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MONOACYLCADAVERINES IN THE BLOOD OF SCHIZOPHRENIC PATIENTS

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SUMMARY

Concentrations of cadaverine, monoacetylcadaverine and monopropionylcadaverine in the blood of schizophrenic and nonschizophrenic subjects were measured. Two groups, one from the U.S.A. the other from Japan, were tested. Monoacetylcadaverine and monopropionylcadaverine were found elevated in the blood of some schizophrenic patients in comparison with those in controls in each group. Their increase could be caused by a reduced monoamine oxidase activity or by an increased acylation in schizophrenic patients.

INTRODUCTION

Monoacetylcadaverine and monopropionylcadaverine were identified in the urine of schizophrenic patients [1]. Their connection with the mental illness, however, was excluded in view of current opinion on the exogenous origin of cadaverine.

It was demonstrated that cadaverine in mammals originates from bacterial decomposition of food in intestines [2] or from tissue putrefaction [3]. Moreover, it was shown that the urine levels of monoacetylcadaverine and monopropionylcadaverine could be substantially reduced by administering broad spectrum antibiotics, indicating that the suppression of intestinal bacterial flora resulted in less cadaverine formed exogenously and consequently in less cadaverine catabolites [1].

Some recent findings indicate that the actual role of cadaverine might be different from that of an exogenous contaminant. Cadaverine is physiolog-

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ically present in the mammalian brain and blood [4, 5]. There is also an uptake system for cadaverine in mammalian brain which is inhibited by cyanide and some polyamines [6]. Neither the blood nor the brain concentrations of cadaverine in mice are lowered by the absence of bacterial flora in the intestine [7]. Biosynthesis of cadaverine in the rat kidney was recently reported [8]. Thus, even though there is no doubt that cadaverine is produced by bacteria, the brain concentration of cadaverine is maintained through mechanisms which are independent of bacterial decarboxylation.

Since monoacetylcadaverine and monopropionylcadaverine were originally identified in the urine of schizophrenic patients [1], a comparison between their concentrations in the blood of schizophrenic and nonschizophrenic subjects was made in this study.

METHOD

Subjects

The measurements were performed on blood samples obtained from two groups of subjects. The first group consisted of patients and controls from the United States (N=37), the second group originated from Japan (N=36). In both groups, the schizophrenic patients were hospitalized at inpatient units. They were admitted in a psychotic episode if the presumptive diagnosis was schizophrenia. They manifested delusions, hallucinations and/or a thought disorder as well as inappropriate affect. The patients were free of known organic disease. Their diagnoses were made using criteria by Feighner et al. [9]. Chronic or acute pattern of the illness and schizophrenia subtypes were not distinguished in this comparison. Most of the schizophrenic subjects (with the exception of two naive patients from the second group) were taking phenothiazine medication.

The control subjects included laboratory personnel and neurological patients hospitalized for stroke. Table I gives the composition and age of both groups.

TABLE I

COMPOSITION OF THE TWO GROUPS OF ASSAYED SUBJECTS

		Males	Females	Age (mean ± S.E.)
Group I	Controls	10	4	59.8 ± 3.9
U.S.A.	Schizophrenics	14	9	39.6 ± 2.6
Group II	Controls	15	0	41.0 ± 3.8
Japan	Schizophrenics	21	0	23.5 ± 1.3

Samples

Cadaverine, monoacetylcadaverine and monopropionylcadaverine were measured in samples of the whole blood from the first group and in the blood plasma from the second group. The sample of the venous blood from the first group was transferred into a glass vial containing an equal volume of

0.2 *M* perchloric acid, and the vial was immediately sealed. In the second group, the blood was collected into tubes containing ethylenediaminetetraacetic acid disodium salt (EDTA), 1 mg/ml of blood, centrifuged (150 *g* for 10 min), the plasma was transferred into a glass vial containing an equal volume of the perchloric acid, and the vial was sealed. With each sample in both groups, a second vial was simultaneously filled with the perchloric acid, sealed, and processed as its blank.

