Vol. 86, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1979 Pages 48-56

THE EFFECT OF β-PHENYLETHYLAMINE ON BEHAVIOR AND POLYRIBOSOMES IN THE RAT

Martin M. Widelitz

Research and Development Service, VA Medical Center, Coatesville, PA 19320 and Departments of Psychiatry and Human Behavior and Pharmacology, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107

Received November 14, 1978

SUMMARY: β-phenylethylamine which has been reported to induce a behavior state in rats similar to paranoid schizophrenia in humans was found to disrupt polyribosomes. The effect on polyribosomes is similar to that found with amphetamine. The behavior response, however, is different and it is suggested that serotonin rather than dopamine may be responsible for this response.

INTRODUCTION: It has been shown that amphetamine can induce psychosis in man as well as a stereotypic state in animals (1, 2, 3, 4). It has further been shown that this class of monoamines has the capacity to disaggregate polyribosomes in animals temporally coincidental with the stereotypic state (5, 6) and inhibit *in vitro* protein synthesis directed by synthetic as well as natural mRNA (7, 8). There have been several explanations for the mechanism found in these events (9, 10, 11, 12) but to date unequivocal data elucidating this problem is lacking.

More recently it has been reported that the naturally occurring sympathomimetic amine β-phenylethylamine (PEA) can also induce behavioral effects similar to amphetamine induced stereotypic behavior (13, 14). We have undertaken a study to verify the behavioral events and observe if PEA could in fact induce polyribosome disaggregation and inhibit protein synthesis. We also investigated the role of several classes of neuroleptics in preventing and/or reversing the behavioral and polyribosomal effects of PEA.

MATERIALS AND METHODS: Charles River CD rats weighing between 250-350 gm were individually housed at room temperature and were maintained on a 12:12 hour (7 a.m. - 7 p.m.) light-dark schedule with Purina laboratory chow and water continuously available. β-phenylethylamine (PEA) (1 gm/ml free base) (Sigma) was

0006-291X/79/010048-09$01.00/0

diluted to selected concentrations with saline and was injected intraperitoneally on a mg/kg basis at a dosage ranging from 25-100 mg/kg. Two neuroleptics, haloperidol and chlorpromazine, were utilized to test inhibition before and reversal after the injection of PEA of the behavioral phenomenon and the disaggregation of polyribosomes. These drugs were also administered intraperitoneally.

When the neuroleptics were administered first the PEA was not injected until the animals were in a very tranquil, hyporeactive state. This took place in about 5-10 min. They did respond to noise such as snapping of fingers, falling of objects, however, and manifested a startle reaction even if somewhat less acute than normally. When the PEA was the initial drug administered the neuroleptics were not injected until a behavioral change had been effected[1]. This behavioral reaction was dose dependent. The effect commenced in 5-10 min. and was maximal at 15-30 min. The animals on the average became quiescent in 5-10 min. after the injection of the neuroleptics. After they were tranquil following injection of the neuroleptics, the animals were then anesthetized and decapitated. Brains were homogenized in buffer containing 25 mM Tris-HCl (pH 7.5), 5 mM Mg $(CH_3COO)_2$, 5 mM 2-mercaptoethanol, 100 mM KCl and 10% Sucrose (w/v). Polysomes were pelleted through 2 M Sucrose as previously described (5), suspended in buffer containing 25 mM Tris-HCl (pH 7.5), 5 mM Mg $(CH_3COO)_2$, 100 mM KCl and 5 mM 2-mercaptoethanol and utilized for velocity sedimentation in sucrose gradients. One to 2 ml polysome samples containing 6.0 to 12.0 A_{260} units were layered on 10-35% linear sucrose gradients of 29 ml volume containing 25 mM Tris-HCl (pH 7.5), 100 mM KCl and 3 mM $MgCl_2$. Centrifugation was carried out in an SW 25 rotor of a Beckman L2-50 ultracentrifuge at 36,000 x g x 2-1/2 hours (3-5° C.). Gradients were fractionated using a model UA 5 ISCO density gradient fractionator and monitored at 254 nm.

RESULTS: A total of 55 CD rats were tested. Twenty-seven rats were examined for a behavioral change pattern and polyribosomal disaggregation after intraperitoneal injection of PEA in the following concentrations: 50, 75, and 100 mg/kg. Several rats were tested at 25 mg/kg but the effects were negative in inducing behavioral change and polyribosomal disaggregation.

