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The Enigma of Conditioned Taste Aversion Learning: Stimulus Properties of 2-phenylethylamine Derivatives

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Summary

The functional aversive stimulus properties of several IP doses of (±)-amphetamine (1.25-10 mg.kg^{-1}), 2-phenylethylamine (PEA, 2.5-10 mg.kg^{-1}, following inhibition of monoamine oxidase with pargyline 50 mg.kg^{-1}) and phenylethanolamine (6.25-50 mg.kg^{-1}) were measured with the conditioned taste aversion (CTA) paradigm. A two-bottle choice procedure was used, water vs. 0.1 % saccharin with one conditioning trial and three retention trials. (±)-Amphetamine and phenylethanolamine induced a significant conditioned taste aversion but PEA did not. (±)-Amphetamine and PEA increased spontaneous locomotor activity but phenylethanolamine had no effects on this measure. Measurement of whole brain levels of these drugs revealed that the peak brain elevation of PEA occurred at approximately 10 min whereas the peak elevations of (±)-amphetamine and phenylethanolamine occurred at approximately 20 min. The present failure of PEA to elicit conditioned taste aversion learning is consistent with previous reports for this compound. The differential functional aversive stimulus effects of these three compounds are surprising since they exhibit similar discriminative stimulus properties and both (±)-amphetamine and PEA are self-administered by laboratory animals. The present data suggest that time to maximal brain concentrations following peripheral injection may be a determinant of the aversive stimulus properties of PEA derivatives.

Key words

2-phenylethylamine • (±)-amphetamine • Phenylethanolamine • Conditioned taste aversion • Locomotor activity • Drug levels

Introduction

When rats ingest a novel flavored fluid that is paired with subsequent delayed administration of drugs, or certain other unconditioned stimuli, a conditioned taste aversion (CTA) may be learned (Goudie 1979). This is usually measured as a reduction in consumption of that fluid relative to water in choice tests, ranging from the standard two-bottle test to the multiple bottle arrays established by Bureš and Burešová (see Greenshaw and Burešová 1982). Direct stimulation of neural pathways may act as a substitute for flavour in this paradigm (Greenshaw *et al.* 1985) and evidence for a common neural substrate of CTA formation (possibly involving glutamate (Bielavská *et al.* 2000) has now been provided (Bielavská and Bureš 1994, Ivanová and Bureš 1990). An enigmatic feature of this CTA phenomenon is the paradoxical observation that normally rewarding stimuli may be effective unconditioned stimuli. For example, amphetamine induces CTA learning but is readily self-administered, decreases thresholds for electrical brain stimulation reward and may induce conditioned place-preferences in rats (Carr and White 1986).

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The characteristics that are necessary for drugs to act as effective unconditioned stimuli in CTA learning are unknown. Although the duration of action of drug stimuli may not be a critical determinant of potency in this paradigm (d'Mello *et al.* 1981, Fletcher 1986) it is possible that pharmacokinetic factors such as time to peak drug levels in brain may be important. The present study was conducted to measure the relative effects of 2-phenylethylamine (PEA) and two structurally similar compounds, (±) amphetamine and phenylethanolamine (PEOH), in the CTA paradigm, and on locomotor activity in rats. The time-course of their whole brain concentrations was measured following injection. PEA alone is quite ineffective as an unconditioned stimulus for CTA learning in rats in contrast to the potent effects of (±) amphetamine in this paradigm (Greenshaw and Dourish 1984a, Kutscher 1988) although both of these compounds are self-administered in animal studies (Shannon and Degregorio 1982). The effects of PEOH in the CTA paradigm have not been reported but this compound was chosen because PEA and PEOH share discriminative stimulus properties (Reid and Goudie 1986). In addition, although there is a paucity of data describing the neuropharmacological profile of PEOH, cortical and caudate nucleus neuronal firing rates may decreased to a similar extent by PEA and PEOH (Henwood *et al.* 1980). With rats PEA has previously been shown to be ineffective in the CTA paradigm at doses of up to 50 $mg.kg^{-1}$ (Greenshaw and Dourish 1984a). For this reason, the monoamine oxidase (MAO) inhibitor pargyline was used to prevent the oxidative deamination of PEA and to extend the time course of effects of this compound in the present study.

