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BRIEF COMMUNICATION

β-Phenylethylamine Effect on Brain and Blood Catechol-O-Methyltransferase Activity[1,2]

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HANNAN, C J , JR *β-Phenylethylamine effect on brain and blood catechol-O-methyltransferase activity.* PHARMACOL BIOCHEM BEHAV 24(4) 1141–1146, 1986 —A significant decrease in catechol-O-methyltransferase (COMT) activity has been found in the striatum (77% of control) and hippocampus (63% of control) of gerbils treated with daily injections of β-phenylethylamine (50 mg/kg) for 10 days This treatment group also exhibited increased (204% above control) COMT activity in a lysed red blood cell preparation There were no changes in COMT activity in groups receiving 10 mg/kg β-phenylethylamine or haloperidol (0 5 mg/kg) *In vitro* β-phenylethylamine has no demonstrable effect on COMT activity.

β-Phenylethylamine Catechol-O-methyltransferase Regional brain enzymes Red blood cell enzyme Gerbil

CATECHOL-O-METHYLTRANSFERASE (COMT, E.C. 2 1 1.6) primarily catalyses the extraneuronal catabolism of catechol containing neurotransmitters and has been evaluated as a biological marker for a number of diseases, including depression and schizophrenia, for which there are theories implicating involvement with catecholamine metabolism. There is evidence from animal and human studies that the activity of peripheral COMT is sensitive to the same influences as the brain enzyme and this has been used as justification in sampling a peripheral tissue for evidence of a central nervous system disease. Specifically, COMT has been measured in rat red blood cells (RBC) and found to be biochemically similar to the enzyme found in the rat brain [1]. Although COMT activity is under a degree of genetic control [16, 17, 41], its variability has also been proposed to reflect endogenous catecholamine transmitter activity in the brain [43]. Pregnancy and a few certain anemias have been reported to be associated with transient changes in RBC COMT activity [7], but activities are generally considered to be stable over time in an individual [11].

β-Phenylethylamine (PEA) is a trace amine (found in the brain in ng/g amounts) which has been called an endogenous amphetamine and in high concentrations has been proposed to induce a psychotic state with a more tentative involvement in the etiology of schizophrenia [5,44]. The biochemical relationship of the trace amines to the major neurotransmitters has been reviewed by Boulton and Juorio [6].

In order to examine the possibility that elevated endogenous PEA concentrations may induce changes in central and peripheral COMT activity, it was decided to determine if exogenous PEA could alter COMT activity in certain brain regions as well as RBCs. It has been determined that PEA readily penetrates the blood-brain barrier of the rat [30] and the demonstrated similar threshold dose for a behavioral response in the gerbil indicates this compound gains access to the gerbil brain as well. A stereotype was reliably produced in the rat [10] by PEA doses of 50 mg/kg or greater and was accompanied with an increased locomotor activity in a photocell monitored test cage. The effect of PEA on locomotor activity in the gerbils used in this study was monitored

[1]In conducting the research described in this report, the individual adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Research Council

[2]The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or Department of Defense

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TABLE 1

CATECHOL-O-METHYLTRANSFERASE ACTIVITY IN BRAIN REGIONS AND RED BLOOD CELLS OF PEA TREATED GERBILS*

	PEA (50 mg/kg)	PEA (10 mg/kg)	Saline
Hypothalamus	102 ± 11.5	93 ± 24.5	103 ± 23.5
Mesocortex	109 ± 22.5	138 ± 55.5	162 ± 44.5
Hippocampus	94 ± 10.5†	131 ± 39.5	148 ± 33.5
Mesolimbic cortex	138 ± 30.5	153 ± 23.4	123 ± 21.5
Striaum	121 ± 15.5†	162 ± 16.5	157 ± 21.4
Brain stem	128 ± 20.5	145 ± 69.5	138 ± 52.5
Red blood cells	98 ± 18.5†	42 ± 15.4	48 ± 7.3

*Tissue analyzed 90 min after the last of a ten daily 50 mg/kg injection of PEA Activity is expressed as ng 3-MT produced/min/gram fresh tissue weight or per ml packed red blood cells (mean value ± SD, number of animals)

