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Phenylalanine, brain phenethylamine and motor activity in the rat

S. ROBERT SNODGRASS

Neurology Research Laboratory, Children's Hospital Medical Center, 300 *Longwood Avenue, Boston, Massachusetts* 02115, *U.S.A.*

Varying doses of L-phenylalanine were administered intraperitoneally to rats and motor activity determined by means of photocells in an activity cage. Control rats, and rats whose brain catecholamines were depleted by reserpine, α-methyl-*p*-tyrosine, or intraventricular 6-hydroxydopamine showed a decrease or no change in motor activity following phenylalanine injections. Injections of the decarboxylase inhibitor, Ro 4-4602, failed to alter the effect of phenylalanine. After injections of [^{3}H]phenylalanine, [^{3}H]β-phenethylamine accounted for less than 5% of brain tritium and radioactivity associated with this amine declined rapidly with time. The proportion of brain radioactivity due to β-phenethylamine was increased by the monoamine oxidase inhibitor, pargyline, and was not affected by the peripheral decarboxylase inhibitor Ro 4-4602. When Ro 4-4602 and pargyline were given together, the increase in radioactive phenethylamine produced by pargyline was prevented, suggesting that a significant proportion of brain phenethylamine may be synthesized in the periphery and can enter brain if monoamine oxidase is inhibited.

β-Phenethylamine (PEA) has been detected in the brain and peripheral tissues of man (Levine, Nirenberg & others, 1964; Inwang, Mosniam & Sabielli, 1973) and animals (Nakajima, Kakimoto & Sano, 1964; Jackson & Temple, 1970; Saavedra & Axelrod, 1973; Snodgrass, unpublished) and has been proposed as a possible neurotransmitter (Fischer & Heller, 1972; Saavedra & Axelrod, 1973; Sabell, & Giardina, 1973). PEA is a weak stimulant with an amphetamine-like effect on motor activity (Mantegazza & Riva, 1963; Fischer, Ludmer, & Sabelli, 1967; Jackson, 1972; Fischer, 1972). Fischer & others (1967) suggested that the motor effects of amphetamine were related, not to catecholamines, but to release of PEA from sites in brain. Fischer & Heller (1972) reported that low doses of PEA (2·5 mg kg^{-1}) increased exploratory motor activity in mice, and that its precursor, L-phenylalanine, also produced an increase in motor activity, as well as antagonizing the depression of motor activity by L-dopa. Fischer & Heller further suggested that PEA has a direct effect on receptors, in addition to possible indirect effects produced by release of endogenous catecholamines (Jackson & Smythe, 1973). Because Fischer's report has stimulated wide interest, and because phenylalanine loading is often used to simulate the abnormalities of the disease phenylketonuria (Knox, 1966; McKean, Boggs & Peterson, 1968; Lowden & LaRamee, 1969), I have studied the effects of injections of L-phenylalanine upon motor activity of rats, as well as the formation of radioactive PEA and phenylethanolamine (OHPEA) from [^{3}H]phenylalanine. Rats were studied under a variety of conditions, but Fischer's observations could not be extended to the rat.

METHODS

Male Charles River rats, 100–200 g were given free access to food and water except while in the motor activity testing apparatus. The apparatus was a model 1497 Lehigh Valley Electronics Corp. activity cage, which measures motor activity by the interruption of light beams. Activity was recorded every 10 min for a total of 50 min and the activity for the last 40 min used in this report. This method of data analysis was used because response rates were more variable in the first 10 min. Rats were always placed in the cage for 15 min on the day preceding any drug testing, to reduce the novelty of the testing apparatus. On the day of testing, rats were first placed in the cage for 50 min for recording of baseline motor activity. They were then removed, injected with the phenylalanine in a volume of 0·4–0·45 ml, and after 3 min were returned to the apparatus for an additional 50 min. Because of variability in the base-line motor activity of rats, post-injection motor activity was always expressed as the percentage change from the baseline activity for that individual animal.