Analytical procedures

A thin-layer chromatography—mass spectrometry (TLC—MS) method was used for determinations. Dansyl derivatives formed in the whole blood homogenate (first group) or plasma (second group) with perchloric acid were separated by TLC, eluted, and quantified by MS. The molecular ions of interest were identified by peak matching against ions corresponding to the internal standards and were quantified by the integrated ion technique.

Dansylation and TLC

The sample was weighed and homogenized in five volumes of 0.2 *M* perchloric acid. The whole homogenate was then submitted to dansylation (reaction with 1-dimethylaminonaphthalene-5-sulfonyl chloride), for six hours at room temperature [10]. Dansyl derivatives of amines were extracted into toluene and separated by TLC on silica gel-coated plates (Merck G). The fraction which co-chromatographed with the dansylated compound in question was scraped off, eluted, and separated with the second chromatography system. The TLC fractions were eluted again and their contents measured by high-resolution MS. The solvent systems for chromatography are shown in Table II.

Quantitative MS

A modified version of the integrated ion current technique [11, 12] with the peak matching circuit [13] and the internal standard of a dansylated compound in question [14, 15] was used. A known quantity of an internal standard was added to each eluted chromatographic fraction and the dried mixture was introduced via the probe into the mass spectrometer (AEI MS-902). The list of internal standards and their molecular ions is shown in Table III. The sample was evaporated over 30—45 sec by heating it to 350° into the source maintained at 220°, and ionized with the electron beam energy 70 eV. The molecular ions corresponding to the dansylated compound in question (Table III) and to its internal standard were recorded using the peak matching circuit of the spectrometer at a resolving power between 2000 and 8000. Their molecular ratio was preset with an accuracy of 2 ppm. If the mass ratio between the two matched peak maxima differed at any time during the evaporation of the sample by more than 40 ppm (due to drift of the instrument, sample contamination, or electrical interference), the sample was disregarded. The evaluation of the accuracy of the peak matching and rejection of samples was done by computer (Xerox Sigma-2). The lower values for the number of reported measurements in Table IV compared with Table I were caused by the rejected samples.

TABLE II

SOLVENT SYSTEMS FOR TLC SEPARATION OF DANSYLATED CADAVERINE, MONOACETYLCADAVERINE AND MONOPROPIONYLCADAVERINE

Compound	First chromatography			Second chromatography		
	Solvent	Runs	Eluent	Solvent	Runs	Eluent
Bis-Dns-cadaverine	Heptane—acetone (1:1)	1	Ethyl acetate	Chloroform—triethylamine (10:1)	1	Ethyl acetate
Dns-acetylcadaverine Dns-propionylcadaverine	Heptane—acetone (1:1)	1	Methanol	Benzene—methanol (14:1)	3	Ethyl acetate—acetone (1:1)

TABLE III

MS INTERNAL STANDARDS FOR QUANTITATIVE DETERMINATION OF DANSYLATED CADAVERINE, MONOACETYLCADAVERINE AND MONOPROPIONYLCADAVERINE

Compound	Composition	*m/e*	Standard	Composition	*m/e*	Ratio
Bis-Dns-cadaverine	$C_{29}H_{36}N_4O_4S_2$	568.2178	Bis-Dns-hexamethylenediamine	$C_{30}H_{38}N_4O_4S_2$	582.2344	1.024666
Dns-acetylcadaverine	$C_{19}H_{27}N_3O_3S$	377.1773	Dns-acetylhexamethylenediamine	$C_{20}H_{29}N_3O_3S$	391.1930	1.037159
Dns-propionylcadaverine	$C_{20}H_{29}N_3O_3S$	391.1930	Dns-propionylhexamethylenediamine	$C_{21}H_{31}N_3O_3S$	405.2086	1.035828

BLE IV

NCENTRATIONS OF CADAVERINE, MONOACETYLCADAVERINE AND MONOPROPION DAVERINE IN BLOOD SAMPLES FROM TWO GROUPS OF CONTROL SUBJECTS AND SCHI RENIC PATIENTS

lues $\times 10^{-12}$ mole/g of wet weight, mean ± standard error; the numbers of subjects are give entheses.

