Arch Psychiatr Nervenkr (1982) 232:463–471

Archiv für Psychiatrie und Nervenkrankheiten
Archives of Psychiatry and Neurological Sciences

Phenylethylamine and Phenylacetic Acid in CSF of Schizophrenics and Healthy Controls

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Summary. Phenylethylamine (PEA) is an endogenous substance with amphetamine-like stimulant properties. On the basis of this ability an abnormal brain PEA metabolism has been proposed as an etiological factor in some forms of schizophrenia.

In the present study 28 schizophrenic patients and 15 healthy controls were investigated. No significant difference from control values was found in PEA concentration in cerebrospinal fluid (CSF) of either untreated or neuroleptic-treated schizophrenics. However, 2 schizophrenics with highest BPRS scores had extremely high PEA concentrations. Free phenylacetic acid (PAA), the major metabolite of PEA, was significantly decreased in ummedicated but not in drug-treated schizophrenics.

Because of the assumed neuromodulatory properties of PEA, it is suggested that lowered PAA concentrations and the tendency for PEA to be elevated may imply that altered central neurotransmission occurs in certain forms of schizophrenia.

Key words: Phenylethylamine – Phenylacetic acid – Schizophrenia – CSF

Zusammenfassung. Phenyläthylamin (PEA) ist eine endogene Substanz mit amphetaminähnlichen, stimulierenden Eigenschaften, die auch im menschlichen Organismus vorkommt. Wegen dieser Wirkungen ist ein veränderter PEA Stoffwechsel bei bestimmten Formen von Schizophrenie zu vermuten.

In der vorliegenden Studie wurde bei 28 schizophrenen Patienten und 15 psychisch gesunden Kontrollpersonen PEA und Phenylessigsäure (PAA) gemessen. Im Liquor cerebrospinalis unbehandelter und behandelter Patien-

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ten mit paranoider Schizophrenie sowie gesunder Kontrollen wurden keine signifikant unterschiedlichen PEA Konzentrationen gefunden. Allerdings zeigten die zwei Patienten mit den höchsten Psychosescores in der Brief Psychiatric Rating Scale extrem hohe PEA Werte im Liquor.

Dagegen war die unkonjugierte Phenylessigsäure (PAA), der Hauptmetabolit des PEA, signifikant bei unbehandelten Schizophrenen erniedrigt ($P<0.05$).

Da PEA vermutlich neuromodulatorische Wirkungen hat, kann angenommen werden, daß schon äußerst geringe und spezifisch lokalisierte Veränderungen im PEA Stoffwechsel (wie in der erniedrigten PAA und der partiellen Erhöhung von PEA zum Ausdruck kommt) veränderte zentrale Neurotransmission in bestimmten Schizophrenieformen bewirken.

Schlüsselwörter: Phenyläthylamin - Phenylessigsäure - Schizophrenie - CSF

Introduction

Amphetamine-induced psychosis exhibits close similarities to the paranoid hallucinatory subtype of schizophrenia (Connell 1958; Kalant 1973). Although clearcut differences exist in the clinical symptomatology (Bonhoff and Lewrenz 1954), this model has been of considerable heuristic value in psychiatric research.

Phenylethylamine (PEA), which is structurally and pharmacologically similar to amphetamine (Fig. 1), is the decarboxylation product of phenylalanine and a preferred substrate of monoamine oxidase (MAO) type B. It seems to possess dopamine and noradrenaline releasing properties (Fuxe et al. 1967) and possibly direct dopamine and serotonin agonistic effects (Jackson 1975; Antelman et al. 1977; Sloviter et al. 1980). PEA has been detected by sensitive methods in various tissues (including brain) of untreated animals (Reynolds et al. 1980; Saavedra 1978) and in the brain (Reynolds et al. 1978) and urine of humans (Jepson et al. 1960; Reynolds and Gray 1976; Schweitzer et al. 1975). In animals, PEA causes stereotypies (Randrup and Munkvad 1966; Sabelli et al 1975) which are increased by MAO B inhibition (Moja et al. 1976) and attenuated by neuroleptics (Borison et al. 1977; Moja et al. 1978) but, in contrast to amphetamine, also by α-adrenergic blockers (Borison and Diamond 1978).

Increased PEA excretion has been reported in the urine of schizophrenic patients by Fischer et al. (1972) but not by Schweitzer et al. (1975). Potkin et al. (1979) reported increased 24 h urinary PEA excretion in paranoid but not in nonparanoid chronic schizophrenics. Sandler et al. (1978) found a significant increase of unconjugated phenylacetic acid (PAA), the major metabolite of PEA, in cerebrospinal fluid (CSF) of schizophrenics as compared to healthy controls. However, metabolic influences of neuroleptics and other psychotropic drugs which can interfere with PEA metabolism (Sabelli et al. 1973) cannot completely be excluded in all of these studies.

