Spermidine Oxidase Activity in Serum of Normal and Schizophrenic Subjects

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Spermidine oxidase activity in human serum is distributed over a relatively wide range, with a highly significant difference between normal and schizophrenic subjects. The enzyme activity showed no age- or sex-related differences. It is largely inhibited by quinacrine and chloroquine.

Additional Keyphrases: polyamine metabolism - schizophre
nia - sex- and age-related differences

The two major polyamines, spermidine and spermine, occur ubiquitously in tissues (1). They may participate in one or more phases of nucleic acid metabolism (2-3). Polyamine concentrations are altered in some clinical conditions such as cancer (4), sickle cell anemia (5), and chronic renal failure (6). Schizophrenia is another disease in which polyamine concentrations are altered (7-8), and the polyamine, spermidine, may be involved (9).

To my knowledge, few data on distribution of polyamine oxidase (PAO) activity in human serum and tissues have been reported (10-11). Because of the possible significance of a polyamine-polyamine oxidase interaction that mediates polyamine catabolism, with formation of active metabolites, I estimated polyamine oxidase activity in the serum of a representative sample of healthy subjects, with attention to sex- and age-related differences. A comparison of spermidine oxidase activity in healthy subjects with that in schizophrenics is also reported here.

Materials and Methods

Samples

Blood was sampled from unselected normal (volunteer) men and non-pregnant women, with the help of the Blood Bank Institute, Mosul.

Blood samples from schizophrenic patients were obtained from Mosul Hospital. These patients manifested delusions, hallucinations, thought disorder, and inappropriate affect, but they were free of known organic disease. Their classification according to DSM-II (12) and their daily drug dosages, at the time of sampling were as follows: schizophrenia chronic, thioridazine, 600 mg, and trihexyphenidyl, 4 mg; schizophrenia chronic hallucinatory, trifluoperazine, 20 mg, and maprotiline, 75 mg; schizophrenia chronic acute episode, thioridazine, 600 mg, and nitrazepam, 5 mg; schizophrenia paranoid, chlorpromazine, 300 mg; schizophrenia acute, trifluoperazine, 20 mg; schizophrenia acute nondifferentiated, trifluoperazine, 20 mg; schizophrenia acute catatonic, chlorpromazine, 75 mg; schizophrenia acute paretic, chlorpromazine, 75 mg, and trihexyphenidyl, 4 mg; and schizophrenia, thioridazine, 300 mg.

The blood was sampled by venepuncture into sterile disposable syringes, transferred into glass tubes, and allowed to clot for 5 min at 37 °C. Sera were separated by centrifugation and assayed on the day of collection. Data on sex and age were recorded.

Procedures

Standard spermidine oxidase assay. I used the assay of Tabor and Kellogg (13) for determination of spermidine oxidase activity in untreated (native) human serum: the activity was assayed spectrophotometrically in a Beckman recording spectrophotometer by following the decrease in

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absorbance at 410 nm owing to reduction of potassium ferricyanide.

Assay and control reaction mixtures. The reaction system used for routine assays consisted of potassium phosphate buffer (12.5 mmol/L, pH 7.4) containing 0.5 mmol of potassium ferricyanide and 277 μmol of spermidine trihydrochloride (substrate) per liter. This mixture was freshly prepared daily. Three milliliters of the mixture was placed in a 3-ML cuvette with a 1-cm lightpath. Enzyme, 100 μL, was added, and the reaction mixture was stirred rapidly. The control cuvette was identical except that the substrate (spermidine) was not added. The reaction rate was followed at 410 nm, at 20 °C, in a Beckman recording spectrophotometer Acta MVII. Two equivalents of ferricyanide are reduced per mole of spermidine. The molar absorptivity of potassium ferricyanide is \( 0.96 \times 10^3 \) at 410 nm. One unit of enzyme catalyzes the oxidation of 1 mg of spermidine in 1 min at 20 °C. The enzyme activity of spermidine oxidase is expressed here as ng/min per mg of protein. Each sample was assayed in duplicate.

Results were analyzed by using the unpaired Student's t-test (14).