†$p<0.01$, different from saline control by Student t-test after ANOVA among all three groups had a $p<0.05$

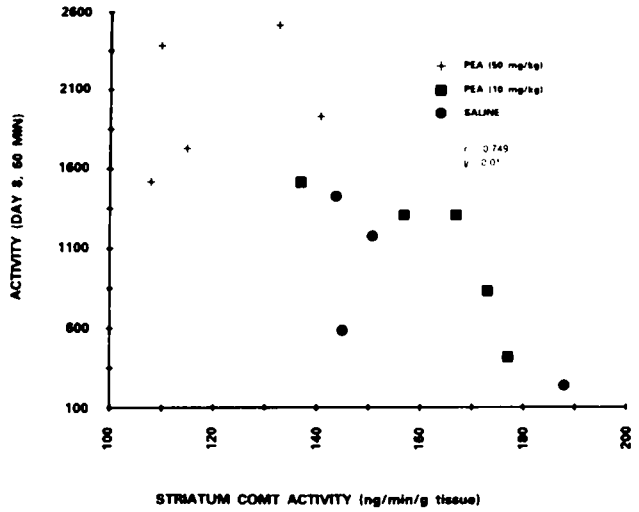

FIG 1 Scattergram of striatal COMT activity versus locomotor activity for 60 min during the 8th treatment day Each symbol is for one gerbil Correlation coefficient (r) and significance of correlation were calculated by the linear regression model software of the Hewlett Packard 9845C computer

during the administration period to provide an indicator of the behavioral potency of this treatment

An additional experiment was done to evaluate the effect of the antipsychotic drug haloperidol on COMT activity This dopamine blocking drug has been reported to not alter RBC COMT after chronic treatment in two independent studies [4,22], although a profound behavioral depression was produced as evidenced by near zero measures of locomotor activity in the present study This study reports regional brain COMT in addition to the RBC enzyme to determine if haloperidol has any effect on COMT in CNS tissues

METHOD

Experimental Procedure

Thirty-eight outbred male Mongolian gerbils (65–90 grams) were obtained from Tumblebrook Farms, Westbrookfield, MA and were caged individually in a temperature controlled room with a 12 hour light-dark cycle Three experiments using 15, 15, and 8 animals respectively were conducted In the first two experiments three groups of five animals were each given 10 morning intraperitoneal injections of either β-phenylethylamine (PEA), 50 mg/kg, PEA, 10 mg/kg or saline (5 ml/kg, as were the volumes to produce specified dosages of PEA) In the first experiment animals were killed 90 min after the last injection, while in the second group animals were killed 24 hr after the last injection. In the third experiment two groups of four animals were each given 10 morning intraperitoneal injections of either haloperidol (McNeil Pharmaceuticals, Spring House, PA), 0.5 mg/kg, or saline Animals in all experiments were placed in photobeam activity boxes (Coulburn Instrument Co , Layfayette, PA) immediately after injections (08.00 hr–10 00 hr) on days 1, 2, 3, 4, 5 and 8 of the 10 treatment days

On the tenth day and 90 min after the daily injection (experiment 1) or on the eleventh day, 24 hr after the last injection (experiment 2) gerbils were placed in a jar with halothane dampened gauze until they lost the righting reflex Approximately 10 seconds were required for this anesthesia, after which the gerbil was immediately removed and the chest opened for cardiac blood collection with a 3 ml heparinized vacuum tube The brain was then removed in approximately 75 seconds and placed in ice cold saline Blood was refrigerated until all specimens were collected, then processed as described below for a lysed red blood cell (RBC) preparation which was used for the COMT assay

Blood and Tissue Preparation

A 1 ml aliquot of gently mixed whole blood was transferred from each sample into 5 ml Nunc freezer tubes (Vangard International, Neptune, NJ) Blood was centrifuged (2,000 g, 10 min 4°C) and the plasma aspirated off RBCs were washed with 4 ml saline and centrifugally separated as above. Cells were lysed with the addition of 3 ml of distilled water mixed and stored at −80°C until use

