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INHIBITION AND ACTIVATION OF CAERULOPLASMIN BY EXTRACTS FROM THE URINE OF SCHIZOPHRENIC PATIENTS

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Abstract—Extracts of some 24-hr samples of urine from schizophrenic patients accelerated the caeruloplasmin-catalysed oxidation of noradrenaline but inhibited the oxidation of 5-hydroxytryptamine; these effects were not observed with extracts prepared in an identical manner from the urine of pre-operative or healthy controls. The oxidation of noradrenaline by caeruloplasmin was inhibited by extracts of urine from some members of each group of people and the oxidation of noradrenaline by cupric ions was catalysed by some of these urine extracts.

THE POSSIBILITY that a biochemical lesion is responsible for mental illness has received considerable attention in recent years[1] and has derived support from the discovery of drugs, such as mescaline and LSD, which modify mood, perception and behaviour in a way which simulates certain clinical aspects of schizophrenia. This possible biochemical basis for mental illness, particularly schizophrenia, has led many workers to search for abnormal chemical constituents in the various body fluids of patients suffering from mental illness. Friedhoff and Van Winkle[2] have demonstrated the presence of β-(3,4-dimethoxyphenyl)ethylamine in the urine of schizophrenics, and Kuehl[3] has tentatively identified tryptamine in the urine of patients suffering from Parkinson's disease. Smythies and co-workers[4] have carefully investigated the urine of schizophrenic patients and also controls, and have demonstrated the presence of a number of "pink spot" precursors one of which may have been monoacetyl cadaverine.

Molecules of higher molecular weight have also been reported to occur in the plasma of schizophrenic patients. Heath and co-workers[5] reported on a factor called taraxein which they claimed was present in the plasma of schizophrenic patients, but attempts to repeat this work have met with only limited success.[6] It has been suggested[7] that plasma levels of caeruloplasmin, an oxidative enzyme normally present in plasma, are higher in schizophrenic patients than in controls, but further studies have cast doubts on the validity of these observations.[8]

Despite considerable effort it must be admitted that the presence of abnormal constituents in the body fluids of schizophrenic patients has not been unambiguously demonstrated. In view of this it seemed worth while to investigate the use of a biological or biochemical assay method for demonstrating the presence of such materials, if

indeed they do exist at all. Previous work by Drysdale *et al.*[9] indicated that extracts of the urine from schizophrenic patients affected the behaviour of the house fly (*Musca domestica*); similar extracts from normal urine had no effect. In view of the previous possible implication of caeruloplasmin in mental illness it was decided to study the effects of extracts of schizophrenic urine on the oxidative activity of caeruloplasmin using noradrenaline (NA) and 5-hydroxytryptamine (5-HT) as substrates. Since caeruloplasmin is a copper-dependent metalloenzyme, the oxidation of noradrenaline and 5-HT by copper ions in the presence of urine extracts but in the absence of any added caeruloplasmin was also investigated. The results of these studies form the basis of this paper.

EXPERIMENTAL

(a) *Subjects*

These investigations were carried out on three groups of individuals, urine samples being collected over 24-hr periods. The whole 24-hr sample was processed as detailed below for the isolation of the extracts which were then subjected to assay.

(1) The acute schizophrenic patients were young, physically healthy males who had not previously received any form of treatment, including drugs, until the urine samples had been collected. Criteria for the diagnosis of schizophrenia were those laid down by Bleuler.[10]

(2) The non-schizophrenic patients were young males hospitalised for surgical correction of mechanical defects such as torn articular cartilages or deviated nasal septa. The urine was collected over the 24 hr immediately preceding the operation. These patients were not receiving drugs.

(3) The third group consisted of young healthy males on the hospital staff.

(b) *Extraction procedure* (modified after Drysdale *et al.*[9])

Each of the 24-hr samples of urine was acidified to pH 2 with concentrated hydrochloric acid and adsorbed onto a Zeo-Karb 225 (H^+) column (2·5 × 30 cm). The column was washed with distilled water until the effluent was neutral and the basic material was then eluted with ammoniacal ethanol (20:80, 180 ml). This eluate was evaporated to a small volume *in vacuo* at 36° under nitrogen and the concentrate, dissolved in distilled water (5 ml), was applied to a diethylaminoethyl cellulose DEII column (1·8 × 8 cm). The material on the column was eluted with distilled water (25 ml) and the eluate, adjusted to pH 2 with concentrated hydrochloric acid, was then passed down a de-acidite FF column (1 × 10 cm). After adjusting the eluate to pH 2 with a concentrated hydrochloric acid, this eluate was re-chromatographed on a Zeo-Karb 225 (H^+) column as described above and was finally purified by chromatography on Whatman No. 3 paper for 18 hr in the descending mode using butanol–acetic acid–water (8:2:2) as the mobile phase. The bands were located with ninhydrin; the band with $R_f = 0{\cdot}48$ was extracted from the paper with dilute hydrochloric acid and concentrated to dryness *in vacuo* as described above. This fraction was assayed for its ability to inhibit or accelerate the oxidation of noradrenaline and 5-HT by caeruloplasmin and to oxidise noradrenaline in the presence of added cupric ions.

