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Pharmacological Changes Induced by Repeated Exposure to Phenylethylamine

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GIANUTSOS, G. AND S. CHUTE. *Pharmacological changes induced by repeated exposure to phenylethylamine.* PHARMACOL BIOCHEM BEHAV 25(1) 129–134, 1986.—Mice receiving daily injections of phenylethylamine (PEA) exhibited an enhanced PEA-induced motor stimulation, beginning on day 21 of administration. The mice receiving PEA were also more sensitive to the stimulatory effect of amphetamine and PCP. There was no change in brain or hepatic monoamine oxidase activity nor in hepatic mixed function oxidase activity after this treatment, indicating that altered metabolism was not a factor in the sensitization. Striatal dopamine receptors, labelled by spiroperidol, were increased after the long-term PEA, suggesting that the sensitization may be due to increased dopaminergic receptor activity.

Phenylethylamine Dopamine Amphetamine Receptors

PHENYLETHYLAMINE (PEA), an endogenous trace amine found in the CNS, has been suggested as a possible mediator of psychiatric diseases [21]. This substance is structurally related to amphetamine and produces amphetamine-like behavioral effects when injected into rodents in large doses. These include motor stimulation [15] and stereotypy [1], which are antagonized by drugs used to treat schizophrenia [13]. In addition to its structural similarity to amphetamine, altered PEA metabolism has been suggested in clinical studies of schizophrenia [20], particularly of the chronic, paranoid type [18].

An unusual response to PEA occurs during repeated administration. Animals receiving frequent injections of PEA develop a syndrome of stereotyped behaviors including continuous gnawing and head-bobbing [1], which have been suggested to serve as an animal model of schizophrenia.

In this study, we further characterized the sensitization to PEA occurring with long-term drug treatment and have initiated preliminary investigations into possible mechanisms for this effect.

METHOD

Drug Administration

Male mice (CD-1, Charles River Farms, Wilmington, MA) were used in all experiments. The mice were injected subcutaneously (SC) with PEA. For long-term experiments, the mice received a daily injection of PEA (50 mg/kg) for up to 28 days (see the Results section); controls received a daily injection of saline. When other drugs were used (i.e., amphetamine or phencyclidine (PCP)), these were also injected SC on day 21, 24 hr after the last (20th) PEA injection. All drugs were dissolved in distilled water immediately before injection.

Motor Activity

Activity of the mice was measured using a Stoelting Activity Monitor. Mice were acclimated to the device for 20 min before testing, after which they were removed, injected with drug or saline and returned to the chamber for 1 (PEA, PCP) or 2 hr (amphetamine). All experiments were performed during the morning (8–11 a.m.). Activity is expressed as counts recorded by the instrument during the period of measurement.

MAO Activity

Brain and liver MAO activity was measured essentially as described by Campbell and coworkers [2], using radiolabelled serotonin as a substrate for the A form of the enzyme and PEA as a substrate for the B form. Briefly, tissue was homogenized in 80 mM phosphate buffer (pH=7.2) and 50 μl aliquot was incubated for 60 min in buffer containing the appropriate ^{14}C-labelled substrate. The deaminated metabolites were separated by ion exchange on Amberlite CG-50 columns and the enzymatic activity was quantified by liquid scintillation spectrometry. A boiled tissue sample served as the blank.

Drug Metabolism

Hepatic mixed function oxidase (MFO) activity was measured using p-nitroanisole as a substrate for O-demethylase activity and aniline as a substrate for hydroxylase activity as described by Netter and Seidel [16] and Kato and Gillette [12], respectively. In brief, livers from mice were homogenized in 0.1 M Na-K phosphate buffer and the crude microsomal supernate derived from a centrifugation at 9500 × g was used in the assay. The formation of p-nitrophenol or p-aminophenol during a 20–30 min incuba-