All drugs were given intraperitoneally unless otherwise indicated. Reserpine was given 18 h before testing; the drug Ro 4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzyl)hydrazine], pargyline, and α-methyl-*p*-tyrosine were given 2 h before testing. Rats treated with 6-hydroxydopamine were given two injections of 250 μg of the base, dissolved in 0·1% ascorbic acid-saline solution three days apart, into a lateral ventricle under ether anaesthesia. Testing was done 12–20 days after the second injection.

Chemical analysis. Brains were removed from rats after decapitation, and were homogenized in 10% trichloroacetic acid containing 0·2% sodium metabisulphite. After a single ether extraction, the aqueous homogenate was applied to a 6 × 25 mm column of Dowex 50 × 4, 200–400 mesh, H^+ form, the column then being washed with distilled water, 50% ethanol, 0·2M sodium phosphate, pH 7·5, to elute phenylalanine, and 3M HCl in 40% methanol to elute PEA and OHPEA. Recoveries were checked by adding [^{14}C]phenethylamine (New England Nuclear Corp.) and phenylethanolamine to brain homogenates and were found to be 89·6 ± 1·8 and 91·1 ± 2·1% respectively (mean ± s.e.m., n=4). Aliquots of the column eluates were added to scintillation vials, mixed with 10 ml of Triton X-100–Toluene–Omniflour (New England Nuclear Corporation) solution and counted in a liquid scintillation counter with quench correction by the external standard channels ratio method. Other aliquots of the eluates were taken to dryness in a Buchler evapo-mix, resuspended in 95% ethanol and spotted on silica Gel G thin-layer plates for chromatography using diethylamine–pyridine–amyl alcohol (10:12:55) as the developing solvent. Phenylacetic acid, phenylalanine, PEA and OHPEA, were located by reference to spots of standards which were run in additional lanes of the chromatogram, and the corresponding areas of the chromatograms scraped into counting vials and counted.

Sources. L-phenylalanine [^{3}H] (1·0 Ci mmol^{-1}) was purchased from New England Nuclear Corporation, 6-hydroxydopamine hydrobromide and α-methyl-*p*-tyrosine methyl ester from Regis Chemical Co., and PEA, OHPEA, and reserpine from K & K Chemical Laboratories, Plainview, N.Y.

Statistical analysis. Because ratios of two quantities are not usually normally distributed (Colquhoun, 1971) and because of the small number of animals per group,

differences between groups were analysed by means of the Mann-Whitney test (Conover, 1971).

RESULTS

Table 1 indicates that administration of L-phenylalanine, either alone, or in combination with various pretreatments, did not increase motor activity as measured in the activity cage. This Table expresses the effect of drug injections as percentage change in baseline motor activity, where absolute values of motor activity are given for the baseline period. Expression of the data as relative change seems preferable because the variability in baseline activity between individual rats may cause absolute values to be misleading. As an example, phenylalanine after α-methyl-*p*-tyrosine caused the group mean activity to change from 14 ± 3 to 18 ± 7 but the mean percentage change was −1·9 ± 5·6%. In high concentrations, phenylalanine produced small but reproducible decreases in motor activity. Phenylalanine did not antagonize the effects of reserpine or α-methyl-*p*-tyrosine, and when combined with a monoamine oxidase inhibitor (pargyline) it did not increase motor activity beyond the modest increases already evident in these pargyline-treated animals when compared to their

Table 1. *Effect of L-phenylalanine upon motor activity in rats.* In part A, motor activity for each group of rats, recorded as described in the text, is expressed as the total number of counts or crossings registered by the photo cells of the activity cage over 40 min. After recording initial motor activity, the rats were given a second drug injection and the relative change in subsequent motor activity is shown in part B.