The purpose of this investigation was to measure both PEA and PAA (conjugated and unconjugated) in the CSF of treated and untreated schizophrenics in comparison with psychiatrically healthy controls.

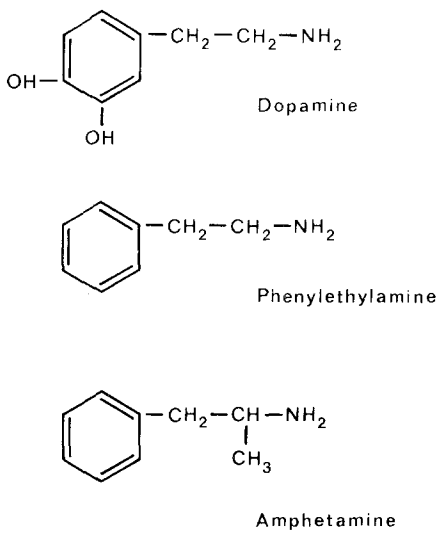

Fig. 1. Structural similarities between dopamine, phenylethylamine and amphetamine

Patients and Methods

The present study is part of a biochemical investigation into the CSF of schizophrenic patients which is being performed in collaboration with the Clinica Borda do Campo (Sao Paulo, Brazil) and has already been described in more detail elsewhere (Gattaz et al. 1982).

The study comprises 28 paranoid schizophrenic patients (all males, mean age 30.6 ± 8.0 years) and 15 controls (13 males and 2 females, mean age 35.0 ± 15.7 years). Since the results obtained in the 2 females were very similar to those in males, data were considered together. Patients were diagnosed according to the Research Diagnostic Criteria (Spitzer et al 1975). Two experienced psychiatrists independently evaluated the psychopathological state by means of the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham 1962). There were 15 patients under treatment with neuroleptic drugs (butyrophenones and phenothiazines) for at least 3 weeks (mean dose $\pm$ S. D. in chlorpromazine equivalents: 585 ± 755 mg/day). The remaining 13 patients had not had any drugs for a period of at least 4 weeks prior to the study. Further data from the patients' group, including psychopathological ratings on the BPRS, are summarized in Table 1.

Controls were subjects with nonspecific neurological symptomatology (headaches, dizziness, etc.) which necessitated a lumbar puncture for diagnostic reasons. Controls were not under drug treatment at the time of lumbar puncture.

Informed consent was obtained from all of the subjects and/or their first degree relatives after the nature of the study and possible complications had fully been explained. No side effects occurred in any of these patients.

CSF was obtained by lumbar puncture in a sitting position between 9 and 10 a. m., after probands had fasted for 12 h and had bed rest for 10 h. The CSF samples (16 ml) were removed without additions. To avoid rostral-caudal gradient effects, samples were gently mixed and then frozen on dry ice and stored.

PEA

After thawing, 25 μl of a standard solution containing 100 ng D_9-phenethylamine and 1 ml H_2O were added to 1 ml CSF and the sample brought to pH 12.5 with 2 *N* NaOH. Thereafter, it was loaded onto a C_{18} Sep Pak cartridge (Waters Ass., Inc., Milford, MA, USA), precleaned by rinsing with 80 ml 70% methanol and 10 ml H_2O. The loaded cartridge was rinsed with 10 ml H_2O and subsequently with 10 ml 30% methanol. Phenethylamine was eluted with 10 ml 70%

Table 1. Comparison of the ethnographic and psychopathological variables between patients with and without neuroleptic drugs

	Schizophrenics with drugs ($n=15$)	Schizophrenics without drugs ($n=13$)
Age*	31.8±10.1	29.3±4.4
Age at the onset of the disease[a]	20.6± 5.4	21.8±3.8
Duration of the disease[b]	10.8± 6.8	8.3±4.5
Number of hospitalisations	13.6±10.0	7.6±7.3
Andp.	7.0± 4.5	5.3±1.7
Aner.	12.9± 4.6	13.8±4.0
Thot.	14.5± 7.6	15.6±5.7
Actv.	3.8± 1.8	6.4±3.1[b]
Host.	6.4± 4.4	8.5±4.7
Total	44.6±11.4	49.7±9.1

Scores of the brief psychiatric rating scale: andp. = anxiety/depression; aner. = anergia; thot. = thought disturbance; actv. = activation; host. = hostile/suspiciousness
[a] In years; [b] $P< 0.05$ (two-tailed Mann-Whitney U-test)

methanol. This fraction was evaporated under reduced pressure and the residue reacted with 100 µl heptafluorobutyric anhydride for 30 min at 60° C. The unreacted heptafluorobutyric anhydride was then blown off with dry N_2 and the residue dissolved in 50 µl toluene. Of this, 2.5 µl were injected into a capillary GCMS system (Carlo Erba fractovap 2150 coupled to a Finnigan 4000 mass spectrometer), operated in the chemical ionization mode with methane as the reactant gas. Gas chromatographic separation was achieved on a 50 m × 0.3 mm glass capillary coated with SE 54. The oven temperature was raised from 80° C at a rate of 5° C/min to 250° C. Hydrogen was used as the carrier gas. The temperatures at the GC/MS interface and in the ion source were 250° C and 150° C resp. Two characteristic „quasimolecular" ions were monitored with a programmable multiple ion monitor (Promim): m/z 318 for phenethylamine heptafluorobutyrate and m/z 327 for the D_9 analog.

