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Discriminative Stimulus Properties of Beta-Phenylethylamine, Deuterated β-Phenylethylamine, Phenylethanolamine and Some Metabolites of Phenylethylamine in Rodents

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REID, D. AND A. J. GOUDIE. *Discriminative stimulus properties of beta-phenylethylamine, deuterated β-phenylethylamine, phenylethanolamine and some metabolites of phenylethylamine in rodents.* PHARMACOL BIOCHEM BEHAV 24(6) 1547–1553, 1986.—The discriminative stimulus (cue) properties of phenylethylamine (PEA) were analysed in rodents in a conventional two lever FR10 operant drug discrimination task. Rats trained to discriminate phenylethylamine at 30 mg/kg showed complete dose-related generalization to PEA and to two potential PEA metabolites: phenylethanolamine (PEOH) and N-Methyl PEA (NMPEA). Only partial (50%) generalization was seen with N-Methylphenylethanolamine (NMPEOH), another potential PEA metabolite. The specificity of PEA's action as a discriminative stimulus was demonstrated by the finding that fenfluramine, a substituted phenylethylamine, failed to generalize to PEA even at high doses with marked behavioural effects which are known to have discriminative stimulus properties themselves. These data suggest that NMPEA and PEOH may be functionally important active metabolites of PEA, particularly if the major pathway of PEA metabolism to phenylacetic acid under the influence of MAO Type B is for any reason impaired. A long acting deuterium substituted form of PEA (α, α, d_2 PEA), which is resistant to metabolism by MAO, produced complete dose-related generalization to the PEA cue but was more potent than PEA, due presumably to its resistance to metabolism by MAO. Deuterated PEA may therefore be a useful agent to use in future studies of the PEA cue, because the discriminability of PEA itself appears to be low due to its very rapid metabolism in vivo.

Beta-Phenylethylamine Deuterated β-phenylethylamine Phenylethanolamine N-Methylphenylethylamine N-Methylphenylethanolamine Fenfluramine Drug discrimination Operant procedures Rats

BETA-PHENYLETHYLAMINE (PEA) is one of a number of so-called trace amines [6,29] which are found in the CNS in concentrations which are generally low relative to those reported for other amines [6]. However, PEA, like other trace amines, is heterogenously distributed in the brain [33], and despite its relatively low absolute concentration in the CNS it has a very rapid rate of turnover due to its preferential metabolism by MAO Type B [33,50]. The rapid turnover of PEA may be a better indicator of its potential functional significance than its low absolute concentration [29]. It is therefore possible that PEA may act as a neurotransmitter or neuromodulator [6, 32, 33]. PEA has been implicated in the control of various aspects of normal behaviour [15, 26, 42, 44] and in the etiology of a variety of behavioural disorders such as schizophrenia, phenylketonuria, depression and Parkinson's disease [14, 34, 35, 38, 39, 48]. Much of the interest in PEA's pharmacological and behavioural actions has resulted from the close structural relationship between PEA and amphetamine (α-methyl PEA) and PEA has even been conceptualised as a potential "endogenous amphetamine" [42,52] which may mediate the actions of psychostimulant drugs [5].

In recent years the drug discrimination (DD) bioassay procedure has become an increasingly popular tool for the in vivo analysis of drug actions (see [11] for reviews). This procedure has also been used to analyse the actions of endogenous constituents of the brain such as the endorphins (e.g., [9, 45, 46]). We have therefore utilised the DD procedure to analyse PEA's actions in vivo in rats. In an early DD study with PEA, Huang and Ho [28] reported that PEA at a very low dose generalized to the amphetamine cue in rats pretreated with a MAO inhibitor to retard PEA metabolism. Subsequently Colpaert *et al.* [10] reported that the relative potencies of MAO inhibitors in generalizing to the cocaine

