Pharmacology Biochemistry & Behavior, Vol. 26, pp. 199–202, 1987. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

BRIEF COMMUNICATION

Behaviourally Specific Interactions Between Naloxone and Beta-Phenylethylamine in an Operant Drug Discrimination Procedure in Rats

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Received 13 December 1985

GOUDIE, A. J. *Behaviourally specific interactions between naloxone and beta-phenylethylamine in an operant drug discrimination procedure in rats.* PHARMACOL BIOCHEM BEHAV **26**(1) 199–202, 1987.—Rats were trained to discriminate phenylethylamine (PEA) at 30 mg/kg (IP). In subsequent generalization tests, it was found that naloxone had no effect on the discriminative stimulus (cue) properties of PEA, but it did potentiate PEA's dose-related rate suppressant effects. Thus the potentiation by naloxone of PEA's effects was behaviourally specific and confined to drug effects on motoric behaviours. These data support the results of previous in vivo and in vitro studies, which suggest that interactions between endogenous PEA and endorphin systems may be functionally important. Such interactions could be of significance in stress-related behavioural disorders.

Beta-phenylethylamine Naloxone Endorphins Drug discrimination

BETA-Phenylethylamine (PEA) is a trace amine [4] which may be a functionally significant neuromodulator or neurotransmitter [15, 17, 21]. PEA has a close structural resemblance to amphetamine and it has been suggested that PEA *may* be an "endogenous amphetamine" [3,31] which may be associated with the etiology of behavioural disorders such as phenylketonuria, schizophrenia and Parkinson's disease [23, 28–30].

A number of in vivo *and* in vitro studies suggest that endogenous PEA may interact functionally with endorphin systems. PEA potentiates the suppressant effects of Met-enkephalin on single cell firing, without itself affecting firing rate [17]. Such data are in accord with reports [11–13] that the analgesia produced by endorphins or by morphine can be potentiated by PEA or by inhibition of MAO Type B, PEA being a preferential substrate for MAO B. Potentiation by PEA of morphine's actions has been confirmed in the isolated mouse vas deferens [6]. Other studies have shown that subconvulsant doses of PEA potentiated the seizure-inducing actions of enkephalins [32]. PEA also produces naloxone reversible analgesia in mice [26]. Furthermore, Dourish and Cooper [9] reported that naloxone produced behaviourally selective potentiating *and* antagonistic effects on different behaviours induced by PEA in rats. All these studies suggest that interactions between endogenous opiate and PEA systems may be functionally significant. We therefore examined the effects of naloxone in rats trained to discriminate PEA in a drug discrimination (DD) task, from which a variety of behavioural measures can be obtained, to analyse further PEA/naloxone interactions. The data show that interactions between naloxone and PEA in the DD procedure are behaviourally specific. Treatment with naloxone potentiated PEA's rate-suppressant effects, but did not modify PEA's discriminative stimulus properties.

METHOD

The animals (14 female albino rats) used in this study were the same as those used in a prior report [27] on the PEA cue. Full details of the housing conditions, apparatus and training procedure for the DD task can be found in this earlier report [27], only a brief summary of the methods used is presented here. Standard operant chambers (Colbourn Instruments, USA) containing two levers were utilised with reinforcement provided by 45 mg food pellets. The DD procedure utilized was similar to a standard FR10 drug discrimination (DD) procedure as described, for example, by Colpaert *et al.* [5]. Operant sessions were of 15 min duration. On all operant sessions the total number of responses on both levers was recorded. Accuracy of lever selection on each session was assessed by the total number of responses ac-

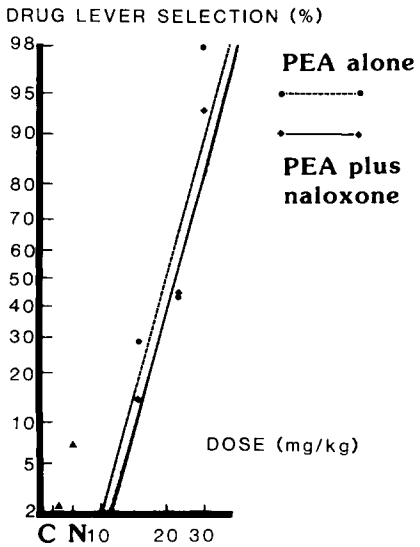

FIG. 1. Dose/response curves obtained in the presence and absence of naloxone treatment. Percent drug lever selection on a probit scale is plotted against dose of phenylethylamine on a log scale. (100% drug lever selection was plotted arbitrarily as 98% and 0% as 2%.) The figure shows the calculated log/probit regression lines and the raw scores obtained at each dose of phenylethylamine tested. Data shown at C represent the effects of control vehicle injections, data shown at N represent the effect of naloxone alone.