Methods

Animals

Male Sprague-Dawley rats weighing 250-300 g were used for these experiments. These animals were individually housed under a 12h light dark cycle (lights on 6am) at 20±1 ºC. Food and water were freely available in the home cages except for CTA studies, when water was available according to the experimental schedule. Animals were randomly allocated to groups (n=6-8). Drug (doses expressed as the .HCl salt) and vehicle (0.9 % saline) were administered IP in a volume of 1 $ml.\ kg^{-1}$.

Conditioned Taste Aversion Learning

Using a two-bottle choice procedure, the effects of a range of doses of (±) amphetamine and PEOH alone and in the case of PEA following monoamine oxidase inhibition with pargyline (50 $mg.kg^{-1}$ IP, 24 h prior to PEA), were assessed. For each experiment separate groups of rats were given daily 30 min two-bottle access to water. The groups were matched for baseline fluid intake and then given 30-min two-bottle access to 0.1 % sodium saccharin solution on the conditioning day. 7Immediately following saccharin availability each animal was injected with drug or vehicle. On each of the following three days, animals were exposed to a 30-min choice test in which one bottle contained 0.1 % sodium saccharin and the other contained water. Under these conditions, saccharin preference is reflected as a percentage of total daily fluid intake (measured to ± 0.1 mL).

Locomotor Activity

The locomotor effects of the highest dose of each drug were assessed in separate groups of rats. Following a 30 min period of exposure to the respective activity test boxes (12 x 12 photobeam array 30x30x20 cm: see Arnold *et al.* 1995) each animal was injected with drug or vehicle and activity was measured in 10-min intervals for 60 min.

Measurement of drug levels in brain

The concentrations of (±) amphetamine, PEA and PEOH in whole brain were measured at 1, 5, 10, 20, 30 and 60 min following their respective administration to separate groups of rats. The animals were killed by cervical dislocation and decapitation and whole brains were immediately removed and stored at –80 ºC until the time of analysis. Drug levels were measured by a sensitive mass spectrometric method using deuterated amines as internal standards (see Paterson *et al.* 1985).

Statistics

The data were analysed by ANOVA followed by Newman-Keuls tests for comparisons between means, in each case the group n=6-8 and the criterion for statistical significance was set at $P \leq 0.05$.