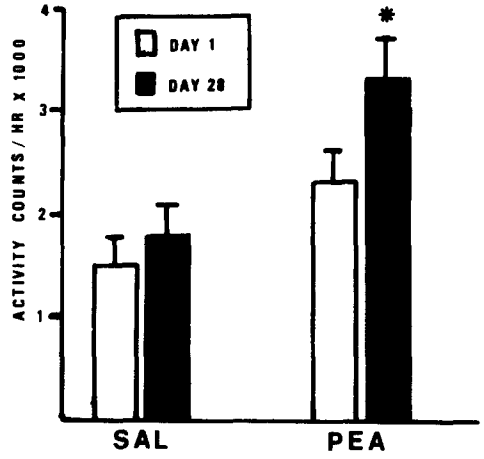

FIG. 1. Effect of repeated injection of PEA on motor activity. Mice were injected with saline or PEA (50 mg/kg) and their motor activity was measured after an acute injection (open bar) or 24 hours after receiving 27 daily injections of PEA (50 mg/kg; filled bar). *Indicates activity which was significantly greater on day 28 than on day 1.

tion of an aliquot of this supernate at 37°C was determined spectrophotometrically, at 410 and 640 nm, respectively.

Dopamine Binding

The binding of ^{3}H-spiroperidol was measured as an index of dopamine (DA) receptor density and was performed essentially as described by Creese and coworkers [3]. Briefly, striatal tissue from identically treated mice was pooled and homogenized in 0.32 M sucrose. After an initial 1000 × g centrifugation, the supernate was centrifuged at 48,000 × g for 20 min. The resulting pellet was resuspended in Tris buffer (pH=7.7 containing Na, K, Mg and Ca chlorides) washed and used in the assay. A 1 ml aliquot of the final tissue preparation was incubated at 37°C for 20 min in the presence of different concentrations (0.2–2.7 nM) ^{3}H-spiroperidol (New England Nuclear, specific activity of 35 Ci/mmole). After incubation, the contents were filtered through a Whatman GF/B filter under reduced pressure and the tissue was washed 3 times with ice-cold buffer. Non-specific binding was defined as label which was not displaced by 1 μM (+)-butaclamol. Binding constants (Bmax and Kd) were calculated by linear regression and represent data derived from at least 8 concentrations of spiroperidol. Values in the results are mean±SEM derived from six separate experiments with different groups of mice assayed at different times.

Statistics

Student's t-test was used for statistical analysis with $p<0.05$ representing the level of significance.

RESULTS

Sensitization to PEA

Mice were injected daily with PEA (50 mg/kg) and the effect of this dose on motor activity after 1 injection was compared with the effect after 28 injections and is summarized in Fig. 1. This dose of PEA, a dose which marginally stimulates activity in normal mice, was significantly more effective in mice receiving the injection on the 28th day.

The time course of this effect is illustrated in Fig. 2. In these experiments, the mice received the 50 mg/kg dose daily, but a non-stimulatory dose (25 mg–kg) was used as the challenge on the designated test day, 24 hr after the last previous PEA injection. In naive mice, this dose does not significantly stimulate motor activity as shown in the group designated day 1. However, when this dose was tested in the mice in the long-term PEA group on the 21st or 28th day after initiating treatment, a significant stimulation was observed. A trend towards sensitization began to emerge by day 14, but this did not reach statistical significance until tested on day 21.

Cross-Sensitization to Other Drugs

The effect of amphetamine and PCP after long-term PEA administration was also investigated. Since significant sensitization was observed to PEA after 20 injections, these drugs were tested in mice on day 21, 24 hr after receiving their 20th (and final) injection of PEA. Results with amphetamine are illustrated in Fig. 3. Mice maintained on PEA were significantly more sensitive to the stimulation produced by amphetamine than were controls; this was observed at both a mildly stimulatory (1 mg/kg) and a normally-inactive (0.5 mg/kg) dose of amphetamine. Similarly, mice in the long-term PEA group were more sensitive to the stimulation induced by PCP, as illustrated in Fig. 4.

