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Deuterium Substitution Enhances the Effects of β-Phenylethylamine on Spontaneous Motor Activity in the Rat

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DOURISH, C. T., A. J. GREENSHAW AND A. A. BOULTON. *Deuterium substitution enhances the effects of β-phenylethylamine on spontaneous motor activity in the rat.* PHARMACOL BIOCHEM BEHAV **19**(3) 471–475, 1983.—The effects of β-phenylethylamine (PEA) and α,α,β,β-tetradeutero-β-phenylethylamine (deuterated PEA) on spontaneous motor activity and conditioned taste aversion learning in the rat were examined. The intensity and duration of certain behavioural components elicited by PEA, namely, sniffing, headweaving, splayed hindlimbs and hyperreactivity, were significantly increased by deuterium substitution. In contrast, deuteration had no effect on the ability of PEA to elicit a conditioned taste aversion. The potentiation of the amine's effects on activity seemed to be directly related to the longer persistence of PEA in the brain due to the kinetic isotope effect since it appears that tetra-deuterated PEA is a poorer substrate for monoamine oxidase than the protonated amine.

β-Phenylethylamine Deuterium substitution Spontaneous activity Conditioned taste aversion

β-PHENYLETHYLAMINE (PEA) is an endogenous trace amine which has been identified and quantified in the human and rodent brain [10,19]. It is similar in structure to the psychomotor stimulant amphetamine and the two compounds have been reported to possess a number of pharmacological [13] and behavioural properties in common [8,22]. It has been suggested by some authors that PEA may be an endogenous amphetamine [20,24] whereas Boulton [4] has proposed that it, like many of the other trace amines, may function as a neurotransmitter or neuromodulator. Recently some doubt has been cast on its proposed endogenous amphetamine status by reports of differential effects of PEA and amphetamine in certain behavioural paradigms [16,17]. A recent study in this laboratory has suggested that PEA and amphetamine possess quite different aversive stimulus properties [15]. In a conditioned taste aversion (CTA) experiment it was demonstrated that behaviourally active doses of PEA administered acutely or chronically were ineffective in inducing a CTA to saccharin, whereas amphetamine produced a pronounced CTA after a single injection. It has been proposed that the duration of action of psychoactive compounds may be an important factor in the CTA paradigm [14]. Since PEA is a specific substrate for type B monoamine oxidase [25] and as such exhibits an extremely short half-life in mammalian tissue [11,23], it seems possible that the failure of PEA to produce a CTA may be related to the compound's short duration of action. Recently it has been reported that specific side-chain substitution with deuterium in the biogenic amines PEA, *para*-tyramine, *meta*-tyramine, dopamine and serotonin, which are catabolized by monoamine oxidase decreases their oxidative deamination [12, 26, 27]. It appears that the probable reason for this is that the carbon-deuterium bond is more stable than the carbon-hydrogen bond and thus more difficult to break by monoamine oxidase (see review by Blake *et al.* [3]). Consequently, in order to investigate further the role of PEA in behaviour we have examined the effects of tetra-deuterated and proteo (i.e., nondeuterated) PEA in the CTA paradigm and on spontaneous activity in the rat.

METHOD

Animals

The subjects were 21 male Wistar rats (200–250 g) obtained from Charles River Canada, Montreal. They were housed in groups under a 12 hr dark/light cycle at a temperature of 20±1°C. Food was available ad lib in the home cages. Water was available according to the experimental procedure.

Apparatus

Testing was conducted in individual Plexiglas cages (40 cm square, 23 cm high) positioned in photobeam-based activity recording devices, which measured horizontal and vertical activity (Opto-Varimex Minor, Columbus Instruments, Columbus, OH). The activity devices were controlled by a microprocessor/microcomputer system (see [9] for details).

TABLE 1

RATING SCALE USED IN THE OBSERVATIONAL ANALYSIS OF COMPONENTS OF SPONTANEOUS ACTIVITY

Score	Frequency
0	Absent
1	Mild intensity or present 1–2 times during time sample.
2	Moderate intensity or present 3–4 times during time sample.
3	High intensity or present 5 or more times during time sample.
4	Severe or present for prolonged periods during time sample.