As we reported previously with amphetamine (5) the PEA treated animals showed increased amounts of 40 S and 60 S subunits and a significant rise in the 80 S monomer. The optical density in the polymeric region is significantly reduced as compared with the control profile. As was also shown previously the treatment and control groups differ to a significant degree as shown in Table 1 and this is again observed to be dose dependent (see Table 1).

[1] Behavioral change is more appropriate than stereotypic state as one notes in the text. A complex behavior state was observed where stereotypies was only one element of a behavioral pattern of our treated rat population.

Table 1. Polyribosomal content of polyribosomal preparations at various doses of PEA

No. of Rats Tested	Dose (mg/kg)	Polymeric Particles (%) (SD)
6	0	78.2 ± 4.7
9	50	61.0 ± 14.6
8	75	47.1 ± 22.9
4	100	46.0 ± 26.3

SUMMARY: Four groups of rats were given PEA in doses of 0, 50, 75, and 100 mg/kg, respectively. The percentage of polymeric particles was calculated for each rat. These percentages are presented with their standard deviations in Table 1. The percentages (X) were then converted to arcsines (ϕ) by the transformation $\phi = 2 \arcsin \sqrt{X}$ in order to stabilize the within-group variances. An analysis-of-variance was applied to the arcsin data (Table 2) and an over-all significant difference between treatment groups was found ($F = 3.80$, $p < .05$). Post hoc comparisons of the group means revealed that the 75 and 100 mg/kg dose groups did not differ from one another and were therefore combined for subsequent comparisons; this combined sample of 12 rats (75 + 100 dose groups) also did not differ significantly in the percentage of polymeric particles from the 50 mg/kg group ($F = 2.71$, $p > .10$) but showed a significantly lower particle rate than did the zero dose group, i.e. than the untreated control group ($F = 10.82$, $p < .01$). Finally, the 50 mg/kg group also showed a lower particle rate than did the zero-dose control group but the difference here was at a lesser confidence level ($F = 3.13$, $p < .10$).

We assessed the protection by the neuroleptics haloperidol and chlorpromazine in inhibiting stereotypic behavior and polyribosomal disaggregation. Haloperidol in doses ranging from 10-30 mg/kg was injected intraperitoneally 30 min. before PEA in doses ranging from 50-100 mg/kg was injected by the same route. We utilized the criteria of greater than 68% absorbance in > 110 S region as intact polysomes. At all dosage levels of PEA given subsequent to haloperidol, 5 of 15 rats were protected, not manifesting any behavioral aberrations. Of 8 rats given haloperidol in doses of 5-25 mg/kg before PEA in doses of 50-75 mg/kg was injected, 3 died, 3 manifested behavioral change while 2 showed no change in behavior. Haloperidol was not as effective as chlorpromazine at the 50 and 75 mg/kg dose levels of PEA in preventing behavioral aberrations as noted in Table 2 (see Table 2). When utilizing chlorpromazine as the protective neuroleptic at 5 and 75 mg/kg dose, only 1 of the 7 rats tested showed an ab-

Table 2. Effect of PEA on behavior and polyribosomes following injection of haloperidol and chlorpromazine

Halo-peridol mg/kg	PEA mg/kg	Abnormal Behavioral Response	Percent Polymeric Particles	Chlor-promazine mg/kg	PEA mg/kg	Abnormal Behavioral Response	Percent Polymeric Particles
10	50	+	60	25	50	0	61
25	50	Convulsion and death	--	25	50	+	57
25	50	+	60	50	50	0	79
10	50	+	57	50	50	0	62
10	50	0	70	--	--	--	--
5	50	0	74	--	--	--	--
25	75	died	--	100	75	0	70
25	75	died	--	50	75	0	75
--	--	--	--	50	75	0	63
10	100	+	46	50	100	+	54
25	100	+	56	50	100	+	60
25	100	0	66	100	100	+	47
30	100	+	50	100	100	+	63
30	100	+	55	100	100	+	72
30	100	0	68	100	100	+	67
30	100	0	70	--	--	--	--

normal behavior pattern. At 100 mg/kg of PEA, however, haloperidol was more effective in preventing behavioral change than chlorpromazine where doses of this neuroleptic up to 100 mg/kg did not prevent severe abnormal behavior patterns in any of the rats tested, while 3 of 7 rats given haloperidol in doses ranging from 10-30 mg/kg did not show behavioral changes.