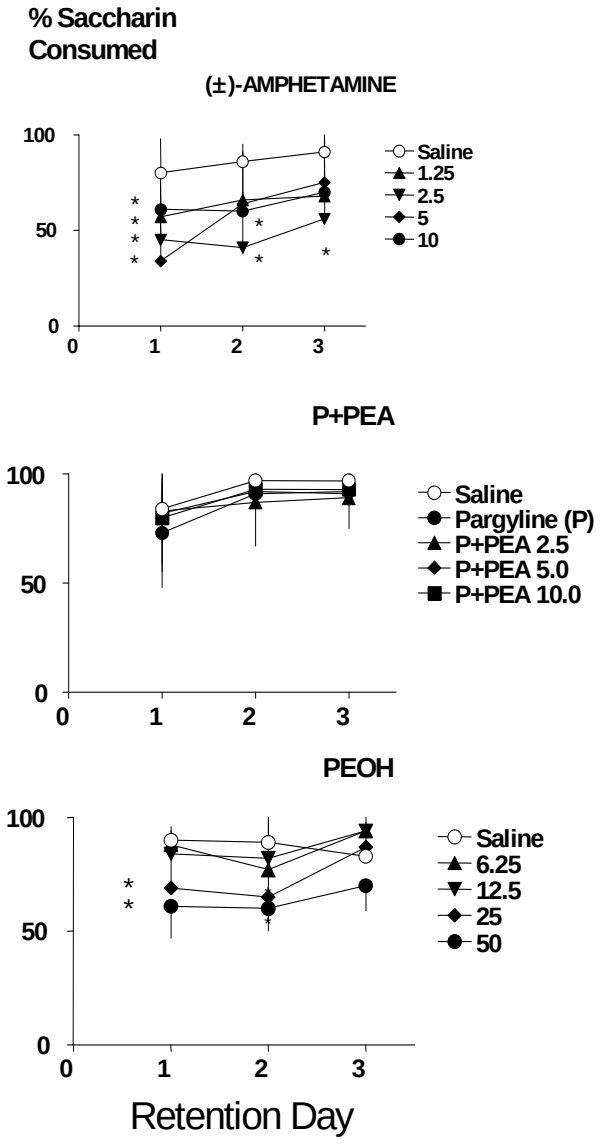

Fig. 1. *Dose-dependent CTA induced by (±)-amphetamine (upper panel) and PEOH (lower panel. PEA in the presence of MAO inhibition with pargyline (P, see text) did not induce a CTA (middle panel). The ordinate scale represents % saccharin consumed. Data are means ± standard errors. *Denotes a significant effect (P≤0.05) compared to saline-treated group. Doses are mg.kg^{-1}.*

Results

CTA learning

In accord with prior studies, (±) amphetamine was effective as an aversive stimulus in the CTA aradigm at 2.5-10.0 mg.kg^{-1}. PEOH did induce at CTA, but only after 50 mg.kg^{-1}. Following inhibition of monoamine oxidase, there was no evidence of formation of a CTA following PEA doses of up to 10 mg.kg^{-1}. Pargyline pretreatment had no effect on CTA learning. These results are illustrated by the data displayed in Fig. 1. Prior experiments in this laboratory have demon-strated that, in the absence of MAO inhibition, PEA is virtually devoid of aversive stimulus properties in this paradigm with rats (Greenshaw and Dourish 1984a, Dourish *et al.* 1983).

Locomotor Activity

(±) Amphetamine at 10 mg.kg^{-1} induced a marked locomotor stimulant response for the 60-min measurement period. Long lasting stimulant effects were also observed following PEA at 2.5-10.0 mg.kg^{-1}. The pargyline pretreatment had no significant effect on locomotor activity. PEA alone at 50 mg.kg^{-1} increased locomotor activity for 40 min post-injection. This group was included as this stimulant dose of PEA was previously shown to be ineffective in the CTA paradigm in rats (Greenshaw and Dourish 1984a) but brain levels had not been measured in this context. PEOH did not have any effects on the present locomotor activity measure at this dose. These effects are illustrated by the data displayed in Figure 2.

Brain concentrations of drugs

The levels of drug achieved in whole brain at different times following injection are displayed in Table 1. With PEA, peak levels were achieved with 50 mg.kg^{-1} after 10 min in the absence of MAO inhibition and at 5 min with 10 mg.kg^{-1} in the presence of MAO inhibition. Both PEOH and (±) amphetamine reached peak levels 20 min after administration of 10 mg.kg^{-1}. Levels of each of these compounds were still elevated in whole brain at 60 min post injection.

Discussion

The present data extend the previous observation that structurally similar compounds may exhibit different potencies in the CTA paradigm. PEA was ineffective as a CTA inducing stimulus in rats, in agreement with previous reports (Dourish *et al.* 1983, Greenshaw and Dourish 1984a,b, Kutscher 1988). Both (±) amphetamine and PEOH were effective as unconditioned stimuli in this paradigm. Pargyline did not induce CTA learning in these experiments because it was administered prior to saccharin exposure. Although PEA and (±) amphetamine induced marked stimulant effects, PEOH did not alter locomotor activity as measured in this experiment. It is difficult to draw comparisons between efficacy in locomotor activity tests and CTA learning. Indeed it is