TABLE 2

DEFINITION OF BEHAVIOURAL COMPONENTS SCORED IN THE OBSERVATIONAL ANALYSIS

Behavioural Component	Description
Sniffing	Distinctive pattern of air inspiration accompanied by vibrissae movements.
Headweaving	Repetitive side-to-side movements of the head, often in one location in the cage.
Splayed Hindlimbs	A dramatic extension of the hindlimbs causing a flattening of the body posture.
Hyperreactivity	Startle response to a pencil tap on the cage top.
Grooming	Purposeful licking and cleaning of the body.
Forepaw Padding	Repetitive placing movements of the forepaws.
Rearing	Defined as both front paws off the ground (also measured automatically as vertical activity).

Procedure

The animals were water-deprived for 23 hours and subsequently given access to two water bottles in the test cages for a period of 30 min per day, for three days. The volume of water consumed by each animal was measured to the nearest 0.5 ml. After the third 30 min drinking session the animals were randomly assigned to three groups of seven. These groups were then adjusted so that the final groups were matched for daily water intake. Each of these groups was allocated to one of the three treatment conditions which were 50 mg/kg PEA, 50 mg/kg deuterated PEA and saline. The 50 mg/kg dose was chosen on the basis of previous studies since it produces significant effects on unconditioned behaviour, without inducing toxic reactions (see Greenshaw and Dourish [15]). On the fourth test day each animal was given access to a 0.1% solution of sodium saccharin (Stanley Drug Products Ltd) in two 100 ml bottles for a period of 30 min. Immediately after the 30 min period of exposure to the saccharin solution each animal was injected according to the treatment allocation described above. The rats were observed continuously for a 30 min period following injection. At 5 min intervals the spontaneous activity of each animal was rated on a 5 point scale according to the intensity of behaviour observed (see Table 1). The behavioural responses rated were those which had been found in previous studies to be induced or increased by PEA (see Table 2 and [7]). For each of the subsequent three days the animals were exposed to two-bottle choice retention tests. During the retention tests each animal was simultaneously given access to one bottle containing water and one bottle containing a 0.1% solution of sodium saccharin for a period of 30 min. This procedure allowed an assessment of any change in the animals preference for saccharin while enabling the subjects to maintain their normal level of fluid intake.

Drugs

β-Phenylethylamine hydrochloride (PEA) was purchased from Sigma Chemical Co. (St. Louis, MO). $\alpha,\alpha,\beta,\beta$-Tetradeutero($d_4$)-$\beta$-phenylethylamine hydrochloride (deuterated PEA) was synthesized in our laboratory as described by Davis and Boulton [5]. The purity of the deuterated compound was determined by mass spectrometric analysis of the dansyl derivatives of the amines and found to be: 98.3% d_4, 1.3% d_3, 0.4% d_2. The drugs were administered intraperitoneally in a 0.9% saline vehicle in a volume of 1 ml/kg. The control group received an equivalent volume of 0.9% saline by the same route.

RESULTS

The animals' saccharin intake during each of the three retention trials was converted to a percentage of their total fluid intake on each of these days. The mean ($\pm$ SD) saccharin consumption (as a percentage of total intake) on retention day one was: saline 79.5$\pm$25.5, proteo PEA 71.7$\pm$25.7 deuterated PEA 62.5+28.4; on retention day two: saline 83.3$\pm$10.7, proteo PEA 80.4$\pm$18.4 deuterated PEA 87.8$\pm$6.8 and on retention day three: saline 86.8+7.7 proteo PEA 82.7+9.4 and deuterated PEA 83.3$\pm$19.3. It is apparent from these data that there was no systematic difference between the relative saccharin preferences of any of the three treatment groups. This was confirmed by two-way analysis of variance (treatments $\times$ trials) with repeated measures on the trials factor, which indicated a significant effect of trials, $F(2,36)=3.95$, $p<0.05$, but no effect of treatments, $F(2,18)=0.45$, $p>0.2$, and no significant interaction, $F(4,36)=0.77$, $p>0.2$. The significant trials effect appears to indicate that the animals tended to consume progressively more saccharin over days, presumably reflecting an attenuation of neophobia.