Treatment	Relative change in motor activity (mean ± s.e.)
A. Baseline motor activity in counts per 40 min period	
Controls (saline injection)	258 ± 26 (9)
Reserpine 3 mg kg^{-1}	41 ± 6 (4)*
Reserpine 10 mg kg^{-1}	20 ± 4 (4)*
Ro 4–4602 100 mg kg^{-1}	227 ± 40 (4)
DL-α-Methyl-*p*-tyrosine methyl ester 250 mg kg^{-1}	14 ± 3 (6)**
Pargyline 75 mg kg^{-1}	328 ± 45 (6)*
6-Hydroxydopamine treated rats	209 ± 37 (4)
B. Change in baseline motor activity	
Control (saline injection)	1·7 ± 2·9 (7)
L-Phenylalanine 25 mg kg^{-1}	4·7 ± 3·9 (5)
L-Phenylalanine 50 mg kg^{-1}	—01·3 ± 2·1 (4)
L-Phenylalanine 75 mg kg^{-1}	—12·9 ± 5·9 (3)
L-Phenylalanine 100 mg kg^{-1}	—25·0 ± 5·9 (5)*
L-Phenylalanine 140 mg kg^{-1}	—48·3 ± 10·5 (5)**
Reserpine 3 mg kg^{-1} + phenylalanine 75 mg kg^{-1}	—09·4 ± 2·4 (4)
Reserpine 10 mg kg^{-1} + phenylalanine 75 mg kg^{-1}	—18·1 ± 5·5 (4)*
Ro 4–4602 100 mg kg^{-1} + phenylalanine 75 mg kg^{-1}	—01·1 ± 2·7 (4)
DL-α-Methyl-*p*-tyrosine methyl ester 250 mg kg^{-1} + phenylalanine 75 mg kg^{-1}	—01·9 ± 5·7 (6)
Pargyline 75 mg kg^{-1} + phenylalanine 75 mg kg^{-1}	—09·5 ± 5·1 (4)
6-Hydroxydopamine rats + phenylalanine 50 mg kg^{-1}	—10·6 ± 4·8 (4)
6-Hydroxydopamine rats + phenylalanine 75 mg kg^{-1}	—29·9 ± 8·1 (4)*
Intraventricular phenylalanine, 300 μg	4·7 ± 4·5 (4)

With rats receiving intraventricular phenylalanine, motor activity is compared against controls receiving the same volume of saline. The numbers in parentheses indicate the number of rats used, and * means that a difference from pre-injection period (or control rats, for part A) is significant at the 0·05 level, ** means significant at the 0·01 level (Mann-Whitney test).

baseline motor activity before injection of pargyline. Phenylalanine was also injected intraventricularly under ether anaesthesia and motor activity then compared to that of controls receiving only saline. Both controls and phenylalanine-injected animals showed increased motor activity for the initial 15 min of recording, probably due to emergence from anaesthesia, but there was no significant difference between these two groups.

Table 2 shows the relative formation of ^{3}H-PEA and ^{3}H-OHPEA from [^{3}H]phenylalanine. Pargyline pretreatment resulted in an increase in the proportion of tritium found in the amine fraction, and increased the ratio of OHPEA to PEA. Pretreatment with the decarboxylase inhibitor, Ro 4-4602, did not produce a significant increase in the amount of ^{3}H-PEA or OHPEA. Intraventricular administration of [^{3}H]phenylalanine did not significantly affect the amount of radioactivity found in the two amines over that obtained after peripheral administration of the precursor ($0{\cdot}10 < P < 0{\cdot}05$).

Combined treatment with pargyline and the peripheral decarboxylase inhibitor blocked the rise in ^{3}H-PEA seen with pargyline alone. When the dose of phenylalanine was increased from 50 to 100 mg kg^{-1} no significant rise in ^{3}H-PEA occurred.