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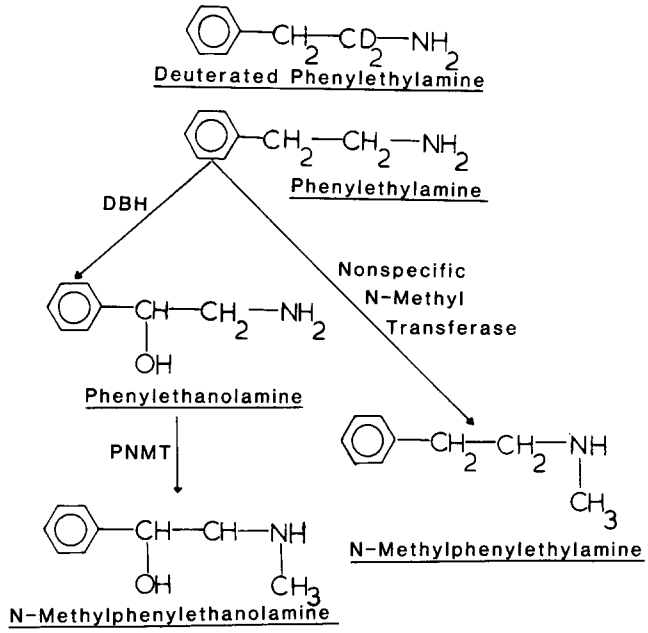

FIG. 1. Structures of compounds studied and their metabolic pathways. Note that deuterated PEA is α, α, d_2 form.

cue depended upon their relative potencies as inhibitors of MAO Type B. This finding was considered to implicate PEA as a potential mediator of the cocaine cue since PEA is a preferred substrate for MAO Type B. Such data are clearly compatible with the more general hypothesis that PEA is an "endogenous amphetamine" [39], which may mediate some actions of psychostimulant drugs [5]. However, in a subsequent DD study [22] we reported that rats trained to discriminate PEA showed only partial (50%) generalization to amphetamine and cocaine, generalization being seen consistently in some rats but not in others, even with very high doses of cocaine and amphetamine. Similarly, we have recently found [23] that rats trained to discriminate cathinone, a stimulant amphetamine congener [27], show only partial generalization in tests with PEA at high doses. Likewise, Glennon *et al.* [20] found that PEA (at relatively low doses) failed to generalize to amphetamine, whilst other researchers [49] have reported that very high doses of PEA produce only partial generalization to the cocaine cue.

Such data are not easily reconciled with the simple notion that PEA is an "endogenous amphetamine," although they do suggest that PEA shares cue properties with psychostimulants, particularly at high doses [24]. To date however, no compounds have been isolated which show complete, as opposed to partial, generalization to the PEA cue. In order to analyse the cue properties of PEA further, we attempted to identify such compounds by examining the actions of potential PEA metabolites in rats trained to discriminate PEA. Previous studies have shown that the DD procedure is an effective assay for detection of active metabolites of psychoactive agents (e.g., [8, 19, 21]). PEA is metabolised predominantly by oxidative deamination to phenylacetic acid [37,50]. However, this is not the only metabolic pathway for PEA, there is good evidence that PEA *can* be metabolised by the alternative pathways shown in Fig. 1.

Hydroxylation of PEA by dopamine-B-hydroxylase results in the production of phenylethanolamine (PEOH) [36,37], whilst PEA and PEOH can be N-methylated to N-Methylphenylethylamine (NMPEA) and N-methylphenylethanolamine (NMPEOH) respectively [4, 31, 37, 42, 44, 48, 50]. Since PEOH, NMPEOH and NMPEA have been reported to have similar, although not identical, behavioural and physiological effects to PEA [7, 30, 41, 42, 43, 44], all of these compounds were tested in animals trained to discriminate PEA, to see if they were potentially important active metabolites of PEA which would generalize completely to the PEA cue. In addition, the tests with PEOH were of interest in their own right since PEOH is also an endogenous trace amine [36]. Any similarities between PEA and PEOH in their discriminative properties might be suggestive of similar in vivo actions of these two trace amines which might be functionally significant. To investigate the pharmacological specificity of the PEA cue in the studies reported here we also determined whether fenfluramine, a non-stimulant substituted phenylethylamine, which is known to be an effective cue in DD procedures [21, 40, 47] would generalize to PEA.