mpound	Group	Control subjects	Schizophrenic patients	t*	P**
laverine	I	14.13 ± 2.78 (10)	15.53 ± 1.74 (18)	0.45101	>0.5
[redacted]					
	II	0.48 ± 0.10 (13)	4.14 ± 0.91 (21)	3.14762	<0.01
nopropionylcadaverine	I	1.70 ± 0.67 (14)	15.75 ± 5.66 (17)	2.23594	<0.05
	II	1.09 ± 0.16 (15)	2.64 ± 0.31 (20)	4.07265	<0.001

= *t* values of *t*-test.
= Level of significance, using two-tailed *t*-test.

The quantity of the substance of interest in a sample was calculated from the ratio between the intensity of the ion of interest and that generated by an internal standard substance. The calibration functions calculated by linear regression for 12 calibration samples containing known picomole quantities of each compound had the correlation coefficients 0.9922, 0.9914 and 0.9865, for cadaverine, monoacetylcadaverine and monopropionylcadaverine, respectively. The reported concentrations were measured as quantities more than three times higher than their blanks. They were not corrected for losses during extraction and TLC.

RESULTS

As shown in Table IV, the concentrations of the measured compounds were lower in the samples of blood plasma from the second group of subjects than in the whole blood of the first group. With the exception of cadaverine in schizophrenic patients, all other concentrations in the second group were lower than in the first group.

There was no significant difference between the mean concentrations of cadaverine in the blood of controls and schizophrenic patients in the first group. In the plasma samples from the second group, there was a significant increase of cadaverine in schizophrenic patients against controls.

Monoacetylcadaverine and monopropionylcadaverine concentrations in both the blood and plasma of schizophrenics showed a larger variance than those of the controls (Fig. 1). While several values were within the region of control concentrations, the others were almost one order of magnitude higher. The mean values were significantly higher in schizophrenics than in controls for both monoacetylcadaverine and monopropionylcadaverine in both the groups (Table IV).