(c) *Assay procedure*

The total extract, prepared as above from a 24-hr sample of urine, was dissolved in distilled water (1·0 ml) to give a stock solution which was fully assayed on the day on

which it was prepared. An aliquot (0·1 ml) of this stock solution was added to noradrenaline (0·1 ml of a $2{\cdot}5 \times 10^{-2}$ M solution), 0·02 ml of caeruloplasmin solution (A.G. Kabi, Sweden) was added and the mixture was made up to 5 ml with pH 5·9 acetate buffer. For assay of the caeruloplasmin-like effect the caeruloplasmin in the above procedure was replaced by copper nitrate (0·05 ml of a 2×10^{-2} M solution). In each case the rate of change in absorption at 490 nm was measured on a Perkin–Elmer 137 u.v. Spectrophotometer at 27°. For assays using 5-HT as the substrate the stock solution (0·1 ml) was added to a mixture of 5-HT (0·2 ml of a $2{\cdot}5 \times 10^{-2}$ M solution) $NADH_2$ (0·025 ml of a $2{\cdot}5 \times 10^{-2}$ M solution) and caeruloplasmin (0·025 ml), the volume then being adjusted to 5 ml with pH 5·9 acetate buffer. The rate of the reaction was obtained by measuring the change in absorption at 340 nm using a Perkin–Elmer 137 u.v. Spectrophotometer at 27°.

Appropriate controls to which urine extracts had not been added were run before and after each assay.

RESULTS

The results of the above assays are given in Tables 1, 2 and 3. In no case was an accelerating effect on the oxidation of 5-HT observed.

DISCUSSION

Caeruloplasmin will oxidise a number of different materials, the most commonly used substrate being *N,N'*-dimethyl-*p*-phenylenediamine. In the present investigations it was decided to use noradrenaline and 5-hydroxytryptamine as the substrates, mainly because they are two of the more important biogenic amines present in the central nervous system. In addition these two compounds are closely related structurally to known hallucinogens. Noradrenaline, for example, is structurally related to mescaline and other hallucinogenic amines,[11] whilst 5-hydroxytryptamine is structurally related to hallucinogenic tryptamines such as psilocybin and psilocin.

The results given in Table 1 were obtained using extracts from the urine of schizophrenic patients. The positive results are scattered throughout all four columns of this table, particularly in columns 2 and 3, indicating the presence, in schizophrenic urine, of a factor or factors which modify the enzymic activity of caeruloplasmin. These results are in marked contrast to those in Table 2 obtained on pre-operative patients, and in Table 3, obtained on controls. In neither of these two tables are there any positive results in columns 2 or 3. This marked difference between Table 1 on the one hand and Tables 2 and 3 on the other is particularly striking and suggests that here may be a lead to a biochemical method which may eventually be of use in the clinical diagnosis of some forms of mental illness.

In addition to the factor which is responsible for the results in columns 2 and 3 of Table 1 only it is clear that there must also be another factor in urine which is responsible for the results in columns 1 and 4 of all three tables. The significance of this factor cannot be defined with any certainty but it seems reasonable to suggest that it may well be related to stress, either mental or physical. This would account for its low occurrence in normal adults (Table 3) and a somewhat higher occurrence in pre-operative patients (Table 2). The presence of this factor in the urine of schizophrenic patients (Table 1) is presumably a result of the stress deriving from the abnormal mental condition of these patients.

TABLE 1. EFFECT OF EXTRACTS FROM URINE OF SCHIZOPHRENIC PATIENTS ON CAERULOPLASMIN CATALYSED OXIDATION OF NORADRENALINE (NA) AND 5-HYDROXYTRYPTAMINE (5-HT)

Schizophrenic patient No.	Inhibition of NA oxidation	Acceleration of NA oxidation	Inhibition of 5-HT oxidation	Oxidation of NA by Cu^{2+}
1	−	−	±	+
2	−	++	+	+
3	++	−	++	+
4	−	−	+	+
5	−	+++	+	−
6	−	+	±	+
7	−	++	++	−
8	+++	−	−	+
9	−	++	++	+
10	−	−	−	+
11	±	−	++	−
12	+	−	±	±
13	−	+	−	−
14	−	+	+	±

− indicates no effect.
+ indicates a measurable effect, the relative magnitudes being indicated by the numbers of plus signs viz. ++ +++ > +++ > ++ > +.
± indicates a doubtful effect.

TABLE 2. EFFECT OF EXTRACTS FROM URINE OF PRE-OPERATIVE PATIENTS ON CAERULOPLASMIN-CATALYSED OXIDATION OF NORADRENALINE (NA) AND 5-HYDROXYTRYPTAMINE (5-HT)

Pre-operative patient No.	Inhibition of NA oxidation	Acceleration of NA oxidation	Inhibition of 5-HT oxidation	Oxidation of NA by Cu^{2+}
1	±	−	−	+
2	++	−	−	+
3	+	−	−	−
4	−	−	−	−
5	+	−	−	+
6	++	−	−	+
7	++	−	−	+
8	+	−		
9	−	−		+
10	−	−		

TABLE 3. EFFECT OF EXTRACTS FROM NORMAL URINE ON CAERULOPLASMIN-CATALYSED OXIDATION OF NORADRENALINE (NA) AND 5-HYDROXYTRYPTAMINE (5-HT)

Normal No.	Inhibition of NA oxidation	Acceleration of NA oxidation	Inhibition of 5-HT oxidation	Oxidation of NA by Cu^{2+}
1	−	−	−	−
2	−	−	−	+
3	−	−	−	−
4	−	−	−	−
5	−	−	−	+
6	−	−	−	+
7	−	−	−	−
8	+	−	−	+
9	−	−	−	+
10	+	−	−	−

The nature of these materials is at present unknown. They give positive stains with ninhydrin and on treatment with hydrochloric acid (10 N) at 100° for 12 hr give three or four ninhydrin-positive spots, one of which gives a colour with ninhydrin very similar to that given by dimethoxyphenylethylamine. It is possible that these factors are small heteropeptides containing a catecholamine residue. It is hoped to separate and isolate these compounds in sufficient quantity for structural studies to be carried out.

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