Quantitation was done by relating the peak height ratios of amine and internal standard which were linear within the range of interest. Each extract was injected three times, and the result represents the mean of the second and third determination. Details of the procedure will be reported elsewhere (Lauber and Waldmeier, in prep.).

PAA

PAA was estimated as its pentafluorobenzyl ester by mass fragmentography, using deuteriated PAA as internal standard in a modification of the method of Fellows et al. (1978).

Non-parametric tests were used for statistical evaluation.

Results

1. PAA (Free and Conjugated)

Free PAA was significantly decreased in the CSF of patients without neuroleptics as compared to controls ($P<0.05$) whereas those with neuroleptics

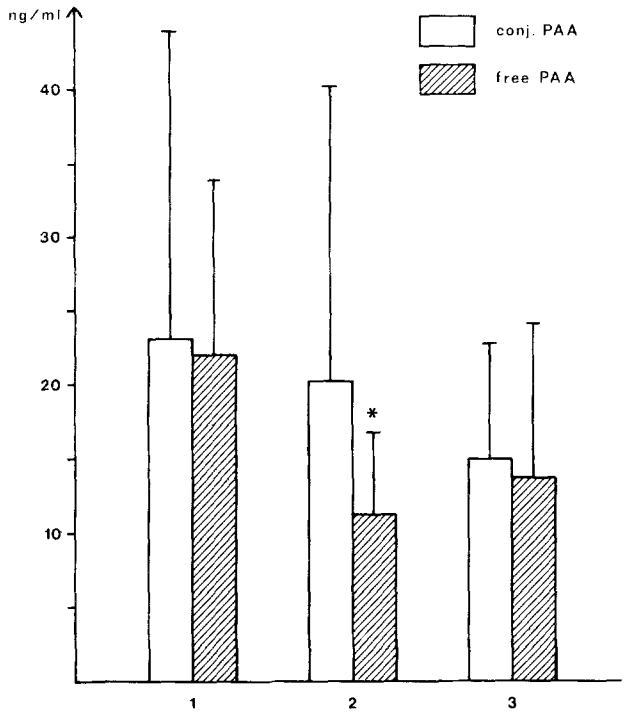

Fig. 2. Free and conjugated phenylacetic acid (PAA) in the CSF of healthy controls (*1*), untreated schizophrenics (*2*) and neuroleptic-treated schizophrenics (*3*). Mean ± SD

showed a non-significant tendency to be decreased below control values ($P<0.10$; Fig. 2). No differences between these three groups were found for conjugated PAA (Fig. 2).

Free PAA correlated significantly (Spearman rank correlation coefficients) with the following items of the BPRS: conceptual disorganisation ($r=0.47$; $P<0.01$, mannerism ($r=0.41$; $P<0.05$), hallucinatory behavior ($r=0.48$; $P<0.01$), unusual thought content ($r=0.33$; $P<0.05$) and blunted affect ($r=0.38=P<0.05$).

Conjugated PAA correlated significantly with hallucinatory behavior ($r=0.52$; $P<0.01$) unusual thought content ($r=0.35$; $P<0.05$).

2. PEA

Although there was a tendency for the group of the unmedicated schizophrenics to have increased PEA concentrations in the CSF, this difference did not reach statistical significance (Fig. 3). There was one patient with an extremely. high PEA level (10 SD above the mean values). After his exclusion there was again no