In previous studies of the PEA cue in rats [22] we encountered considerable difficulty in training subjects to discriminate this agent. We were only able to train half our experimental subjects to discriminate PEA after an extended period of training relative to that typically used in DD studies. We suggested that the apparent poor discriminability of PEA was due to PEA's well known very rapid metabolism in vivo [50], since subjects probably have considerable difficulty discriminating the presence or absence of an agent which has very rapidly changing levels in the brain and plasma. Indeed, in our earlier studies [22] we showed that the PEA cue was of very short duration; after treatment with a very high dose of PEA the cue began to decay significantly within 15 to 30 minutes post-injection. We therefore tested deuterated PEA (α, α, d_2 PEA—see Fig. 1) for generalization to PEA itself, since deuterated PEA is effectively a long acting (and more potent) form of its parent compound because substitution of the stable isotope deuterium on the alpha carbon atom of the amine side chain renders PEA resistant to metabolism by MAO [2, 3, 13]. Thus we were interested in analysing the cue properties of deuterated PEA, to compare them quantitatively and qualitatively with those of PEA itself and thus to determine whether deuterated PEA might be a useful tool to use in future studies of the PEA cue due to its reported prolonged duration of action relative to PEA [13].

In summary, in the experiments reported here we were concerned with: (1) Further analysing the actions of PEA as a cue in the DD procedure; (2) Demonstrating the specificity of PEA's actions in this bioassay; (3) Investigating the possibility that potential PEA metabolites might generalize to the PEA cue; and (4) Comparing the actions of PEA and deuterated PEA.

METHOD

Animals

Twenty four female Albino rats (178–227 g) were individually housed in a temperature (21°C) controlled room. Each subject was maintained at about 80% of its ad lib body weight by restricted daily feeding. Subjects were run in operant sessions on 4 or 5 days each week. During the training phase of the study they were run twice daily (in the morning and afternoon), with at least 4 hours separating training sessions. This procedure was used to speed up the training of our

subjects; it was based on the results of our previous studies of the PEA cue [22] which showed that the cue induced by a 40 mg/kg dose of PEA in rats was almost totally absent 60 min post-injection. Thus training sessions spaced at least 4 hours apart could not be confounded by any possible residual drug effects from prior training sessions with this rapidly metabolised agent.

Apparatus

Standard operant chambers (Colbourn Instruments, USA) containing two levers were utilized. A food chamber was located between the levers. Reinforcement consisted of 45 mg food pellets. A light in the food chamber came on for 160 msec during the presentation of each food pellet, providing secondary reinforcement. The presentation of light stimuli and food pellets was controlled by a NOVA 3 Data-General computer programmed in ACT-N which was located in an adjoining room. The software was also used to record various aspects of subjects' behaviour.

Training Procedure for Acquisition of PEA/Saline Discrimination

The procedure utilized was similar to a standard FR10 drug discrimination (DD) procedure as described, for example, by Colpaert *et al.* [10]. Subjects were initially shaped to press either lever for food reward. Subsequent operant sessions were of 15 min duration, only one lever being operative on any one day. The single operative lever was determined by whether or not subjects were injected with drug (PEA) or saline. Injections were administered prior to each session in a balanced pseudorandom sequence. The sequence of operative levers was randomized between successive subjects in each operant chamber to avoid the data being confounded by inter-animal olfactory cues [17]. For half the subjects the drug lever was the left lever and for half the subjects it was the right lever. The schedule of reinforcement on the operative lever was escalated progressively over sessions 1 to 20 from FR1 to FR10, drug or saline injections preceding each session. Subsequent to session 20, the FR10 schedule was used for the remainder of the study. The training procedure over initial sessions was intended to introduce a predictive drug cue before subjects had extensive experience of responding on both levers for food reward, since such experience is known to retard the speed of acquisition of DD tasks. On all training and test sessions the total number of response on both levers was recorded. Accuracy of lever selection on each session was assessed by the total number of responses accumulated on both levers prior to the delivery of the first reinforcement (the FRF value). If the FRF was less than twice the Fixed Ratio value in operation on any specific session a correct lever selection was defined as having occurred. The training dose of PEA was 30 mg/kg, the choice of this dose being based on our previous studies of PEA discrimination [22]. After 50 training sessions only 14 out of the 24 subjects had been trained to a criterion of 10 consecutive sessions of correct lever selection ($p<0.01$ for each subject; Binomial test). The remaining 10 subjects were discarded from the study at this point as they seemed unlikely to reach the training criterion without extensive further training.

Generalization Test Phase

Test sessions after session 50 were always run on a Tuesday or a Friday. On test days subjects were reinforced throughout the 15 min session for responding on the first lever on which they accumulated 10 responses—the "selected lever." On intervening days baseline training sessions were continued, but these were only run once a day, rather than twice daily as in the training phase of the experiment.