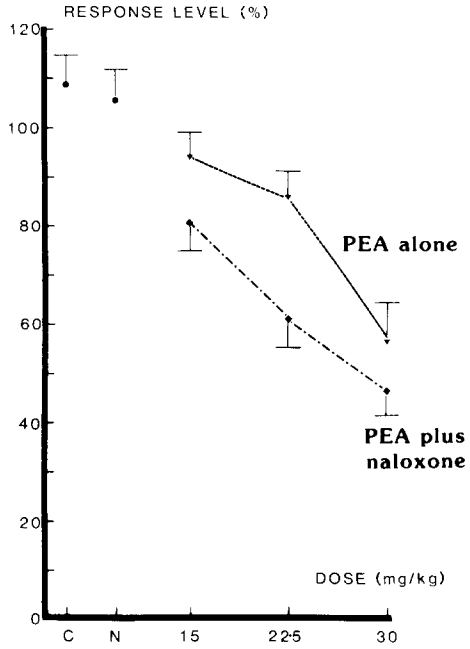

FIG. 2. Effects of control vehicle injections (C), naloxone (N) and PEA at doses between 15 and 30 mg/kg in the presence and absence of naloxone. The response level (mean±S.E.) was derived by dividing the total number of responses made in each test session by the total number made on the most immediately preceding saline training session.

cumulated on both levers prior to delivery of the first reinforcement (the FRF value). We also recorded the time (latency) after each animal was put into the operant chamber before it obtained its first reward. The training dose of PEA was 30 mg/kg, the choice of this dose being based on our previous studies of PEA discrimination [14]. Over 50 training sessions 14 subjects were trained to a criterion of 10 consecutive sessions of correct lever selection ($p<0.001$ for each subject. Binomial test). Test sessions were run on a Tuesday or a Friday. On test days subjects were reinforced for responding on the first lever on which they accumulated 10 responses—the "selected lever." On intervening days baseline training DD sessions were continued. Fifteen min before test sessions subjects received two injections administered within 30 seconds of each other on either side of the peritoneal cavity. The first injection was either saline or naloxone (10 mg/kg), the second injection was either saline or PEA at one of three doses (15, 22.5 and 30 mg/kg). Thus the experiment included a control vehicle (C) test (saline/saline injections), a test for the effects of naloxone (N) and saline together (naloxone/saline injections) and two sets of substitution tests conducted with various doses of PEA either after saline or after naloxone pretreatment. Each combination of drug injections was tested once in all 14 trained animals.

All drugs were administered IP in 0.9% saline at 2 ml/kg of rat. Drugs were phenylethylamine hydrochloride and naloxone hydrochloride; they were made up as salts.

Lever selection data were analysed by probit regression analyses [10] to obtain ED_{50} values and their 95% confidence limits. Drug effects on rates of responding were determined by expressing the effect of each treatment as a percentage of the total responses made on the most immediately preceding saline baseline day—this was termed the "response level" (cf. [5,27]). Drug effects on response rates and latency to first reward were analysed by repeated measures ANOVAs.

RESULTS

The trained animals showed a high level (ca. 95%) of correct lever selections after training. The effects of test treatments on lever selection are shown in Fig. 1. Following injections with the saline/saline vehicle control (C) all rats selected the saline lever; following saline/naloxone treatment (N) only one rat (7.1%) selected the PEA lever.

The ED_{50} values (and associated 95% confidence limits) were 19.8 (16.3 to 23.1) and 21.7 (18.6 to 25.1) mg/kg for the dose/response curves obtained in the absence and presence of PEA respectively. Thus, naloxone alone (N) or vehicle injections alone (C) produced saline lever selection, PEA induced a dose-related discriminative stimulus and pretreatment with naloxone had no effect on the ED_{50} or the slope of the PEA generalization curve.

The effects of saline or naloxone treatment in conjunction with PEA, of naloxone alone (N) and of control vehicle injections (C) on response level are shown in Fig. 2.