Inhibition of spermidine oxidase activity. Quinacrine [3-chloro-7-methoxy-9-(1-methyl-4-diethylaminobutylamino) acridine]; chloroquine [7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline]; isoniazid (4-pyridine carboxylic acid hydrazide), each at 0.2 mmol/L final concentration, and EDTA disodium salt, at 30 μmol/L final concentration, were used in inhibition studies. Serum samples were pre-incubated with one or other of these inhibitors for 20 min at 20 °C, then assayed for enzyme activity.

Determination of protein, urea, and creatinine. Protein in serum samples was estimated by use of the biuret reaction (15). Serum urea was estimated indirectly by determination of ammonia as a product of urease action on urea (16), serum creatinine by the Jaffé reaction (16).

Results

Spermidine oxidase activity in serum of normal adults and schizophrenic patients. Serum spermidine oxidase activity was estimated in serum from 31 normal adults ranging in age between 18 and 55 y. The activity was found to be distributed over a wide range (see Figure 1). The mean enzyme activity plus or minus the standard error of the mean, calculated for the entire group of normal adults, was 63.3 ± 3.5 ng/min per mg of protein. Within this group, the mean for men was not statistically different from that for women, nor was there any statistically significant difference by age.

Values for the 18 schizophrenic patients were markedly higher than that in normal serum (Figure 2). The mean (±SEM) was 229.6 ± 34.3, and the difference in the enzyme activity of the means between schizophrenic and normal was statistically highly significant, \( P < 0.001 \).

Values for serum urea and creatinine were within the normal reference interval, for both normal and schizophrenic subjects.

Reproducibility. To test reproducibility and reliability of the assay procedure, I repeated the estimations on five subjects, using fresh sera. The interval between the first and this second assay ranged from one to four weeks. The difference in the average values for measured activities between the first (62.2 ± 9.5) and repeated (75.8 ± 10.1) assays was not significant (\( P > 0.05 \)). The coefficient of variation for eight repeated assays of identical samples was 15.1%.

Substrate specificity. Under the standard assay conditions, the enzyme oxidizes spermidine, spermine, and histamine, the respective relative rates of oxidation being 100, 75, and 25, respectively. The enzyme did not oxidize the diamine, putrescine, or 1,3-diaminopropane.

Inhibition of spermidine oxidase activity. I examined the effect of various compounds on the oxidation of spermidine. Isoniazid showed no inhibitory effect on spermidine oxidase; quinacrine inhibited the enzyme activity by 80%, as did chloroquine. EDTA inhibited it by about 20%.

Discussion

An amine oxidase characterized by its high activity with spermidine as substrate has been detected in serum of human subjects. The assay of Tabor and Kellogg (13), which I used here for determination of spermidine oxidase activity in human serum, is suitable for this purpose.

The activity is greater in serum from schizophrenics than in non-schizophrenic subjects. This observed difference does not seem to be pharmacologically induced, because five of the schizophrenic patients were receiving medication.
(phenothiazine derivatives) on their first day of sampling. To minimize the effects of geographic, cultural, and genetic variables, which may contribute to fluctuations in absolute values for serum spermidine oxidase, normal and schizophrenic subjects were selected from the same environment.

I saw no correlation between spermidine oxidase activity and serum urea and creatinine concentrations, either in normal or schizophrenic subjects.

A report by Richardson (9) indicated a link between spermidine and schizophrenia. The higher spermidine oxidase activity in schizophrenics indicated in the present report is further evidence for the involvement of the polyanine, spermidine, in schizophrenia.

The activity in serum was appreciably inhibited by quinacrine, usually considered to be specific inhibitor of flavin-containing compounds (17). In this respect, spermidine oxidase seems to resemble amine oxidases found in mammalian tissues and in plants and microbial organisms (10, 13, 17, 18). Chloroquine had a profound inhibitory effect on spermidine oxidase. Quinacrine and chloroquine contain spermidine as a common moiety in their structure, which suggests that a competitive inhibition by these compounds. Unlike monoamine oxidase in human serum (19), spermidine oxidase is not inhibited markedly by isoniazide, a pyridoxal-phosphate enzyme inhibitor, or by EDTA.

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References