	8	0	22
PEA 300 μg	5	0	24*

Each score is median of 7 rats rated on a 0–4 scale during a 30 min test Ratings were recorded at 5 min intervals (maximum score is $6\times4=24$) Significant differences were determined by 2 tailed Wilcoxon test *$p<0.02$

Analysis of the observational data by Wilcoxon test revealed that 300 μg PEA significantly increased sniffing (see Table 1). Sniffing was also increased by 200 μg PEA but this effect did not achieve statistical significance. PEA had no effect on grooming and did not elicit stereotyped head movements (see Table 1).

DISCUSSION

Bilateral application of 200 and 300 μg PEA to the rat caudate nucleus elicited significant increases in ambulation This is the first reported evidence of a stimulant action of intracranially administered PEA, without concurrent MAO inhibition, and contrasts with a previous failure to observe locomotor stimulation in rats pre-treated with an MAO inhibitor and injected intrastriatally with 100 μg PEA [6]. This discrepancy can probably be attributed to differences in drug dosage and methodological differences in the two studies. Costall *et al* [6] used a global rating scale to assess hyperactive/stereotyped behaviour, a method which has subsequently been shown to produce misleading results [12,16]. In the present study, the use of a photobeam system which is sensitive to ambulatory movements, coupled with direct observation of individual elements of behaviour revealed that intrastriatal PEA application significantly increased ambulation and sniffing. Intrastriatal application of 300 μg PEA also influenced rearing and this effect was biphasic consisting of an initial depression and a subsequent elevation of this behavioural response. The cause of this biphasic response is unclear at present

The PEA-induced stimulant effects on ambulation and sniffing observed in the present study closely resemble those produced by 12 5–25 0 mg/kg PEA given IP [4,9] Therefore, it appears likely that the stimulant action of systemically administered PEA may be mediated, at least in part, by striatal mechanisms. In this regard, it is noteworthy that 12.5–50.0 mg/kg PEA injected IP in rats produces a thousand fold increase in striatal PEA concentrations, during the first 15 minutes following drug treatment ([11] and L. E. Dyck, personal communication). Although the striatum seems to play an important role in mediating the PEA response, this involvement is by no means exclusive The nucleus accum-

bens also appears to be involved in the expression of PEAs' stimulant effects and 12.5–50.0 μg PEA applied bilaterally to the accumbens of rats pre-treated with the MAO inhibitor nialimide produces locomotor stimulation in a photocell cage [7,13]. It is likely that similar stimulant effects to those observed in the present study could be elicited by the intracaudate application of lower PEA doses in rats pre-treated with an MAO inhibitor, since PEA is catabolized extremely rapidly by type B MAO [2]

The present demonstration of PEA-induced locomotor stimulation after intracaudate injection is consistent with the observation of Costall *et al* [6] that intrastriatal application of amphetamine produces locomotor stimulation in MAO inhibitor-pretreated rats, and thus extends the evidence of a striking similarity in the unconditioned behavioural effects of the two compounds. The stimulant effects of PEA and amphetamine in the striatum are probably dopamine-mediated and this interpretation is supported by the recent observation that both compounds facilitate the release and block the reuptake of dopamine in the striatum of freely-moving rats [15]

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