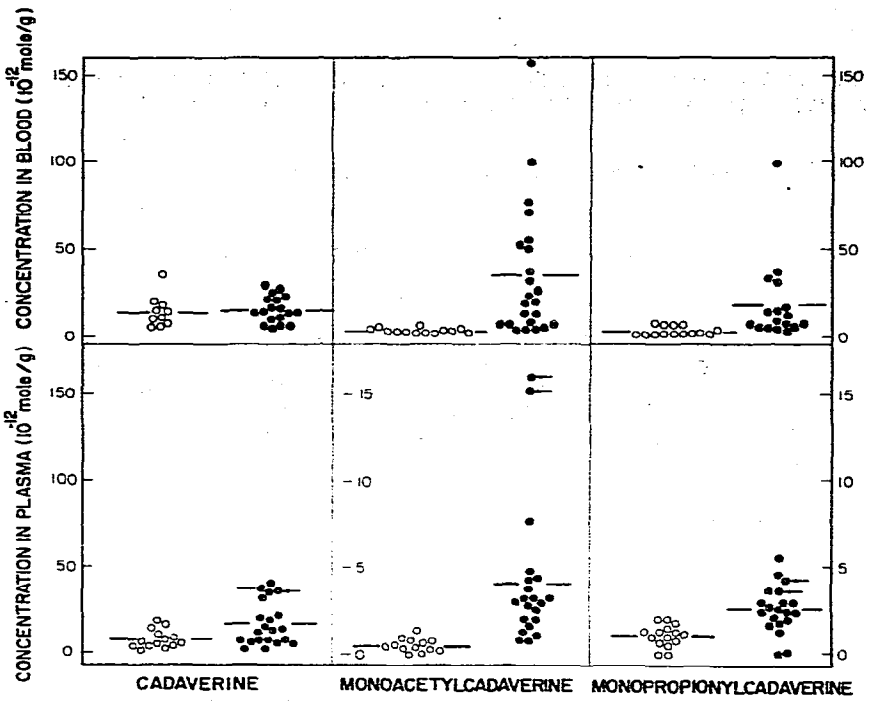

Fig.1. Concentrations of cadaverine, monoacetylcadaverine and monopropionylcadaverine in the blood samples from the two groups of control subjects and schizophrenic patients. Group I in the upper, group II in the lower part of the diagram. Note the expanded concentration scale for monoacetylcadaverine and monopropionylcadaverine in group II. ○, Controls; ●, schizophrenic patients; mean concentrations are indicated by the horizontal lines. The arrows in group II are pointing to values obtained on two newly admitted patients having no medication.

DISCUSSION

This report is based on a relatively small number of subjects and the conclusion about an increase in the blood concentrations of monoacetylcadaverine and monopropionylcadaverine in some schizophrenic patients should not be generalized. Further analysis of elevated monoacylcadaverines in the blood of schizophrenic patients will require an extensive and detailed study.

The lower values of all measured compounds in the plasma of the second group are puzzling. They are not caused by relatively higher concentrations in the blood cells or by the different processing of the samples. Table V shows values of monoacylcadaverines found in test samples processed as the whole blood or plasma in the same manner as were the samples from the first and second group, respectively. The fact that there are different concentration ratios between cadaverine and monoacetylcadaverine or monopropionylcadaverine in the two groups, and that there is a significantly higher mean cadaverine concentration in the plasma of schizophrenics than in controls from the second group while there is no difference in the blood samples of the first group, seems to indicate that the observed differences between the groups might have some biological importance.

TABLE V

CONCENTRATIONS OF MONOACYLCADAVERINES IN IDENTICAL BLOOD SAMPLES PROCESSED AS WHOLE BLOOD OR BLOOD PLASMA

Values × 10^{-12} mole/g of weight, mean ± standard error; the numbers of blood samples are given in parentheses.

	Monoacetylcadaverine	Monopropionylcadaverine
Whole blood (Group I)	4.00 ± 0.42 (9)	3.97 ± 1.19 (9)
Blood plasma (Group II)	3.20 ± 0.30 (9)	3.98 ± 0.96 (9)

There is a genetic polymorphism in man for metabolism of some drugs (niazid, sulphadimidine) [16—18]. The two major phenotypes, rapid and slow inactivators, differ in the rate of their hepatic acetylation [19]. In Caucasian and Negro populations the slow allele is approximately three times more frequent than the rapid allele, but among Japanese exactly the reverse proportion was found [20, 21]. In a way similar to niazid and sulphadimidine, the observed differences in concentrations of monoacylcadaverines between the first and second group in this study might reflect genetic variations in the acylation of cadaverine, and the distribution of acylator phenotypes for cadaverine in American and Japanese populations, respectively. The use of independent controls for hypothetical slow and fast allele in each population could possibly increase the differences, and their significance, found between the schizophrenic and control subjects, assuming that either group, or both, have genetically controlled bimodal distribution of monoacylcadaverine concentration in the blood.

The elevated blood levels of monoacylcadaverines in schizophrenic patients do not seem to be pharmacologically induced. In contrast, it is possible that phenothiazines are decreasing the elevated concentrations of monoacylcadaverines in the blood of schizophrenics because the concentrations found in samples from the two schizophrenic patients without medication (Fig. 1, arrows) were among the highest in that group.

Both the role and origin of cadaverine, monoacetylcadaverine and monopropionylcadaverine in human blood are unknown. Cadaverine concentrations in the blood and brain have been reported to fluctuate during sleep in mice and during hibernation in molluscs [4, 22].

It has been demonstrated that 1,4-diaminobutane is preferentially acetylated by the rat brain tissue [23]. Because of the low substrate specificity of enzymes metabolizing diamines [2] it seems reasonable to consider the possibility that a similar mechanism might metabolize both 1,4-diaminobutane and 1,5-diaminopentane (cadaverine) in humans. The increase of monoacetylcadaverine and monopropionylcadaverine in blood could be caused by a higher rate of acylation or by a lowered catabolism of monoacylcadaverines.