Six brain regions were dissected from each hemisphere using the approach described by Heffner and co-workers [21]. The regions (1) hypothalamus, (2) mesocortex (frontal cortex and septum), (3) hippocampus, (4) mesolimbic cortex (nucleus accumbens and olfactory tubercle), (5) striatum (caudate-putamen and globus pallidus), (6) brain stem (pons-medulla) were used for COMT analysis Tissue for COMT assay was frozen (−80°C) in 1 5 ml polypropylene tubes for later assay Within one month these samples were prepared for assay by the addition of 0.5 ml cold 0 15 M KCl and sonicated approximately 10 sec with a model W185D Cell Disrupter (Heat Systems Ultrasonics, Plainview, LI, NY) at a 40 watt power setting The supernatant, after 8,800 g centrifugation for 10 min, provided the COMT enzyme used for assay

Enzyme Assay

A modification of the HPLC method described by Shoup

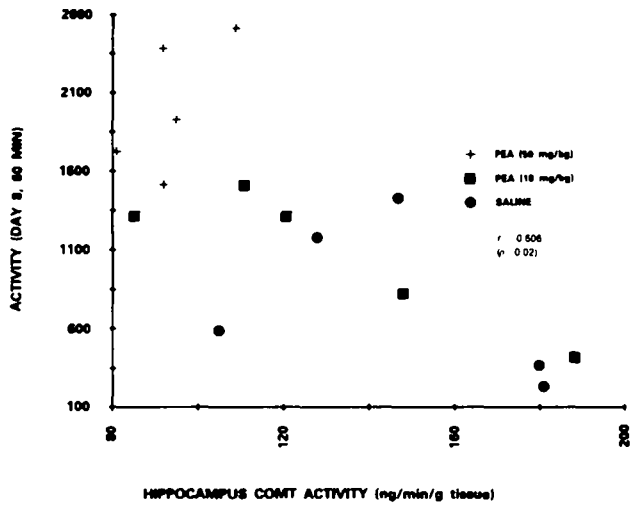

FIG 2 Scattergram of hippocampal COMT activity versus locomotor activity for 60 min during the 8th treatment day See Fig 1 for more details

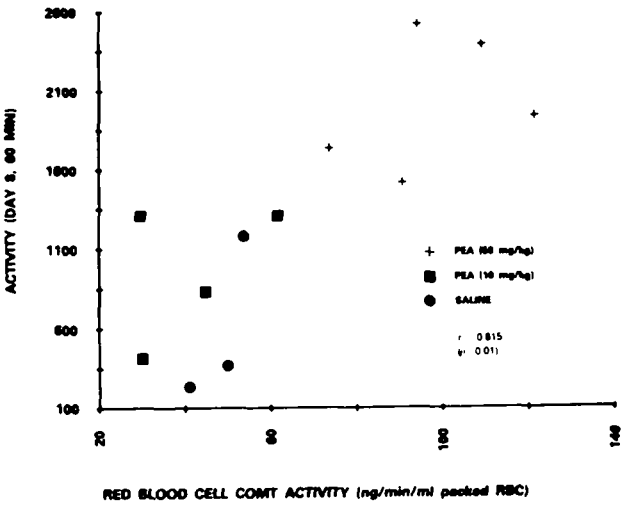

FIG 3 Scattergram of red blood cell COMT activity versus locomotor activity for 60 min during the 8th treatment day See Fig 1 for more details

and co-workers [38] was employed to estimate COMT activity. The assay incubation mixture consisted of 400 μl of enzyme preparation from either the brain or blood as described above, 50 μl of 0.05 M phosphate buffer pH 7.8, 25 μl of 10 mM SAM, 25 μl adenosine deaminase (3U), 25 μl of 25 mM pargyline, and 25 μl of 10 mM $MgCl_2$. The reaction was begun with 25 μl of 50 mM dopamine and incubated at 37°C for 100 min. Termination of the reaction with the addition of 50 μl of 4 M HCl containing 4.66 nmoles of 4-hydroxy-3-methoxybenzylamine as internal standard and mixing was followed by centrifugation at 8,800 g for 10 min. It should be noted that this preparation does contain membranes which have recently been shown to contain COMT activity [36] as well as the predominant soluble COMT activity. Due to the low Vmax of the membrane fraction COMT, it has been impossible to detect its product formation with this HPLC assay when the substrate concentration is optimized for its Km which is much lower than the soluble fraction COMT.