Initial test sessions were conducted to obtain a dose/effect curve for the PEA cue. Subjects were injected with PEA at 4 doses between 0 and 30 mg/kg. Doses were administered in a non-systematic random order. Generalization tests were then conducted with deuterated PEA (α, α, d_2), phenylethanolamine (PEOH), N-Methyl PEA (NMPEA), N-Methylphenylethanolamine (NMPEOH) and fenfluramine (trifluromethylphenylethylamine). Generalization tests were conducted with 3 or (usually) more doses of each compound, except in the case of fenfluramine when only two high dose (1 and 3 mg/kg) tests were conducted with doses of this drug which are known to have potent cue properties [21, 40, 47]. Generalization tests were conducted with all other drugs until they caused either at least 70% generalization to PEA or until the tested drug suppressed the rate of operant responding to less then 20% of the baseline level, so that higher doses could not be tested. Drugs and drug doses were tested in non-systematic random orders. In some generalization tests with high drug doses a few subjects failed to select either lever. However, this never occurred with more than 2 subjects at any dose, thus generalization test data were obtained from at least 10 trained animals in all cases.

Drugs

All drugs were injected as hydrochloride salts, dissolved in 0.9% saline and injected IP at a volume of 2 ml/kg. All injections were given 15 min prior to operant sessions. Drug sources were: PEA (Sigma Chemicals, UK), Phenylethanolamine (Sigma Chemicals, UK), NMPEA (Sigma Chemicals, UK), NMPEOH (Aldrich Chemicals, UK), Fenfluramine (Servier Laboratories, London, UK) and deuterated PEA (supplied by Drs. B. A. Davies and A. J. Greenshaw of Saskatoon, Saskatchewan, Canada).

Data Analyses

Lever selection data were analysed by probit analysis using maximum likelihood methods [18]. Drug effects on rates of operant responding were determined by expressing the effect of each dose as a percentage of the total responses made on the most immediately preceding saline baseline session (cf. [10,22]).

RESULTS

For the rats trained to criterion within 50 sessions the median STC value was 38 sessions (range=24–47). For the rats that failed to reach criterion, a systematic tendency towards learning the PEA discrimination *was* seen, but the mean overall proportion of correct lever selections shown by these rats over the last 10 training sessions (41–50) was only 69.5%, therefore these subjects were not used in the generalization test phase of the experiment. In addition, 2 of the 14 rats that reached criterion failed to show more than 80% correct lever selections post-criterion, so that generalization tests were conducted on only 12 of the original 24 subjects. For these subjects, during the post-criterion discrimination

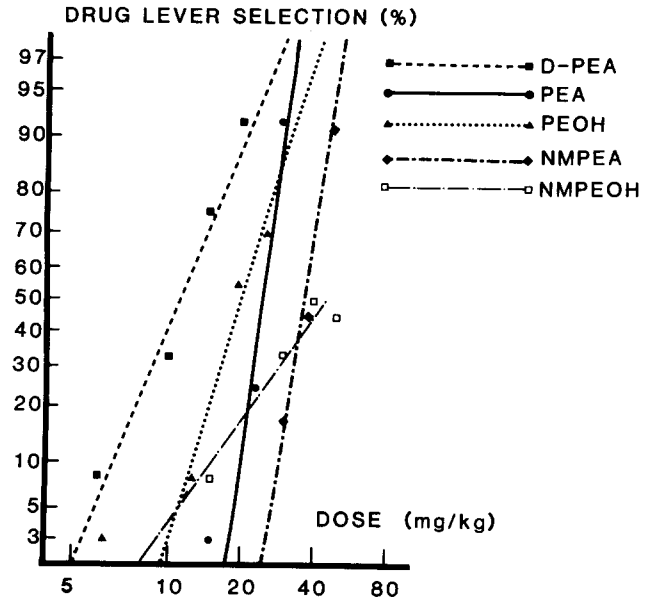

FIG. 2. Log/probit summary plot for all drugs showing at least 50% generalization to the PEA cue. The percentage of rats selecting the drug lever is plotted on a probit scale on the abscissa against dose on a log scale on the ordinate. Straight lines indicate theoretical (i.e., calculated) lines of best fit, observed data points are also plotted for each drug. D-PEA=Deuterated PEA, PEOH=Phenylethanolamine, NMPEA=N-Methyl PEA, NMPEOH=N-Methylphenylethanolamine.