Metabolism

In order to investigate a possible mechanism for these effects, consideration was first given to potential changes in drug metabolism. Since it is known that the stimulatory effects of PEA are intensified by inhibition of the B form of MAO [8], it could be rationalized that the apparent sensitization is the result of a decrease in MAO activity with a concomitant elevation in brain PEA levels. Data summarized in Table 1 suggests that alterations in MAO are not responsible for the increased effect of PEA. There was no change in MAO-B activity in either the brain or liver of the mice receiving PEA. Similar results were obtained with MAO-A (data not shown).

Similarly, the apparent sensitization to amphetamine or PCP could have been due to a drug-induced inhibition of hepatic microsomal drug metabolism. As summarized in Table 2, this also appears to be an unlikely mechanism for the increased pharmacological effect of the drugs. There was a small, statistically significant increase in aniline hydroxylase activity in livers obtained from the PEA groups with no change in O-demethylase activity. Clearly, there was no evidence for an inhibition of drug metabolism which might account for the observed behavioral effects.

DA Receptors

Since metabolic factors could be ruled out as explanations for the heightened pharmacological effects resulting from long-term PEA, a neurochemical rationale would appear more likely. In this regard, the binding of spiroperidol to putative DA receptors in the CNS was compared in the control and PEA group. These results are illustrated in Table 3. As the analysis indicates, the number of spiroperidol-labelled binding sites was increased by the long-term PEA administration. There was no change in the apparent affinity of spiroperidol for its binding site after this treatment.

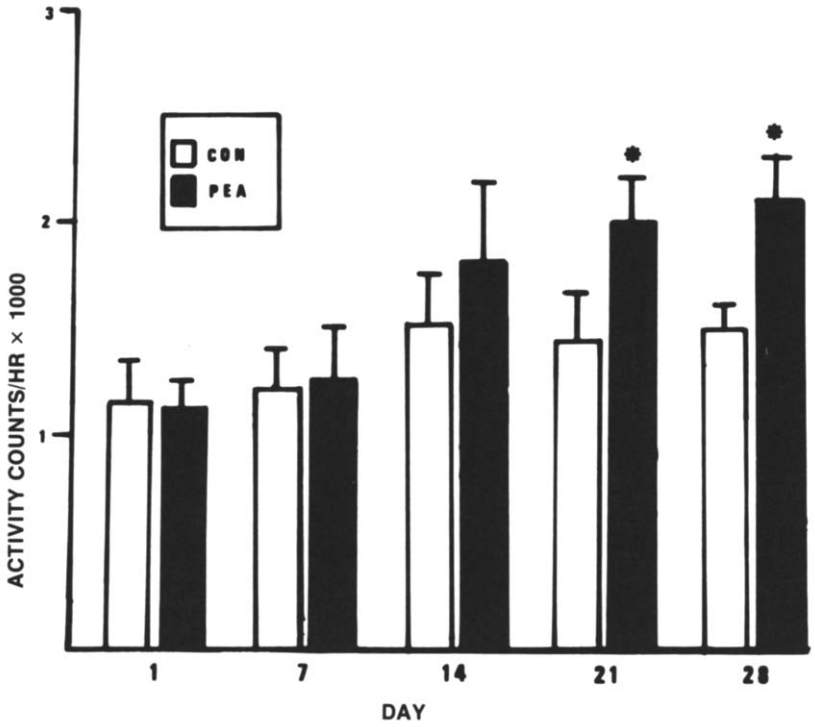

FIG. 2. Time course of sensitization to PEA-induced increase in motor activity. Different groups of mice were injected daily with saline (open bar) or PEA (50 mg/kg; filled bar). On the test day (indicated in the abscissa), they were challenged with PEA (25 mg/kg, SC) and motor activity was measured. *Indicates groups in which the activity after the PEA injection was significantly greater than in the corresponding control group.

DISCUSSION

The results of this investigation illustrate that mice receiving daily injections of PEA became sensitized to the motor stimulation produced by the drug and that the sensitization is generalized to amphetamine and PCP as well. Previously, Borison and coworkers [1] showed that daily injections of PEA to rats resulted in the development of stereotyped behaviors. We also observed stereotyped behaviors, particularly head-bobbing and chewing, in our mice in the latter stages of the experiment.