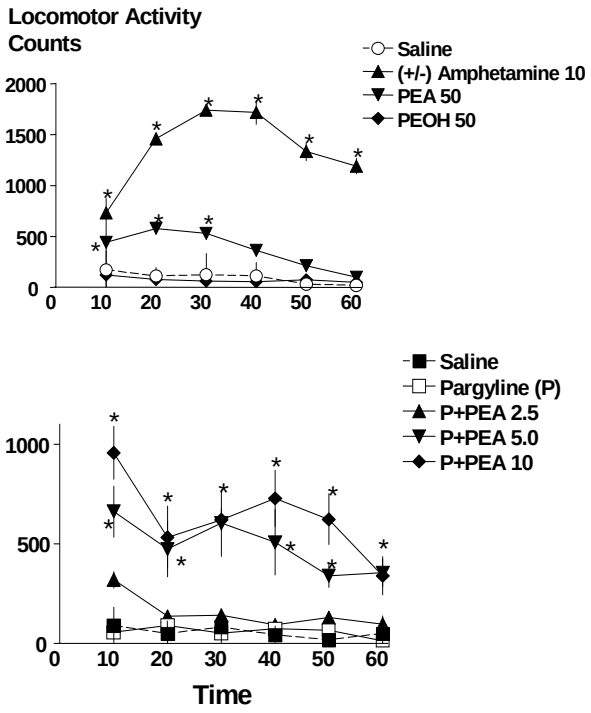

Fig. 2. *Locomotor stimulant effects of (±)-amphetamine, PEA alone (upper panel) and several doses of PEA (lower panel) in the presence of MAO inhibition with pargyline (P, see text). The ordinate scale represents number of photobeam interruptions per time period. Data are means ± standard errors. * Denotes a significant effect (P≤0.05) compared to saline-treated group.*

apparent from the present data with PEOH that locomotor activity changes do not correlate with potency in CTA learning (also see Arnold *et al.* 1995). Nevertheless the present contrast is important as it demonstrates a lack of CTA induction at doses of PEA that are functionally significant, as they induced significant changes in locomotor activity.

An examination of the time course of changes in brain concentrations in the present study indicates that time to peak brain levels may be a determinant of drug potency in CTA learning. Both (±) amphetamine and PEOH reached their relative peak levels at around 20 min, which is significantly later than the time to peak levels of PEA. PEA alone (previously shown to be ineffective in the CTA paradigm with rats, Greenshaw and Dourish 1984a) reached a peak level at around 10 min post injection. Following pargyline pretreatment peak levels of PEA were achieved by around 5 min post injection.

These data suggest that the time to maximal brain concentration may be a critical determinant of aversive stimulus properties of PEA derivatives. Further work will be necessary to test this hypothesis by measuring the relative pharmacokinetic profiles of drugs that differ in their CTA inducing potencies

We previously demonstrated that very high doses of (±) amphetamine were necessary for CTA induction when this drug was administered by the icv route (Greenshaw and Burešová 1982) this was also observed for intracranial administration of carbachol (see Bureš and Burešová 1989). At that time a possible explanation was that central effects of amphetamine may be necessary but not sufficient for CTA induction. The present "time to peak effect" hypothesis could provide a more parsimonious explanation for the low potency of intracerebroventricular (icv) administration of (±) amphetamine in the CTA paradigm. This could be tested by varying the interval between saccharin exposure and icv administration of amphetamine.

As Burešová and Bureš (1984) have demonstrated, MAO inhibitors such as harmaline, pargyline and clorgyline may induce comparable CTA learning using intracranial doses 500, 400 and 250 times lower than IP doses respectively. These data appear to be inconsistent with the proposal that time to a maximal drug effect may be a critical general determinant of aversive stimulus properties of drugs in the CTA paradigm. Nevertheless it is possible that this factor may be critical for the aversive properties of systemically administered drugs. Bureš and Burešová (1989) have described an intracerebral gradient of the central drug effects in this context. They originally targeted the lower medulla (inferior olive, raphé nuclei) as the critical brain region and serotonin as a neurotransmitter participating in the aversive labeling of the gustatory stimulus with centrally administered drugs as unconditioned stimuli. Subsequent studies have revealed the parabrachial nuclei (PBN) as a critical primary site (Ivanová and Bureš 1990), with possible glutamate mediation of CTA formation (Bielavská *et al.* 2000). An earlier regional brain mapping study reported that amphetamine may elicit a CTA but not a conditioned place preference when injected into the area postrema and a conditioned place preference but not a CTA when injected into the nucleus accumbens (Carr and White 1986). For the intracerebroventricular route this adds the potential complexity of sudden onset of (rewarding vs. aversive) action at two contrasting central sites.