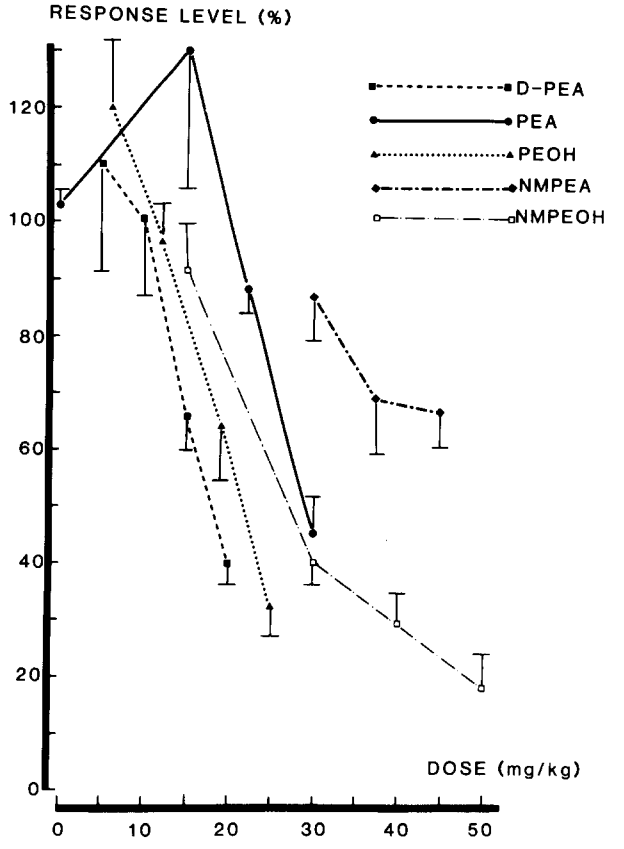

FIG. 3. Mean (±S.E.) response level (% of baseline) as a function of dose for all drugs that showed at least 50% generalization to the PEA cue. Drug codes are as for Fig. 2.

TABLE 1

MEAN (± S.E.) LATENCY TO FIRST REWARD IN SEC

Drug	Dose	Latency	Estimated dose required to prolong latency to 150 sec* (mg/kg)
Saline	0	15.8 ± 1.5	
PEA	15	21.2 ± 2.6	29.6
	22.5	22.5 ± 3.6	
	30	156.5 ± 29.9	
D-PEA	5	14.9 ± 1.5	14.5
	10	37.0 ± 8.0	
	15	86.0 ± 15.0	
	20	321.8 ± 60.5	
PEOH	6.33	17.2 ± 2.2	16.0
	12.6	23.2 ± 3.1	
	19	172.8 ± 77.5	
	25.33	516.3 ± 91.5	
NMPEA	30	20.2 ± 2.9	49.9
	37.5	111.2 ± 7.2	
	45	113.3 ± 72.0	
NMPEOH	15	19.7 ± 3.3	28.5
	30	162.8 ± 59.0	
	40	208.4 ± 55.6	
	50	438.7 ± 81.8	

*This dose was estimated by least squares regression analyses of the linear portions of log dose/response curves.

maintenance sessions interspersed between generalization tests the overall level of percent correct lever selections averaged 89.5%. Errors in these maintenance sessions occurred more often following PEA injections (16.5%) than following saline injections (4.4%). The differential error rate seen in PEA versus saline sessions was highly significant, $\chi^2(1)=16.35$, $p<0.001$. Thus subjects effectively showed saline lever "bias" following training to criterion. All of the compounds tested except fenfluramine produced at least 50% generalization to the PEA cue (see Fig. 2).

For each of the agents shown in Fig. 2 generalization was dose-related, the respective ED_{50} values (mg/kg) determined by probit analyses being: Deuterated PEA (11.0), Phenylethanolamine (19.7), PEA (24.7), M-Methyl PEA (36.1) and N-Methylphenylethanolamine (46.7). It should however be noted that at the highest dose that could be tested, NMPEOH produced only partial (50%) generalization to the PEA cue.