Since deuterium substitution increased the duration of action of PEA, the results of the various activity measures are displayed as scores for each of six time bins of 5 min during the 30 min test (Figs. 1 and 2). Two-way analysis of variance of horizontal activity (treatments and trials, see Fig. 1) with repeated measures on the trials factor, revealed a significant effect of treatments, $F(2,18)=5.94$, $p<0.05$, but no effect of trials and no interaction. Since there was no significant effect of trials, individual comparisons (2-tailed t-test for independent means, $\alpha=0.05$) were carried out on the pooled 30 min data for each treatment. This analysis revealed that horizontal activity was significantly increased by deuterated PEA compared to saline but not by the protonated drug.The apparent difference in horizontal activity between the protonated drug and saline (see Fig. 1) approached significance but did not reach criterion due to large

individual differences in response to PEA. There was no significant difference between the effects of the deuterated drug and the protonated drug on horizontal activity and, similarly, analysis of variance of vertical activity scores revealed no significant effects on rearing.

The data obtained from the observational analysis are displayed in Fig. 2A–E as total scores on the rating scale from the seven subjects in each treatment group during each 5 min bin (max=28). The scores for each behavioural component during each 5 min bin were analysed using the Kruskal-Wallis one-way analysis of variance. Where this test yielded a significant result, individual between-group comparisons were carried out using the Mann-Whitney U test (2-tailed). It can be seen from Fig. 2 that immediately after injection both deuterated and proteo PEA produced increases in the behavioural responses of sniffing, headweaving, splayed hindlimbs and hyperreactivity while virtually abolishing grooming. In addition, both compounds elicited occasional forepaw padding during this time.

There appeared to be a clear difference in the duration of the behavioural stimulation induced by deuterated and protonated PEA. By 15–20 min post-injection the responses of sniffing, headweaving, splayed hindlimbs and hyperreactivity elicited by the unsubstituted compound were not significantly different from control. In contrast, the intensity of the same responses produced by deuterated PEA at this time were still significantly greater than control and were also significantly greater than the responses produced by the protonated amine (see Fig. 2). It is also interesting to note (see Fig. 2E) that the appearance of grooming, which in our experience marks the end of the PEA syndrome, seemed to be later for deuterated PEA than for the undeuterated compound. By combining the observational data we determined that the duration of the behavioural syndrome induced by proteo PEA was approximately 15–20 min whereas the syndrome induced by deuterated PEA had a duration of approximately 20–25 min. This represents a difference in the duration of action of the two compounds which is in the order of 33%.

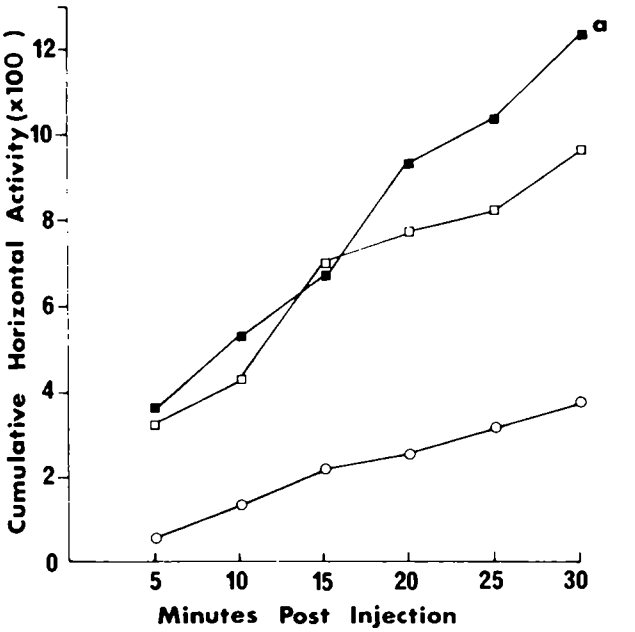

FIG. 1. The effect of proteo PEA and tetra-deuterated PEA on horizontal activity in the rat. Significant differences were determined by 2-tailed independent *t*-test ($\alpha=0.05$). a=significant difference from control. ○–○ saline; □–□ PEA 50 mg/kg; ■–■ deuterated PEA 50 mg/kg.