Four hundred microliters of the deproteinized supernatant were analyzed for 3- and 4-O-methyldopamine (3-MT and 4-MT) formation by adding it to 2.5 ml 4% borate/1% EDTA solution (pH 7.5) and adjusting to pH 6.5 with 2 N HCl. This was poured onto CG-50 cation exchange resin and washed with 3 ml distilled water. The column was washed with 0.8 ml and then eluted with 3 ml of 0.7 M H_2SO_4. This eluant was then injected on the HPLC column. The separation on a reversed phase HPLC column (Spherisorb 5 ODS, HPLC Technology, Palos Verde Peninsular, CA) and detection with an electrochemical detector (LC-4A from Bioanalytical Systems, Lafayette, IN) of the COMT metabolites were as described elsewhere [38] and were facilitated by a 3390A Recording Integrator (Hewlett Packard, Avondale, PA).

Evaluation of this method, which was originally developed for rat tissue, was made with pooled samples of gerbil brain and RBC lysate. The concentration of dopamine employed was five times greater than that necessary to produce the maximum rate of 3-MT production, while the SAM concentration was twice the estimated necessary. The incubation mixture was demonstrated to produce product linearly with time for up to 100 min. The 4.3×10^{-4} M concentration for Mg^{+2} was the optimum found in examining a range of from 10^{-3} to 10^{-6} M and agrees with other findings [19].

RESULTS AND DISCUSSION

The isolated brain regions of hypothalamus, mesocortex, hippocampus, mesolimbic cortex, striatum and brain stem had mean wet weights of 19, 71, 58, 24, 46 and 82 mg respectively. All COMT activity will refer to the amount of 3-O-methyldopamine (3-MT) produced per min per gram wet weight of tissue or per ml of packed RBC. The amount of 4-O-methyldopamine produced by COMT was also determined in each assay, but is not reported because it was found to not vary significantly among treatments. Activity of COMT did not vary significantly among the different brain regions in the saline treated group (Table 1). To evaluate the *in vitro* effect of PEA on the COMT assay, from 2 to 200 ng/ml final concentration PEA was added to the assay. No changes in COMT activity were observed.

No changes were observed in treatment groups measured 24 hr after the last of the ten daily PEA doses. All brain regions except the mesolimbic cortex from gerbils killed 90 min after treatment with the high dose (50 mg/kg) of PEA had lower average COMT activities than did those from saline control group animals. Statistical evaluation (ANOVA) of tissue COMT activity revealed significant differences among treatment groups ($p<0.05$) for RBC, hippocampus and striatum (Table 1). COMT activity from PEA (50 mg/kg) injected animals was lowered to 63% of the control values ($p=0.00390$, t-test) in the hippocampus and to 77% of control values ($p=0.00986$, t-test) in the striatum In contrast to the brain, COMT activity found in RBC preparations from these animals increased 204% over saline control ($p=0.00223$, t-test) The 10 mg/kg PEA treated group was not different from controls in either COMT activity or in the measure of

ambulation Because of plasma monoamine oxidase which rapidly inactivates systemic PEA, it is necessary to administer relatively high doses or use monoamine oxidase inhibitors in order to obtain behavioral effects

COMT activity in either the brain regions or RBC was not significantly different from saline control gerbils when haloperidol (0 5 mg/kg) was administered for 10 days. Locomotion in the haloperidol group was significantly lower ($p<0$ 05) than the saline control

Locomotion of the saline control group during the first day in the photobeam activity box was approximately twice that of all the subsequent days where it stabilized near 700 beam crossings in one hour The PEA (50 mg/kg) group exhibited significantly ($p<0.05$) increased locomotor activity by the third daily injection and continued to exceed the control group on all subsequent test days. A significant ($p<0$ 02) negative correlation was found between photobeam crossings measured on day 8 and COMT activity in the striatum and hippocampus (Figs 1 and 2) The COMT activity in RBCs was also highly correlated ($p<0$ 01) to photobeam crossings on the final test day, however, this was a positive correlation (Fig. 3)