The sensitization to PEA developed slowly, with significant changes observable after 21 days of injection. Previously, sensitization to amphetamine with long-term treatment has also been reported [6]. Since cross-sensitization to amphetamine and PCP was observed after PEA treatment, it may suggest that a common mechanism underlies the effects of these agents.

Since metabolic factors (i.e., changes in either MAO or in hepatic mixed function oxidase activity) would not appear to be responsible for the sensitization, it is reasonable to expect a change in the function of a CNS neurotransmitter. We examined the dopamine (DA) system because of its role in the pharmacology of CNS stimulants [14]. The binding of spiroperidol to putative DA receptors in the striatum was increased in the long-term PEA group. This suggests that the DA receptor supersensitivity may develop as a result of the PEA treatment.

If the PEA treatment does increase the number of available DA receptors, then drugs which increase presynaptic DA release might be expected to produce greater behavioral activity in mice chronically exposed to PEA. Since increased DA release has been suggested as a mechanism for PEA [17], amphetamine [14] and PCP [10], it could, therefore, explain the increase in activity which we observed. Furthermore, Karoum and coworkers [11] have found that DA turnover is enhanced by chronic PEA treatment, suggesting that multiple factors (both pre and postsynaptic) may contribute to heightened dopaminergic synaptic activity.

Other factors must also be taken into consideration before accepting this explanation. For example, it is not yet known whether the striatum represents the site of action for PEA-induced motor stimulation. Consequently, DA receptors in other brain regions may show different effects. However, it is interesting to note that stereotyped behavior induced by PEA is also increased after prolonged treatment, suggesting that striatal DA receptors may indeed be functionally altered by PEA administration. The potential involvement of other neurotransmitters, such as serotonin [5], also cannot be ignored.

It is more difficult to explain why DA receptors would increase in response to chronic administration of PEA, which should be equated with persistent stimulation of the receptor by DA. Other indirectly acting DA agonists have been reported to decrease spiroperidol binding after long-term administration [4,19]. On the other hand, DA agonist binding has been reported to increase with long-term amphetamine [19], while chronic amantadine increases spiroperidol binding [9]. It is also interesting to note that

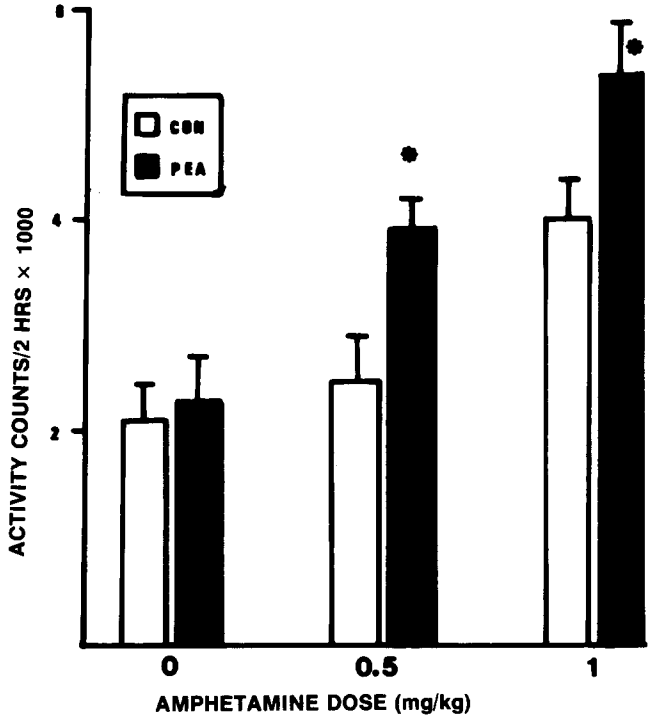

FIG. 3. Effect of amphetamine on motor activity in mice after chronic PEA treatment. Mice were injected daily with saline or PEA (50 mg/kg) for 20 days. On day 21, they were challenged with saline (0 mg/kg dose) or amphetamine and motor activity was measured. *Indicates activity which is significantly different in the chronic PEA group compared with the same dose in the chronic saline group.