We note excellent correlation between the behavioral changes and polyribosomal disaggregation in utilizing haloperidol as the neuroleptic. This is not quite as consistent with chlorpromazine where we note some disparity between the behavioral response and the state of aggregation of polyribosomes in 4 of the 13 rats tested.

Utilizing chlorpromazine in doses ranging from 25-50 mg/kg after inducing an abnormal behavior state in all the rats tested we saw a return to the tranquil state in only 50% of the rats (see Table 3). The correlation between the behavior pattern and percentage of polymeric particles was consistent in 6 of the 8 cases or 75%. When one utilized haloperidol as the neuroleptic in attempting to reverse abnormal behavior we see a return to a tranquil state in only 2 of 6 cases or 33%. Again utilizing 68% absorbance in greater than 110 S region we note good correlation between percentage of polymeric particles and the behavioral state in 66% of the rats tested.

DISCUSSION: It has been shown that phenylethylamine (PEA) can induce a stereotypic behavioral state in animals when given both acutely and chronically (13, 14). Our experience relates solely to the acute administration of PEA to rats. We as well as others have found (13) that in comparing this substance with amphetamine the onset of the behavior state with PEA is more rapid (3-5 min.) as compared to 10-30 min. for amphetamine and of shorter duration (15-30 min.) as compared to 2-3 hours for amphetamine. Moja reported (13) they could not elicit a stereotypic state in rats with doses of PEA less than 64 mg/kg when given alone. In our experience utilizing a single intraperitoneal dose of as little as 50 mg/kg we could consistently induce behavioral change. Borison and Diamond (14) administering 50 mg/kg of PEA and 3.75

Table 3. Effect of neuroleptics on reversal of behavior and disaggregation of polyribosomes following induction of these events by PEA

Haloperidol					Chlorpromazine				
PEA mg/kg	Haloperidol mg/kg	Abnormal Behavior	Behavior Reversal	Percent Polymeric Particles	PEA mg/kg	Chlorpromazine mg/kg	Abnormal Behavior	Behavior Reversal	Percent Polymeric Particles
50	5	+	yes	50	75	50	+	no	30
50	5	+	yes	26	75	50	+	no	60
50	5	+	no	28	75	25	+	no	73
100	10	+	no	62	75	25	+	yes	67
100	10	+	no	64	100	50	+	no	55
75	10	+	no	30	100	50	+	yes	82
--	--	--	--	--	50	25	+	yes	73
--	--	--	--	--	50	25	+	yes	58

mg/kg of amphetamine chronically on a daily basis produced a sensitization to their behavioral actions even when a single dose may be subthreshold.

Our experience with the acute single dose administration of PEA suggests differences in the behavior pattern elicited by amphetamines and PEA. In the amphetamine treated animals initially we usually observed increased sniffing, occasionally licking the cage, then head bobbing, pressing against the cage, and with very toxic doses even retropulsive activity. With PEA one of the early effects is straubing of the tail. This refers to a rigid plasticity where the tail is extended full length and often raised perpendicular to the horizontal plane. Almost simultaneously with this effect we see an abduction of the hind quarters of the animals, as well as tremor. At times we also observed more classical signs of stereotypies such as increased sniffing and head motion already described as well as pressing against the side of the cage. Straubing of the tail, abduction of hind limbs as well as tremor have not been previously described with PEA. This has been noted, however, by Trulson and Jacobs (15) as well as Zemlan et al (16). This behavior syndrome (straubed tail, tremor, abduction of hind limbs, rigidity, reciprocal forepaw treading and lateral head weaving) was attributed to activity at central serotonin mediated synapses. These investigators utilized p-chloroamphetamine (PCA) to induce the serotonin release. Trulson and Jacobs (15) cite the following evidence supporting this thesis. L-tryptophan as well as 5-hydroxytryptophan in a monoamine oxidase inhibitor pre-treated animal both produce the syndrome. These precursor effects are blocked by serotonin synthesis inhibition while it is unaffected by catecholamine depletion. They also state that nialamide, an MAO inhibitor which increases brain serotonin produces the syndrome. The effects described above which were noted in 51% of our rats given PEA is dose dependent and was only seen where doses of PEA were 75 mg/kg or higher. One sees both classical stereotypic behavior such as sniffing, wire biting, licking of