The reported increase in rat brain dopamine metabolism after PEA administration [9,12] has also been observed in this laboratory with the gerbil (unpublished results) and is central to the proposal of PEA in models of psychiatric disease [5,44] Association between catecholamine turnover and COMT activity may seem likely based only upon their presence in the same metabolic pathway Indeed investigators have proposed that such a relationship may exist [2, 13, 20], although no evidence was presented

Our findings of lower extraneuronal COMT activity after chronic PEA administration appear unrelated to changes in catecholamine turnover produced by PEA in the striatum and is consistent with most reported evidence Specific dopaminergic system lesions in the striatum of the rat by either 6-hydroxydopamine [39] or coagulation of the substantia nigra [27] demonstrate no influence over COMT activity Central COMT activity is not altered by selective dopaminergic attenuation [27,39], or stimulation as illustrated in the present and other studies [4,22] Scant evidence that COMT activity may be regulated by substrate availability comes from reduced activities in submaxillary glands subsequent to reserpine or α-methyl-p-tyrosine administration [26] These two treatments involve at least both dopaminergic and noradrenergic neurons Although norepinephrine content in the striatum is relatively low, increases in its concentration after acute but not chronic PEA administration have been reported [23]

In view of the finding of intraneuronal membrane-bound COMT recently described by Roth and coworkers [36], activity changes of this enzyme must be evaluated to account for the total O-methylating capability Because the assay used in the present work measured only soluble, extraneuronal COMT, it remains to be determined if there is a response to PEA by intraneuronal COMT Influences other than substrate feedback of dopamine must be responsible for altering the level of soluble COMT activity The present work demonstrated that PEA produced no *in vitro* effect on soluble COMT activity

Genetic factors are important in determining the widely variable COMT activity in RBCs from a normal human population [17] Frequency distribution studies have shown that human COMT activity is bimodal [41] Two forms of COMT have been identified based upon thermolability [4,37] with the more thermolabile enzyme restricted to the low activity portion of the sample distribution The range of COMT activity in the gerbil is less than in the human The wide range of normal values, and sensitivity to genetic and post translational control mechanisms, make it difficult to evaluate different COMT activities as a biological marker in humans Biochemical variability among outbred animals such as the gerbil could be responsible for findings that appear independent of a treatment, however, the finding of significant effects makes the results more robust There was no evidence of a bimodal distribution of COMT activity in the gerbil RBC preparations as has been found in humans

It would seem from indirect evidence that the correlation between each of the PEA induced changes in RBC, striatal and hippocampal COMT activity with changes in locomotor activity (Figs 1, 2 and 3), are not involved with concentrations of the major amine neurotransmitters Measurement of turnover or levels of biogenic amines in the brain of various strains of rat at different times during the day have shown no relationship with the level of motor activity [24] Also, there is no relationship between plasma catecholamine concentrations and COMT activity [15] Although locomotor activity in the gerbil is much greater than in the rat [31], the circadian rhythm of biogenic amine concentrations is similar to that of the rat and is not correlated with activity [28]

Our finding that COMT can be experimentally altered both centrally and reciprocally in the RBC in response to PEA, indicate a possible association of COMT activity and those psychiatric diseases associated with high PEA production A variety of findings point to an association between PEA and psychotic disorders, such as the report of high PEA concentrations in the urine of paranoid schizophrenics [35] or the stress induced increased excretion of PEA occurring simultaneously with psychotic symptoms in predisposed individuals [31] Considering our demonstration of PEA altered COMT activity, it is disappointing to find a history of conflicting results when COMT was estimated in eight studies of psychotic patients reported in recent literature two studies found increased COMT activity [34,42], two found decreased activity [33,40], and four found no changes [3, 12, 18, 25] The two most carefully controlled studies [3,18] found no changes Part of the explanation for these varied results may be the diagnostic classification system used for clinical studies of psychiatric disease which may include different disease entities under a single name [14,29]

The regional brain changes in soluble COMT activity are interesting preliminary findings which must be further examined to include estimation of the intra-neuronal species of COMT The administration of PEA directly into the CNS may provide a better model in which to evaluate biochemical changes It is remarkable that PEA should alter the activity of soluble COMT which is so resistant to change

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