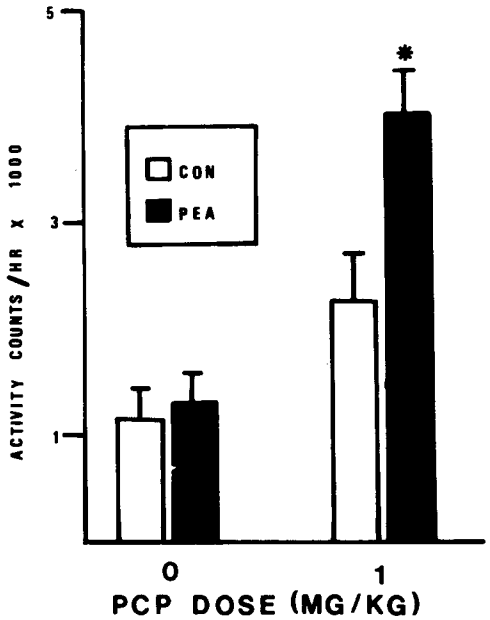

FIG. 4. Effect of PCP on motor activity after chronic PEA treatment. Mice were injected daily with saline or PEA (50 mg/kg) for 20 days. On day 21, they were challenged with saline or PCP (1 mg/kg) and motor activity was measured. *Indicates that significantly greater stimulation was observed in response to the PCP injection in the group receiving daily PEA injections than in the group receiving daily injections of saline.

TABLE 1

EFFECT OF REPEATED ADMINISTRATION OF PEA ON HEPATIC AND BRAIN MAO-B ACTIVITY*

Group	MAO Activity (nmoles/mg/hr) Liver	Brain
Control	838 ± 398	526 ± 123
PEA	758 ± 87	482 ± 42

*Mice were injected daily with saline or PEA (50 mg/kg/day) for 21 days as described in text. Brains and livers were removed 24 hr after the last injection for measurement of MAO activity using ^{14}-C PEA as substrate (N = 5).

TABLE 2

EFFECT OF REPEATED PEA ADMINISTRATION ON HEPATIC MIXED FUNCTION OXIDASE ACTIVITY*

Treatment	MFO Activity (μg product/mg pro/hr) O-Demethylase	Aniline Hydroxylase
Control	33.9 ± 2.8	6.9 ± 0.3
PEA	33.4 ± 2.0	9.3 ± 0.9†

*Mice were treated for 21 days as described in the text and livers were removed for measurement of enzymatic activity 24 hr after their last injection. †Designates values significantly different ($p<0.05$) from control (N = 5).

TABLE 3

EFFECT OF REPEATED ADMINISTRATION OF PEA ON STRIATAL 3H-SPIROPERIDOL BINDING*

Treatment	Spiroperidol Binding Bmax	Kd
Control	410 ± 35	0.33 ± 0.07
PEA	476 ± 27†	0.39 ± 0.07

*Mice were treated for 21 days as described in text and striata were removed 1 day later for measurement of specific spiroperidol binding. Bmax is expressed as fmoles bound/mg protein while Kd is nM. Values represent means ± S.E.M. from 6 separate experiments on different pools of mice; †designates values significantly different ($p<0.05$) from control.

Karoum and coworkers [11] have suggested that PEA and amphetamine may have opposite effects on DA turnover following chronic administration. These curious phenomena require further investigation.

Although more speculative, it is tempting to add to the suggestion that PEA may be involved in the development of schizophrenia. If an increase in endogenous PEA activity was to occur in patients, due to for example decreased MAO activity [7,22], altered DA receptor function might result. On the basis of the results described with exogenous PEA administration, this effect would be to increase DA receptors. This outcome would, of course, be ameliorated by DA antagonist neuroleptic drugs.

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