Figure 3 shows the effect of compounds that produced at least 50% generalization on the level of operant responding. ED_{50} values (i.e., doses that suppressed operant responding to 50% of the baseline level) were calculated for each drug by least squares regression analyses of the linear portions of the log/dose response curves, the respective ED_{50} (mg/kg) values being: Deuterated PEA (19.9), Phenylethanolamine (22.2), PEA (29.5), N-Methyl PEA (58.3) and N-Methylphenylethanolamine (28.4).

TABLE 2

RESULTS OF GENERALIZATION TESTS CONDUCTED WITH FENFLURAMINE

Dose (mg/kg)	Percent PEA Lever Selection	Mean (± S.E.) Response Level (% of baseline)	Mean (± S.E.) Latency to first reinforcement (sec)
0	0	102.2 ± 2.5	15.8 ± 1.5
1	8.33	111.4 ± 5.7	13.9 ± 1.2
3	16.67	47.8 ± 8.0	57.0 ± 13.3

Table 1 shows the effects of the various drugs that showed at least 50% generalization to the PEA cue on the time taken to earn the first reward. In previous work [22] we established that a characteristic effect of PEA in the FR10 DD procedure as used in this study is to produce a dose-related increase in latency to the first reward. This effect of PEA was replicated in the present study (see Table 1) and it was also seen in tests with all of the other drugs that generalized to PEA. In order to allow further potency comparisons to be made between these agents, a theoretical dose was calculated (by least squares regression analyses of the linear portions of the log/dose response curves) which estimated the dose of each agent that would have prolonged the latency to first reward to an arbitrary value of 150 sec. These estimated values are shown in Table 1.

Table 2 shows the data obtained in the generalization tests conducted with fenfluramine. These data show clearly that doses of fenfluramine which had marked effects on the rate of operant responding *and* on the latency to first reinforcement failed to produce more than 16.7% generalization to the PEA cue.

DISCUSSION

A number of related lines of evidence indicate that, in confirmation of our previous suggestion [22], the absolute level of discriminability of PEA in the FR10 DD procedure is low. Firstly, it only proved possible to train 14 out of a total 24 rats to criterion within 50 training sessions. Secondly, even in the most efficient 12 of these 14 selected subjects the overall accuracy of post-criterion lever selection (89.5%) in trained rats was relatively low compared to that which is typically reported for well trained subjects in studies with the FR10 DD procedure. Finally, post-criterion errors in lever selection were typically made after PEA rather than after saline injection. As we have previously suggested [22] the low level of PEA discriminability is most plausibly attributed to the very rapid metabolism of PEA by MAO Type B. It seems probable that experimental subjects encounter difficulty in learning to discriminate the presence or absence of an agent (such as PEA) which has a rapidly changing blood or brain level, especially since the level of PEA in the body may be changing significantly *during* DD training and maintenance sessions. Thus the finding that the longer acting deuterated form of PEA [13], which is resistant to metabolism by MAO, showed complete dose-related generalization to the PEA cue is of considerable interest, because it suggests that future studies with deuterated PEA as a training drug could be of value since the discriminability of deuterated PEA may prove to be higher than that of PEA due to its resistance to metabolism and prolonged duration of action. The potency of PEA relative to deuterated PEA in producing drug lever selection was 1:2.2; whilst the relative potencies of these two compounds in suppressing operant responding and in prolonging the latency to first reward were 1:1.5 and 1:2.0 respectively. Thus on all three measures deuterated PEA was more potent than PEA itself, in agreement with a previous report on the greater potency of deuterated PEA in its gross behavioural effects on motor activity [13]. Such findings are presumably attributable to the relative resistance of carbon-deuterium bonds to enzymatic cleavage [3], and they resemble previous behavioural studies with N,N, dimethyltryptamine [1] and phenelzine [12] which have demonstrated that the behavioural effects of amines which are subject to enzymatic deamination at the alpha carbon atom of the amine side chain can be potentiated and prolonged by substitution of the alpha carbon atom with deuterium rather than hydrogen. The potential clinical value of the use of deuterium substitution as a means of increasing the potency and prolonging the duration of action of amine based drugs has already been noted in studies with the MAO inhibitor phenelzine [12].