Following control vehicle injections (C) the response

TABLE 1

EFFECTS OF DRUG TREATMENTS ON (MEAN ± S.E.) LATENCY TO FIRST REWARD (SEC)

Drug Treatments					
			PEA		
Control Vehicle	Naloxone Alone		15	22.5	30
17.6 ±2.5	16.2 ±3.1	After Saline Pretreatment:	28.8 ±4.1	27.43 ±5.0	172.3 ±39.3
		After Naloxone Pretreatment:	25.1 ±3.8	44.8 ±12.9	179.1 ±41.3

level was approximately 100%, as it was following injection of naloxone alone (N). A repeated measures ANOVA on these data indicated that the effect of naloxone did not differ significantly from that of control vehicle injections ($F<1$) thus naloxone itself had no effect on responding. The response level data obtained with PEA in the presence and absence of naloxone were analysed with a two factor (PEA levels ×3, naloxone levels ×2) repeated measures ANOVA which indicated that there was a significant effect of PEA dose level, $F(2,26)=31.1$, $p<0.001$, a significant effect of naloxone level, $F(1,13)=14.2$, $p<0.005$, but no significant interaction, $F(2,26)=1.17$, $p>0.20$. Thus, whilst PEA produced dose-related suppression of operant responding (cf. [14,27]), this effect was potentiated by naloxone which did not itself effect rates of operant responding. The absence of a naloxone × PEA interaction indicates that potentiation of the effect of PEA was seen at all doses, as shown in the figure.

Table 1 shows the effects of vehicle injections, naloxone alone and PEA at various doses in the presence and absence of naloxone on latency to first reward. The two latency measures obtained after control vehicle and naloxone treatment were not significantly different (repeated measures ANOVA, $F<1$). A two factor repeated measures ANOVA on the data obtained with varying doses of PEA in the presence and absence of naloxone indicated that there was a significant effect of PEA dose, $F(2,26)=10.75$, $p<0.01$, there was no significant effect of naloxone , $F(1,13)<1$, nor was there a significant interaction ($F<1$). Thus, PEA produced a dose-related increase in latency to first reward (cf. [14]). However, this effect of PEA was *not* potentiated by naloxone, in contrast to the findings obtained when total session responses were considered.

In summary, naloxone failed to potentiate the discriminative stimulus properties of PEA. However, naloxone potentiated the rate-suppressant effects of PEA at a dose which did not itself reduce responding. Naloxone did not, however, potentiate PEA's effect in enhancing latency to first reward. Thus the effect of naloxone was limited to potentiation of PEA's motoric effects once subjects had actually started responding.

DISCUSSION

The pattern of lever selection and response level data reported above indicates that the potentiating effect of naloxone on PEA's actions was behaviourally specific, being limited to drug effects on response rate. No potentiation was seen of PEA's discriminative stimulus properties. Such data highlight the value of taking multiple behavioural measures in operant drug discrimination procedures. The fact that a treatment which potentiated PEA's rate-suppressant effects did not affect the discriminative stimulus properties of PEA suggests that the PEA stimulus is *not* related to this agent's well-known effects on motoric behaviours [7,8]. These data are in accord with previous findings [14,27] which indicated that the rate-suppressant and cueing properties of agents which produced partial or complete generalization to the PEA cue did *not* co-vary. Collectively, the data suggest that the PEA cue is not mediated by the systems involved in PEA's gross behavioural (motoric) effects.

The fact that naloxone did not potentiate PEA's actions on latency to first reward (Table 1) suggests strongly that the potentiation observed on the response rate measure was not simply a consequence of naloxone-induced prolongation of PEA's actions, PEA being known to be very rapidly metabolised in rats [33].

The data presented here are therefore similar to the findings of Dourish and Cooper [9] who reported that naloxone potentiated some, but not all, of the behavioural effects of PEA in rats. Dourish and Cooper [9] suggested the PEA resembled apomorphine in terms of its response to naloxone since there is evidence, from a variety of assays, that naloxone potentiates a number of behavioural and physiological effects of apomorphine [1, 24–26]. In particular Harris *et al.* [16] reported that naloxone potentiated the effects of apomorphine on operant responding in a manner similar to that reported in this study. Such findings raise the possibility that the effects reported above *may* be due to indirect or direct dopaminergic effects of PEA itself [2,8], since dopaminergic and endorphinergic systems interact in the control of motoric behaviors (e.g., [19]). We suggest that these data add to a growing body of literature which indicates that interactions between endogenous PEA and endorphin systems may be functionally important. In particular, the data reported here suggest that such interactions may be important for the control of motoric behaviours. In humans stress acts to mobilise in parallel endogenous PEA and endorphin systems [22]. Since stress is often considered of some significance in the etiology of behavioural disorders and since both PEA and endorphin systems have been implicated in such disorders, the possibility arises that stress-related activation of interacting PEA and endorphin systems may be of some significance in the development of these disorders, and that endorphin/PEA interactions consequently merit further analysis.

ACKNOWLEDGEMENTS

This work was supported by The University of Liverpool Research Grants Scheme. Thanks are due to Anne Halliwell and Dorothy Foulds for patiently typing the manuscript, and to M. Taylor, Karen Sheehan and Sue Courtney for care of the animals.

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