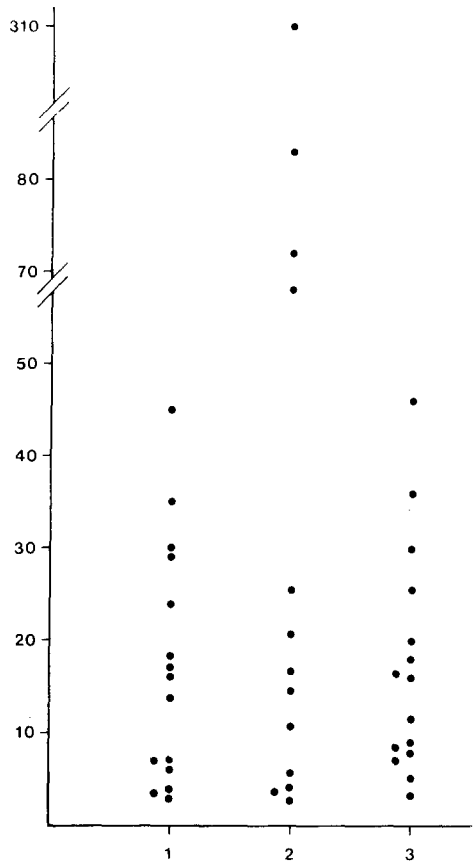

Fig. 3. Phenylethylamine (PEA) in the CSF of healthy controls (*1*), untreated schizophrenics (*2*) and neuroleptic-treated schizophrenics (*3*). Mean ± SD

statistically significant difference. The CSF PEA values of controls and neuroleptic-treated patients were all in the same range.

The two patients with the highest PEA values (310 and 82 ng/ml) had the highest BPRS scores (68 and 63 respectively) which is more than 2 SD above the mean for the total group (45.5 ± 9.4).

The patient with the highest CSF PEA was a 40-year-old male whose disease had lasted for more than 15 years and who had an unusually high number of hospitalisations ($n=23$). His major symptoms were autism, blunted affect, thought disturbances, auditory hallucinations, delusions of persecution and feelings of being influenced.

The second patient with an extraordinary high CSF PEA concentration (83.7 ng/ml) was a 27-year-old male who suffered from emotional withdrawal, paranoid ideation, conceptual disorganization, mannerisms, auditory hallucinations and delusions of persecution. It was his first hospitalisation. Both patients had free PAA values not deviant from the group mean.

Discussion

The possibility of an altered PEA metabolism in certain forms of mental illness is intriguing in that this endogenous substance has stimulant amphetamine-like properties in various laboratory experiments in different species.

In contrast to the results of Fischer et al. (1972) and of Potkin et al. (1979), who reported increased urinary PEA levels in schizophrenics, our data on CSF PEA levels do not indicate an alteration of the metabolism of this amine in such patients, in line with the report of Schweitzer et al. (1975). It could be argued that changes in urine need not be reflected in CSF. However, PEA is able to pass the blood-brain barrier relatively freely, and it can be anticipated, therefore, that the relative proportions of PEA of peripheral and cerebral origin are similar in both body fluids. It should be noted that in the group of unmedicated patients these two with the highest PEA levels had the highest BPRS global scores. As both patients suffered from severe chronic productive psychosis of the paranoid type, at least some association might be assumed between altered PEA concentrations and paranoid features. However, this should not be overemphasized.

The occurrence of significantly decreased free PAA levels in the CSF of unmedicated, but not in drug-treated schizophrenics is at variance with the results of Sandler et al. (1978) who, conversely, reported elevated concentrations of free PAA in CSF, predominantly in female schizophrenics. All but two of our probands were males. Even though control values of both study groups lie in a similar range and clinical and laboratory methods are comparable, it is not possible to pronounce on this difference without analysing further samples from untreated female schizophrenics. Now that an assay procedure for conjugated PAA is available, it would be instructive to measure this material in female schizophrenics. Our patients taking neuroleptics did not show increased PAA levels but had a tendency to lower levels.

It is not clear from these and other data whether decreased PAA concentrations in the lumbar CSF point to an alteration in PEA turnover in the brain. However, whereas PEA and its amino acid precursor phenylalanine pass the blood-CSF barrier fairly easily, this had been thought not to apply to PAA, at least in the cat (Pedemonte et al. 1976). Recently, however, Sandler et al. (1982) have noted a significant degree of correlation in man between CSF and plasma PAA concentrations. Lower CSF PAA in drug free schizophrenics would be consistent with lowered platelet MAO activity in chronic schizophrenics (Wyatt et al. 1973). Demisch et al. (1977) who used PEA as substrate found reduced MAO for the group of paranoid schizophrenics. However, substantial doubts have recently been raised on the solidity of the reported MAO findings (Gattaz 1982).

Although a direct neuronal action of PEA has been suggested (Jackson 1975), other pharmacological evidence points more to a modulatory role in the postsynaptic response to neurotransmission (Reynolds, 1979). In the light of this, subtle changes as measured by lowered PAA levels and a tendency for PEA concentrations to be elevated may have some significance for altered central neurotransmission in certain forms of schizophrenia.

We have not been able to find significant correlations with age and sex. Nevertheless, other influences such as diet, physical activity etc., upon the concentra-

tions of both PEA and PAA in human CSF are to be studied in further clinical experiments.

Acknowledgements. We thank W. Stramke for his valuable statistical advice. We are indebted to Dr. Daher Gattaz from the Clinica Borda do Campo (Brazil) for his help in the experimental work.

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Received September 3, 1982