In any DD study the issue of the pharmacological specificity of the assay developed is of paramount importance. In this specific study, this issue was addressed by conducting substitution tests with fenfluramine, a non-stimulant substituted phenylethylamine which has cue properties which differ from those of CNS stimulants [21]. Even at relatively high doses, which are themselves known to have potent discriminative properties [21, 40, 47] and which have potent behavioural effects (see Table 2), fenfluramine failed to cause more than a trivial level of drug lever selection. Further evidence for the specificity of the PEA cue comes from previous evidence [22] that in rats even *very* high doses of cocaine and amphetamine fail to produce more than partial (50%) generalization to the PEA cue. Thus the cue properties of PEA in the DD procedure show pharmacological specificity. Agents that produce complete, dose-related generalization to PEA are therefore assumed to share a specific common cue. Phenylethanolamine and N-Methyl PEA are the first compounds to be described which produce such complete generalization to the PEA cue. They differ in this respect from N-Methylphenylethanolamine which only produced partial (50%) generalization at the highest dose that could be tested (Fig. 2). The similarities between the cue properties of PEOH and NMPEA suggest that these compounds may be potentially important active metabolites of PEA. NMPEOH seems less likely to be an important active metabolite. Generally, PEOH was *more* potent than PEA in its behavioural actions, the potency ratios for this pair of compounds being 1:1.25, 1:1.33 and 1:1.85 for effects on drug lever selection, suppression of operant responding and prolongation of the latency to first reward respectively. In contrast, NMPEA was *less* potent than PEA, the relative potency ratios for the three behavioural measures being 1:0.68, 1:0.51 and 1:0.59 respectively. Such data might be taken to suggest that PEOH is more likely to be a functionally significant active metabolite of PEA than NMPEA, although it should perhaps be stressed that PEOH is a longer acting compound than PEA and NMPEA [7, 25, 41, 42] and that differences in the pharmacokinetic properties of these agents may well influence potency comparisons to some extent. It is also important to note that whilst PEOH resembled PEA in its actions in the DD procedure as reported above,

other studies with these agents [43,44] indicate that the physiological and behavioural effects of PEOH *can* differ from those of both PEA and NMPEA. Thus *general* conclusions about the possible role of PEOH and NMPEA in PEA's actions are clearly precluded at present. The precise functional significance of PEOH and NMPEA as active metabolites of PEA would clearly depend on the rate of biosynthesis of these compounds at the specific neuroanatomical and synaptic sites at which they may exert specific effects. However, it is of some interest to note that recent studies [31] have shown that PEA can be metabolised to NMPEA in vitro in post mortem human brain tissue. Thus the suggestion that NMPEA may play a role in human psychopathology, although speculative, merits consideration. Since PEA is usually metabolised predominantly to phenylacetic acid under the influence of MAO Type B [50], it is most likely that any behavioural effects that may be attributable to metabolites of PEA would be most pronounced under conditions where metabolism by MAO was retarded. In this context it is relevant to note that reduced levels of platelet MAO activity have tentatively been described as a biological marker for chronic schizophrenia [51]. Presumably, under such conditions relatively large levels of NMPEA and PEOH would be produced and might have functionally significant actions.

Finally, it is worth noting that these data have implications which allow a potential reinterpretation of the data reported by Colpaert *et al.* [10]. These authors suggested that endogenous PEA may mediate the cue properties of cocaine since the relative potencies of MAO inhibitors in generalizing to the cocaine cue depended upon their relative potencies as inhibitors of MAO Type B, PEA being a preferred substrate for this form of MAO. However, since PEOH is also preferentially metabolised by MAO Type B [16], and since we have found that PEOH posseses cue properties in common with those of PEA, it is possible that Colpaert *et al.*'s [10] data can be interpreted just as parsimoniously as being suggestive of a mediating role for endogenous PEOH rather than PEA in the cue properties of cocaine.

In summary, the data reported here go some way to further characterising the nature of the PEA cue by demonstrating its specificity and by isolating two metabolites, NMPEA and PEOH, which show complete generalization to PEA. Previous studies with stimulant drugs [20, 22, 23, 49] have to date only been able to demonstrate partial generalization to the PEA cue. The two metabolites which generalize to the PEA cue (NMPEA and PEOH) *may* be functionally important agents. The finding that deuterated PEA is more potent than its parent compound in the DD procedure extends previous reports of potentiation by deuterium substitution of the behavioural effects of drugs containing amine groups which are metabolised by MAO [1, 12, 13] and also suggests that deuterated PEA might be a valuable tool to use in future analyses of the nature of the PEA cue.

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