It is probable that monoacetylcadaverine and monopropionylcadaverine are catabolized by monoamine oxidases, since their four-carbon analogue (monoacetylputrescine) is a substrate for monoamine oxidase in the rat [24]. In order to test this assumption, we have measured brain concentrations

of monoacylcadaverines in mice treated with monoamine oxidase inhibitors. The results in Table VI indicate that monoacetylcadaverine and monopropionylcadaverine are probably the substrates for monoamine oxidases in the mouse.

TABLE VI

THE EFFECT OF MONOAMINE OXIDASE INHIBITORS ON CONCENTRATIONS OF MONOACETYLCADAVERINE AND MONOPROPIONYLCADAVERINE IN THE MOUSE BRAIN

Values × 10^{-12} mole/g of wet weight, mean ± standard error; the numbers of subjects are given in parentheses. The mice were injected for 7 days with 25 mg/kg/day of Nialamide or Pargyline intraperitoneally. Controls were injected with saline.

	Controls	Nialamide	Pargyline
Monoacetylcadaverine	2.7 ± 0.7 (8)	19.8 ± 7.8 (7)*	12.5 ± 2.8 (8)**
Monopropionylcadaverine	3.4 ± 1.0 (8)	17.7 ± 4.4 (6)**	30.4 ± 4.9 (8)***

Levels of significance using two-tailed *t*-test:
*$P<0.05$.
**$P<0.01$.
***$P<0.001$.

The observed higher values of monoacetylcadaverine and monopropionylcadaverine in the blood of some schizophrenic patients could therefore be caused by an inefficient monoamine oxidizing system. Several studies have indicated that an altered activity of blood platelet monoamine oxidase is an accompaniment to mental illness [25—31]. The investigators were also searching for alterations of monoamine oxidase activity in the brain corresponding to those found in blood platelets. Disappointingly, no changes were found in brain monoamine oxidase activity of mental patients as evaluated post mortem [32, 33]. The attempts to search for an endogenous substrate for monoamine oxidase displaying concomitant changes with lowering of blood platelet monoamine oxidase activity were also unsuccessful [34]. Monoacylcadaverines in the blood and their changes might be a promising step in that direction. Irrespective of whether the blood concentrations of monoacylcadaverines are actually dependent on, or a reflection of, brain concentrations of the same compounds they might be sensitive indicators of the functional activity of monoamine oxidizing systems. Ultimately, it would be interesting to see whether by using monoacylcadaverines as substrates, changes in brain monoamine oxidase activity could be detected in mental patients.

The potential use of blood levels of acylcadaverines as biological markers for schizophrenia could be twofold:

(i) Monoacetylcadaverine and monopropionylcadaverine could be simply blood metabolites having no connection with the physiology of the central nervous system or with etiopathogenesis of mental illness. Their concentrations may or may not depend on the overall monoamine oxidase activity and/or on rate of acylation of cadaverine in tissues of the body. Yet, if a significantly high correlation is found between their blood levels and some forms of mental illness, they could be successfully used as an epiphenomenon for a more "objective" form of clinical diagnosis.

(ii) Monoacetylcadaverine and monopropionylcadaverine could be metabolites of cadaverine, preferentially formed in the brain, their blood levels reflecting an equilibrium with the corresponding concentrations in the brain tissue. Their increase in the blood of schizophrenic patients would be caused by a lowered activity of a monoamine oxidase isoenzyme specific for monoacylcadaverines in the brain, or by an increased rate of acylation which might be genetically controlled. This type of enzymatic anomaly could be directly connected with the etiology of some forms of mental illness.

The two types of conditions described above delineate the extremes of a range of different possibilities for connections between the elevated blood levels of monoacylcadaverines and schizophrenia. The actual relationship will be probably somewhere between those two extremes.

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