the cage as well as the complex behavioral pattern described above. The PEA was found by mass spectrometry to be pure and thus one cannot attribute the complex syndrome we have seen to impurities or different chemicals other than PEA. It would appear that PEA may affect both dopaminergic and serotonergic systems. One must be cautious about observations made in depicting what appears to be involvement of two distinct systems. These two systems which are apparently involved after giving PEA may be important in over-all behavior patterns. It is thought that some brain function may be the result of a relative balance of activity between two or more regulators. This relationship is well known concerning cholinergic and dopaminergic activity in Parkinson's disease. There is some suggestion that in schizophrenia clinical manifestations might result from imbalance between dopamine and any of several neurotransmitters including γ-aminobutyric acid, acetylcholine and serotonin. As stated by Barchas et al (17), "A balance hypothesis involving dopamine and serotonin (or a methylated derivative of serotonin) has the effect of combining the two major hypotheses of schizophrenia into one." Each of the balance hypotheses about schizophrenia posits that while absolute activity of the dopamine system is either unchanged or increased its activity relative to some other system is relatively increased. Speculation as to the etiology of the observations noted here are possibly premature but one is intrigued by the possibility of activating both dopaminergic and serotonergic systems which may be based on the amount of PEA injected. At lower doses, 50-70 mg/kg, one might activate a more sensitive dopaminergic system leading to stereotypic behavior while with a higher dose serotonergic receptors may be activated leading to a behavioral pattern described above which is distinct from stereotypies.

ACKNOWLEDGMENTS: (1) This research was supported by U.S. Veterans Administration Project No. 9391-01. (2) The author thanks Dr. Charles C. Thornton for the statistical analyses.

REFERENCES

1. Randrup, A., Munkvad, I., and Usden, P. (1963) Acta Pharmacol. Toxicol., 20, 145-157.
2. Beamish, P. and Kiloh, L. G. (1960) J. Ment. Sci., 106, 337-343.
3. Ellinwood, E. H., Jr. (1967) J. Nerv. Ment. Dis., 144, 273-283.
4. Connell, P. H. (1958) In: Amphetamine Psychosis, Chapman and Hall, London. Pp. 11-133.
5. Widelitz, M. M., Coryell, M. R., Widelitz, H., and Avadhani, N. G. (1975) Brain Res., 100, 215-220.
6. Moskowitz, M. A., Weiss, B. F., Lytle, L. D., Munro, H. N., and Wurtman, R. J. (1975) Proc. Natl. Acad. Sci., USA, 72, 834-836.
7. Widelitz, M. M., Coryell, M. R., Widelitz, H., and Avadhani, N. G. (1976) J. Neurochem., 27, 471-475.
8. Baliga, S. B., Zahringer, J., Trachtenberg, M., Moskowitz, M. A., and Munro, H. N. (1976) Biochim. Biophys. Acta, 442, 239-250.
9. Taub, F. and Johnson, T. C. (1975) Biochem. J., 151, 173-180.
10. Appel, S. (1966) Trans. N. Y. Acad. Sci., 29, 63-70.
11. McKean, M. J., Boggs, D. E., and Peterson, N. R. (1968) Neurochemistry, 15, 235-241.
12. Baliga, S. B., Pronczuk, A. W., and Munro, H. N. (1968) J. Mol. Biol., 34, 199-218.
13. Moja, E. H., Stoff, D. M., Gillin, J. C., and Wyatt, R. J. (1976) Biol. Psychiat., 11, 731.
14. Borison, R. L. and Diamond, B. I. (1978) Biol. Psychiat., 13(2), 217-225.
15. Trulson, M. E. and Jacobs, B. L. (1976) Europ. J. Pharmacol., 36, 149-154.
16. Zemlan, F. P., Trulson, M. E., Howell, R., and Hoebel, B. G. (1977) Brain Res., 123, 347-356.
17. Barchas, J. D., Akil, H., Elliot, G. R., Holman, R. B., and Watson, S. J. (1978) Science, 200, 964-973.