Table 2. *Brain content of tritiated amines after injection of [^{3}H]phenylalanine.* For PEA and OHPEA, the values given are the means of amine radioactivity divided by the total brain radioactivity, with the mean radioactivity per gram net weight in d min^{-1} given in parentheses. All values are the means of 4–6 experiments. Animals received intraperitoneal [^{3}H]L-phenylalanine, 15 μCi, diluted with cold phenylalanine to a final dose of 50 mg kg^{-1}, except for the last group which received 100 mg kg^{-1} phenylalanine and 30 μCi of [^{3}H]phenylalanine, and were killed at the times indicated following injection. Pargyline and Ro 4-4602 were given intraperitoneally 2 hours before the phenylalanine injection. In the case of intraventricular injections, rats were given 1 μCi of [^{3}H]L-phenylalanine, 10 mM concentration, in 25 μl of saline. Total brain radioactivity was 147 000 ± 19 000 for the rats receiving phenylalanine i.p. and killed at 30 min. * Difference from the 30 min control group is significant at the $P<0{\cdot}05$ level by Kruskal-Wallis analysis of variance (Conover, 1971).

Pretreatment	Time to killing	^{3}H-PEA	^{3}H-OHPEA
None	15 min	3·8 ± 0·5% (9340)	0·1 ± 0·1% (220)
None	30 min	2·8 ± 0·4% (4830)	0·9 ± 0·3% (870)
None	60 min	1·8 ± 0·3 (2040)	0·9 ± 0·4% (870)
None	90 min	0·8 ± 0·2 (840)	0·3 ± 0·1% (330)
Pargyline 75 mg kg^{-1}	30 min	6·0 ± 1·0% (9820)*	2·4 ± 0·4% (3220)*
Ro 4–4602 100 mg kg^{-1}	30 min	3·5 ± 1·2% (6290)	0·5 ± 0·4% (830)
Ro 4–4602 100 mg kg^{-1} + pargyline 75 mg kg^{-1}	30 min	3·0 ± 0·7 (4850)	1·7 ± 0·5% (2670)
Ro 4–4602 100 mg kg^{-1} + pargyline 75 mg kg^{-1} + phenylalanine 100 mg kg^{-1}	30 min	3·6 ± 0·8 (6120)	2·4 ± 907% (4100)
Intraventricular phenylalanine	30 min	4·9 ± 1·7% (18 700)	0·7 ± 0·4% (2790)

DISCUSSION

These data indicate that PEA and OHPEA are found in rat brain after administration of labelled phenylalanine. The work of Oldendorf (1971) indicates that PEA enters brain slightly more readily than does phenylalanine, when both are injected into the rat carotid artery. The possibility exists that PEA and OHPEA are formed peripherally and then enter the brain. However, it is likely that most PEA and OHPEA formed in peripheral tissues would be degraded by monoamine oxidase, for which both are good substrates (Yang & Neff, 1973). Comparison of intraperitoneal and intraventricular administration (Table 2) shows that PEA and OHPEA account for a greater proportion of brain radioactivity after intraventricular than after systemic administration of [^{3}H]phenylalanine. The fact that Ro 4-4602 blocked the increase in ^{3}H-PEA associated with pargyline administration ($P < 0{\cdot}05$) suggests that significant quantities of brain PEA are formed peripherally and then enter the brain if protected from monoamine oxidase. The fact that ^{3}H-OHPEA in brain was not altered by adding Ro 4-4602 may mean that only PEA synthesized in brain can be converted to OHPEA. Edwards & Blau (1973) found a much greater increase in brain PEA after pargyline administration than I have found, but these workers gave much larger doses of phenylalanine (1g kg^{-1}).

The work of Mantegazza & Riva (1963) indicated that PEA was much more potent as a motor stimulant in mice than in rats. Possibly, other metabolites are produced which negate any stimulatory effect of PEA in the rat. An obvious possibility is OHPEA, which appears to mildly inhibit motor activity in the rat (Snodgrass, unpublished observations). The present study provides no evidence for a direct effect of PEA produced from phenylalanine injections on motor activity in the rat and suggests that phenylalanine is not a factor in regulating motor activity in this species. The apparent difference in the motor response of different rodents to PEA remains unexplained. Because phenylalanine is involved in so many metabolic pathways, the failure to observe an effect of phenylalanine upon any behaviour, such as motor activity, does not exclude a role for PEA and OHPEA in the behaviour.

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