DISCUSSION

Deuterium substitution in the alkyl side-chain of PEA enhanced certain behavioural effects of the compound. Deuteration increased the potency of PEA's effects on unconditioned behaviour but did not augment the amine's ability to elicit a CTA to saccharin.

The unconditioned responses of stereotyped sniffing, headweaving, splayed hindlimbs, hyperreactivity and forepaw padding were produced by both proteo and deuterated PEA at a dose of 50 mg/kg. Concurrently, grooming was virtually abolished. This is consistent with the findings of a number of previous studies of the effects of PEA on spontaneous activity in rodents which have demonstrated that the amine elicits both dopamine-related and serotonin-related behavioural responses at this dose [6, 7, 21]. The intensity and duration of sniffing, headweaving, splayed hindlimbs and hyperreactivity were significantly greater after injection of deuterated PEA than after administration of the protonated amine. Similarly, deuterated PEA significantly increased horizontal activity (as measured by photobeam interruptions) whereas proteo PEA did not. In this respect, deuteration appeared to have a similar effect on spontaneous activity to that observed following administration of PEA in combination with a monoamine oxidase inhibitor (see [18]).

The potentiation observed in the present study can probably be attributed to the longer persistence of PEA in the brain due to the kinetic isotope effect. Recently Yu *et al.* [26,27] have shown that $\alpha,\alpha,\beta,\beta$-$d_4$-PEA is less easily oxidised by monoamine oxidase than proteo-PEA. Most interestingly, these authors have also demonstrated that α,α-d_2-PEA is even more resistant to deamination than $\alpha,\alpha,\beta,\beta$-$d_4$-PEA whereas β,β-d_2 substitution actually enhances PEA's catabolism by monoamine oxidase. Consequently a comparison of the behavioural effects of α,α-d_2-PEA and β,β-d_2-PEA might be quite illuminating. We have work currently in progress utilizing this approach.

The greater potency of deuterated PEA in producing increases in the frequency and duration of certain components of spontaneous motor activity is consistent with the observation that deuterium substitution in the alkyl side-chain of the monoamine-oxidase inhibiting-antidepressant phenelzine potentiates this drug's effects on unconditioned behaviour [9]. Similarly, it has been established for a number of years that deuterium substitution enhances the sympathomimetic effects of the endogenous trace amine *para*-tyramine [2]. In addition, a recent study by Beaton, Barker and Liu [1] demonstrated that deuterated N,N-dimethyltryptamine produced a greater disruption of operant responding, and had a longer duration of action than its undeuterated analog.

Although deuterium substitution increased PEA's duration of action in the present study by approximately 33% (as determined by effects on unconditioned behaviour), deuteration had no effect on the ability of PEA to elicit a CTA. It has been previously shown that increasing the duration of the effect of a pharmacological stimulus can augment its ability to induce a CTA [14]. Therefore, it is possible that in the present study the increase in the duration of action of