It is difficult to compare the consequences of direct activation of brain areas involved in CTA learning with the consequences of gradual syndromal effects such

as those observed following IP drug injection. This comparison raises complex questions concerning the interval between the unconditioned and conditioned stimulus presentation and mechanism for initiation of an aversive association in relevant brain structures.

Table 1

Minutes post injection	**1**	**5**	**10**	**20**	**30**	**60**
(±)-amphetamine						
10	0.42 ± 0.10	4.01 ± 0.82	10.81 ± 1.96	17.97 ± 6.49	8.10 ± 2.02	6.26 ± 1.02
PEA 50	1.13 ± 0.34	11.90 ± 3.27	26.83 ± 5.54	5.68 ± 4.25	2.22 ± 0.81	0.13 ± 0.07
PEA 10 + P	0.83 ± 0.21	10.11 ± 2.14	6.85 ± 1.54	9.33 ±0.71	5.22 ± 1.05	1.17 ± 2.20
PEOH 50	0.30 ± 0.08	3.93 ± 0.23	3.66 ± 0.09	9.69 ± 2.45	6.22 ± 2.01	6.83 ± 1.76

Whole brain levels of (±)-amphetamine, PEA, (alone and following pargyline pretreatment, P see text) and PEOH at different times following IP injection. Doses are mg.kg -1 and values are µg g-1 wet tissue, means ± standard errors (n= 6-8 determinations).

Grigson (1997) has proposed an interesting "reward comparison theory" to explain the apparent "aversive" actions of drugs with rewarding properties such as amphetamine. There is significant support for this theory (Grigson 2000; Grigson *et al.* 2000) although it is not clear in this context why PEA is a poor stimulus for CTA learning in rats. Nevertheless, CTA learning is disrupted by tetrodotoxin-induced (TTX) blockade of the PBN elicited after ingestion of the gustatory CS and before administration of the visceral US regardless of whether LiCl or amphetamine acts as the unconditioned stimulus. This indicates a common neural mechanism for memory encoding in this learning paradigm regardless of the "motivational" interpretation of the phenomenon (Bielavská and Bureš 1994).

The fact that some drugs with abuse potential (e.g. PEA in rats) may not readily elicit CTA learning while others do remains enigmatic. In relation to the original interpretation of CTA effects, in terms of the ecological significance of CTA learning or "bait shyness", it makes sense that a slower onset of effects would reflect post-ingestive phenomena (Grant 1987). The issue of delay in delivery of the unconditioned stimulus has been studied extensively in the area of "long-delay learning" (Bureš and Burešová 1989). In the drug literature, the time to onset of maximal effects has not been examined in detail and this may be an important determinant of efficacy of a systemically administered drug stimulus in this context.

Appendix

As a PhD student working in the Laboratory of Professor Derek Blackman at University College Cardiff, Andy Greenshaw worked under the supervision and mentorship of Dr. Jan Bureš in the Laboratory of Neurophysiology of Memory at the Czechoslovak Academy of sciences in 1980 and 1981. Following a suggestion from Professor JP Huston, during a visit to his laboratory in Düsseldorf, Andy wrote to Dr. Bureš and was encouraged to apply to the European Training Program in Brain and Behaviour Research to support his further training under Dr. Bureš guidance in Prague. This led to a wonderful period of research experience for Andy and a rich cultural experience for his young family. Working with Drs. Bureš and Burešová and the group, Andy gained exposure to a variety of innovative multidisciplinary techniques. The scientific and cultural environment in the Laboratory of Neurophysiology of Memory was wonderfully interesting and enjoyable. Dr. Jan Bureš and Dr. Olga Burešová provided tremendous support and encouragement that were instrumental in directing Andy on the path of his academic career. That nine-month period, working on a number of experiments together with several of Dr. Bureš international visitors, yielded several publications. These involved electrical brain self-stimulation, conditioned licking responses and conditioned taste aversion learning. For Andy, Dr. Bureš and Dr. Burešová remain exemplary role models and working under their guidance established the city of Prague as a conditioned approach stimulus that remains

very highly resistant to extinction after more than 20 years.

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