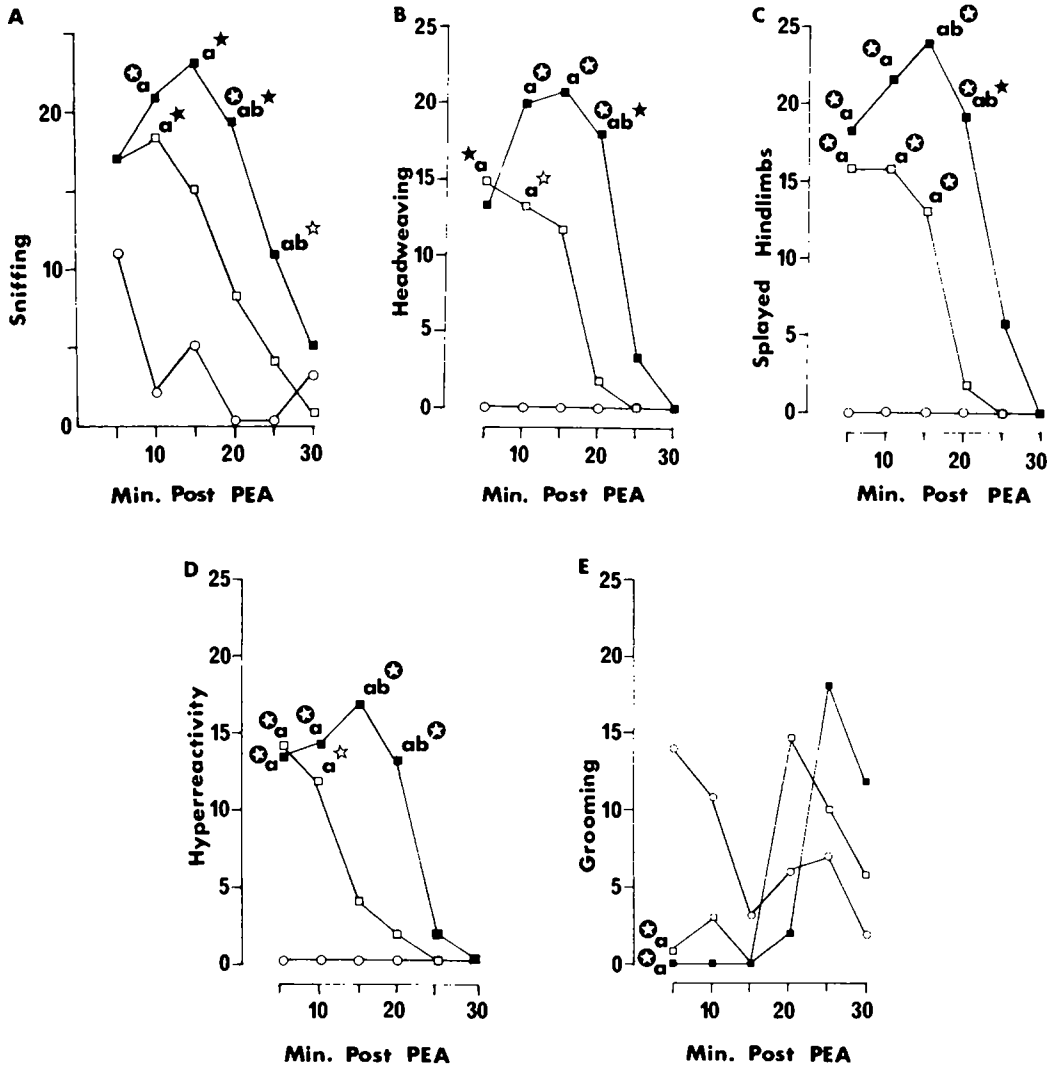

FIG. 2. The effect of proteo PEA and tetra-deuterated PEA on various components of spontaneous motor activity. Significant differences were determined by 2-tailed Mann Whitney U test (☆=$p<0.05$; ★=$p<0.02$; ✪=$p<0.01$). a=significant difference from saline; b=significant difference from proteo PEA. Other details are as described in Fig. 1. (A) sniffing; (B) headweaving; (C) splayed hindlimbs; (D) hyperreactivity; (E) grooming.

PEA produced by deuteration was simply insufficient to make the compound an effective stimulus for CTA learning.

In conclusion, we have demonstrated that a stable isotope-labelled analog of PEA is a more potent drug in terms of its effects on unconditioned behaviour than protonated PEA. Currently, there are a number of drugs and precursors being used clinically (e.g., L-dopa) which are severely limited in usefulness by their rapid catabolism and short duration of action. Consequently, the further study of the behavioural and biochemical effects of deuterated compounds appears likely to be of relevance for chemotherapy.

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